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Trichopus Zeylanicus Leaf

Serum Biochemical Alteration

Highlights

Effect of Emblica Officinalis

Knowledge and Epidemiological

Discovering Thoughts, Inventing Future

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Effect of Trichopus Zeylanicus Leaf Extract on Acute Stress Induced Anxiety in Mice

By Raghu Ram. A, S.N. Sri Harsha, D. Yashwanth Kumar & K.S.S.N. Neelima
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Abstract- Trichopus zeylanicus Gaertn. (Dioscoreaceae) [TZ] leaf is traditionally used as a general health tonic in tribal regions of south India. In the present study the effect of alcoholic extract of TZ leaves was evaluated on acute stress induced anxiety in mice at oral doses of 100mg/kg, 250mg/kg and 500mg/kg. Acute stress was induced by restraint stress method and the stressed rodents were evaluated in light and dark model and elevated plus maze. The extract at the doses of 250mg/kg and 500mg/kg showed a significant increase in the number of crossings and reduced time spent in the dark chamber in light and dark model. Further, it significantly reduced the time spent in the closed arm in elevated plus maze as compared to stressed mice. Moreover, TZ significantly reduced stress induced increased plasma corticosterone levels and hyperglycemia in rats.

Keywords: *acute restraint stress, anxiety and trichopus zeylanicus.*

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Effect of Trichopus Zeylanicus Leaf Extract on Acute Stress Induced Anxiety in Mice

Raghu Ram. A [α], S.N. Sri Harsha [σ], D. Yashwanth Kumar [ρ] & K.S.S.N. Neelima [ω]

Abstract- Trichopus zeylanicus Gaertn. (Dioscoreaceae) [TZ] leaf is traditionally used as a general health tonic in tribal regions of south India. In the present study the effect of alcoholic extract of TZ leaves was evaluated on acute stress induced anxiety in mice at oral doses of 100mg/kg, 250mg/kg and 500mg/kg. Acute stress was induced by restraint stress method and the stressed rodents were evaluated in light and dark model and elevated plus maze. The extract at the doses of 250mg/kg and 500mg/kg showed a significant increase in the number of crossings and reduced time spent in the dark chamber in light and dark model. Further, it significantly reduced the time spent in the closed arm in elevated plus maze as compared to stressed mice. Moreover, TZ significantly reduced stress induced increased plasma corticosterone levels and hyperglycemia in rats.

Keywords: acute restraint stress, anxiety and trichopus zeylanicus.

I. Introduction

Severe stressful conditions are responsible for the etiopathogenesis of various psychosomatic disorders. Homeostasis which is maintained by the various neurotransmitters is challenged during stressful conditions. These alterations in neurotransmitter activity result in behavioral changes as well as a cascade of hormonal release from the hypothalamus–pituitary–adrenal (HPA) axis. The imbalance of these monoamines due to prolonged stressful conditions has been associated with a wide range of central and peripheral disorders like anxiety, depression, obsessive compulsive disorder, eating and sleeping disorders, hyperglycemia and decreased immune response (Kalia, 2005; Rashid et al 2008).

The present day life style has increased the physical and psychological demands resulting in an up rise in various stress- related disorders which further necessitates an urgent need to develop agents to overcome these conditions. Traditional medicines are rich in non-specific anti-stress agents which are of increasing clinical significance.

Trichopus zeylanicus, variety Gaertn. (Family: Dioscoreaceae) is an herbaceous, perennial and rhizomatous wild plant grown in Kerala. It is also known as Arogyappacha meaning, greener of health and is used as a health tonic by the tribal population. It is reported that TZ enhancement the swimming performance of rats in forced swimming test. Further, TZ is reported to have several pharmacological activities such as choleretic activity, hepatoprotection, aphrodisiac property and mast cell stabilizing activity (D. A. Evans et al 2002 and A K. Sharma et al, 1989).

In the light of the above information and folklore use, the present study evaluates the effect of Trichopus zeylanicus leaf extract on acute stress induced anxiety in mice.

II. Materials and Methods

Shade dried leaves of TZ were purchased from the local market for the whole batch of experiments. The leaves were authenticated by matching with the reference specimen no. 2129 at the Botany Department, Government Science College, Durg, India.

a) Preparation of Extract

The powdered leaves, (250 g) were loaded in a soxhlet extractor and were defatted with petroleum ether (60–80). The marc was dried and further extracted with 70% ethanol by maceration (Riebling and Walker, 1975). The extract was concentrated on rotary flash evaporator and vacuum dried over anhydrous sodium sulphate. The dried material (34.9%) was stored under refrigeration at 4–8 ◦C until its use.

b) Animals

Male, Swiss albino mice (20-30 g) were used for behavioral studies, whereas biochemical estimation was performed in male Wister albino rats. Each experimental group consisted of at least six animals. The animals were housed for a minimum of five days prior to the pharmacological experiments, with free access to standard rodent pellet diet (Lipton India Ltd) and tap water, and maintained on a 12/12 h light-dark cycle.

All experiments were conducted in accordance with institutional Animal Ethics Committee guidelines. The experimental protocols were approved by the institutional animal ethics committee. The minimum number of animals and duration of observations required to obtain consistent data were employed (IAEC) - SIP/CPCSEA/IAEC/2013/I/02.

c) Extract and standard drug

The hydroalcoholic extract was formulated as suspension using 0.1% Sodium carboxymethyl cellulose (CMC). Ginseng 100mg/kg (Revital™) was used as reference drug. The extract was adjusted to give a fixed

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volume of 10 ml/ kg orally in doses of 100mg/kg, 250mg/kg and 500mg/kg.

d) Acute restraint stress model

In the present study stress was induced using acute restraint stress (Masood et al., 2003) model with minor modifications. The mice were divided into six groups of six animals each of either sex. Stress was induced by restraining the animals in PVC restrainers for a period of 4 hours. Group 1 animals served as normal control were administered 0.5% Sodium CMC in water and were not exposed to stress. Group 2 animals served as negative control as untreated stress induced; Group 3 animals were administered ginseng 100mg/kg orally. While, Group 4, 5 and 6 were administered TZ extract orally at the doses of 100mg/kg, 250mg/kg and 500mg/kg respectively. The animals were pretreated with the extracts and the reference drug for a period of seven days before the induction of stress.

Following the induction of stress the male Swiss albino mice were evaluated for behavioral changes on the Elevated plus maze model, open field test and Light and dark model. A different set of male Wistar rats, treated as above, were used for biochemical analysis. The animals were sacrificed post stress induction by cervical decapitation, the blood was withdrawn from the jugular vein and serum glucose and corticosterone levels were determined.

e) Elevated plus-maze test (EPM)

This test has been widely used to measure anxiety in rodents (Morra et al., 2006). The wooden apparatus, consisted of two open arms (50 cm×10 cm each), two enclosed arms (50 cm×10 cm×40 cm each) and a central platform (10 cm×10 cm), arranged in such a way that the two arms of each type were opposite to each other. The maze was elevated 70 cm above the floor. Immediately after the induction of stress, each animal was placed at the center of the maze, facing one of the enclosed arms. During the 5-min test period, the number of open and enclosed arms entries, plus the time spent in open arms, was recorded. Entry into an arm was defined as the point when the animal places all four paws onto the arm.

f) Open Field Behavior

This behavioral model is based on the induction of anxiety by exposing the animal to a highly novel field environment. The open field area is a circular arena (diameter 48cm) made of thermocol. It has four radial arms projecting from the center (a small circular region) and each arm has slots of equal area to monitor the animal's exploratory behavior. The model is placed at a height of 70 cm. After the induction of stress, each animal was placed at the center of the open field. During the 5 min test period, spontaneous ambulation (number of segments crossed with four paws) was recorded. (Kulkarni et al 1999).

g) Light and Dark model

In Light Dark Model, exploration of rodent is inhibited by bright illuminations. The animals are placed on brightly lit side of a two-compartment chamber and number of crossings between the light and dark side is recorded. One-third of chamber (40 x 60 cm) is darkened with a cover and separated with a wall from otherwise brightly illuminated area. An opening (Diameter 13 cm) allows the animal to pass from illuminated to darkened compartment. At the start of the test, the mouse was placed in the middle of illuminated part of the cage. The number of crossings and time spent in the open arm was registered during 5 minutes (Vogel et al., 1997).

h) Blood Collection

A different set of male Wistar rats, treated likewise, was used for biochemical analysis. The animals were sacrificed immediately after acute stress induction. The blood was collected and separated in a refrigerated centrifuge at 4°C. The serum was stored at -80°C until further analysis of corticosterone and glucose.

i) Estimation of Corticosterone

Serum corticosterone levels were determined by fluorimetric method (Glick D et al 1964) with minor modifications. Briefly, 500 μL of serum was extracted with 2mL of chloroform. The chloroform was further extracted with 1ml of acid alcohol and the fluorescence was measured at 462 nm and 518 nm.

j) Estimation of serum glucose

The serum glucose level was determined using the (GOD–POD method) glucose oxidase–peroxidase–aminoantipyrine and phenol method (Glucose determiNATion kit, Merck) where the quinonemimine dye formed is estimated spectrophotometerically at 540 nm (Philip et al 1994).

k) Statistical Analysis

The data was analyzed using Prism Graph Pad software and showed as mean±S.D. Comparison between control and drug treated groups were made by one-way analysis of variance (ANOVA) followed by Dunett's test, P values of less than 0.05 were considered to be significant.

III. Results

a) Light and dark model

The statistical analysis revealed a significant ($P<0.05$) increase in the number of crossings between the light and dark compartments in mice pretreated with TZ at 250mg/kg and 500mg/kg as compared to the stress control animals. The effect of ginseng (100 mg/kg) was not significantly different from that observed after TZ 500 mg/kg. Further, TZ treatment significantly increased the time spent in the light chamber as

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compared to the stressed rats. The results are showed in the table 1.

b) Elevated plus-maze model (EPM)

The ANOVA revealed a significant increase in the number of entries in the open arm in the normal, ginseng treated and TZ (250 and 500mg/kg) treated animals as compared to the stress control ($P<0.05$). Further, TZ treated animals significantly increased ($P<0.05$) the time spent in the open arm at the doses of 250 and 500mg/kg. The results are shown in table 2.

c) Open field behavior

The results of open field behavior are depicted in table 3. Statistical analysis showed a significant increase in the ambulatory behavior at the dose of 250 and 500mg/kg ($P< 0.05$). However no significant changes in the behavior were observed at 100mg/kg of TZ as compared to the stress control animals.

d) Effect of TZ extract on serum glucose level

The induction of stress by restraining in mice was confirmed by measuring the serum glucose levels. The animals on exposure to acute restraint stress showed a significant increase in blood glucose levels as compared to the normal mice ($P< 0.05$). Further, treatment with TZ extract at a dose of 250 and 500mg/kg significantly countered this elevation in blood glucose level. The results are depicted in the table 4.

e) Effect of TZ extract on serum corticosterone level

Exposure to acute restraint stress resulted in a significant elevation in serum corticosterone level. Further, treatment with TZ at 250mg/kg and500mg/kg significantly reduced the elevated levels of serum corticosterone. The results are depicted in the table 4.

IV. Discussion and Conclusion

The elevated plus maze is considered to be an etiologically valid animal model of anxiety which uses natural stimuli like fear of a novel open space and fear of balancing on a relatively narrow, raised platform that can induce anxiety in mice (Dawson and Tricklebank, 1995). However it was observed after measurement of anxiety states post acute restraint stress induction that the animals showed further pronounced anxious behavior even in other models like open field and light and dark model. Trichopus zeylanicus leaves have shown significant pharmacological effects like enhancement in swimming performance of rats in forced swimming test and aphrodisiac activity (Subramoniam et al, 1997), which further proposes evaluating its effects on stress and stress induced neuropsychological conditions. The present study investigated the effects of hydroalcoholic extract of Trichopus zeylanicus leaves on the acute stress induced anxiety in mice.

Typically a stress response is characterized by the activation of HPA axis resulting in an increase in blood corticosterone levels which in turn lead to an increase in serum triglycerides levels and hyperglycemia. The study indicated that administration of TZ extract significantly countered altered blood glucose and corticosterone levels in animals exposed to acute restraint stress and proves to be a potential antistress agent.

Further, administration of TZ extracts and evaluation of these stress induced animals in models of anxiety revealed a significant lowering of anxiety response such as increase in ambulatory behavior in the open field. The extract also showed a significant increase in the number of crossings in the EPM and light and dark model. Moreover, the results were comparable to Ginseng at 500mg/kg of TZ.

As reported by Sharma et al, TZ has shown significant adaptogenic activity in forced swimming test and milk induced leucocystosis. Likewise the effect of TZ in alleviating symptoms of stress induced anxiety may be attributed to its adaptogenic potential.

Although this study does not suggest anything about the mechanism of antistress potential yet it proves to be a potential lead in this class of drugs and further relates with the works reported by others on its adaptogenic effect which needs to be further evaluated and optimized.

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Table 1 : Effect of TZ on average number of crossings in Light and dark model following acute restraint stress

Groups	Total number of crossings	Time spent in the light chamber (s)
Normal	11.67 ±2.10*	143.22 ±10.34*
Stress control	2.10± 0.24	38.57± 5.13
Ginseng 100mg/kg	10.75±1.10*	156.31±11.89*
TZ 100 mg/kg	4.13±0.97	48.11±2.19
TZ250 mg/kg	7.14±0.86*	105.47±3.77*
TZ500 mg/kg	10.7±0.74*	141.37±10.27*

*, P< 0.05; in comparison to stress induced animals (n = 6 animals in each group)

Table 2 : Effect of TZ on Elevated plus maze behavior following acute restraint stress in mice

Groups	Time spent in the open arm(seconds)	Number of entries in the open arm
Normal	268.97±10.34*	11.24±0.60*
Stress control	37.88±2.14	2.14±0.36
Ginseng 100mg/kg	260.13±14.80*	10.34±0.90*
TZ 100 mg/kg	42.13±3.87	4.21±0.43
TZ250 mg/kg	167.42±15.40*	6.12±1.70*
TZ500 mg/kg	246.31±12.70*	8.41±1.90*

*, P< 0.05; in comparison to stress induced animals (n = 6 animals in each group)

Table 3 : Effect of TZ on open field behavior following acute restraint stress in mice

Groups	Number of segment travelled
Normal	25.2±2.40*
Stress control	5.67±0.37
Ginseng 100mg/kg	22..27±1.60*
TZ 100 mg/kg	6.41±1.0
TZ250 mg/kg	17.18±1.61*
TZ500 mg/kg	20.31±1.47*

*, P< 0.05; in comparison to stress induced animals (n = 6 animals in each group)

Table 4 : Effect of TZ on serum glucose and corticosterone level following acute restraint stress in mice

Groups	Serum glucose levels (mg/dl)	Serum corticosterone levels(mg/100ml)
Normal	90.28± 8.59 **	126.9±20.31
Stress control	150.46 ± 9.64	311.20±47.26
Ginseng 100mg/kg	101.51 ±6.47 **	157.3±17.88***
TZ 100 mg/kg	140.31±6.41	263.62±23.14
TZ250 mg/kg	126.34 ±11.12 *	198.51±18.77*
TZ500 mg/kg	115.74±10.13**	166.47±23.14**

*, P< 0.05; in comparison to stress induced animals (n = 6 animals in each group)

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Pregnancy Related Biometrical Changes in the Ovaries and Uterus of the Balami Sheep

By Alhaji Zubair Jaji, A.I. Akanmu, R.A.Buduwara, N. Elelu , E. Kigir , M.B. Mahre & B. Gambo
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Abstract- A pregnancy related biometrical study was carried out on ovaries and uterus of the Balami sheep in Maiduguri, Nigeria, with the aim of documenting information on it. Dimensions of 10 non-pregnant and 30 apparently normal pregnant Balami sheep ovaries and uteri obtained from the Maiduguri Municipal abattoir were analyzed. The length, diameter, thickness and weight of left ovary showed no significant increase along the stages of gestation while only the length of the right ovary showed very significant ($p<0.01$) and extremely significant ($p<0.001$) increases during the second and third stages of gestation. The length and diameter of the left uterine horn showed levels of significant increases ($p<0.05$ – $p<0.001$) during the last two stages of gestation while those of the right uterine horn showed extremely significant increases ($p<0.001$) during these periods.

Keywords: balami sheep, biometry, ovary, pregnancy, uterus.

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PRGNANCYRELATEDBIOMETRICALCHANGESINTHEOVARIESANDUTERUSOFTHEBALAMISHEEP

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Pregnancy Related Biometrical Changes in the Ovaries and Uterus of the Balami Sheep

Alhaji Zubair Jaji [α], A.I. Akanmu [σ], R.A.Buduwara [ρ], N. Elelu [ω], E. S. Kigir [¥], M.B. Mahre [§] & B.Gambo [χ]

Abstract- A pregnancy related biometrical study was carried out on ovaries and uterus of the Balami sheep in Maiduguri, Nigeria, with the aim of documenting information on it. Dimensions of 10 non-pregnant and 30 apparently normal pregnant Balami sheep ovaries and uteri obtained from the Maiduguri Municipal abattoir were analyzed. The length, diameter, thickness and weight of left ovary showed no significant increase along the stages of gestation while only the length of the right ovary showed very significant ($p<0.01$) and extremely significant ($p<0.001$) increases during the second and third stages of gestation. The length and diameter of the left uterine horn showed levels of significant increases ($p<0.05$ – $p<0.001$) during the last two stages of gestation while those of the right uterine horn showed extremely significant increases ($p<0.001$) during these periods. The length of the uterine body showed extremely significant increase ($p<0.001$) towards the last two stages of pregnancy while the cervical diameter showed extremely significant increase during the last stage of gestation. It was concluded that in the Balami sheep, pregnancy does not seem have significant effect on the dimensions and weights of the left ovary but do have levels of significant increase ($p<0.01$ – $p<0.001$) in the length of the right ovary during the last two stages of gestation. Conversely, pregnancy has levels of significant effect on the dimensions of the horns, body and cervix of the uterusfrom mid gestation to full term.

Keywords: balami sheep, biometry, ovary, pregnancy, uterus.

I. Introduction

The domestic sheep, Ovis. aries, is a ruminant Chordate of the Bovidae, subfamily in the Artiodactyla, family, order Mammalia. It shares a common Class, Caparinae, and Phylogenetic origin, with the goat. (Geoffrey, et al., 2005).

Sheep have most likely descended from the wild Mouflon of Europe and Asia, one of the earliest animals to be domesticated for agricultural purposes (Sheep (2012). Sheep are important part of the global agricultural economy (Weaver, 2005).

Sheep are raised for fleece, meat (lamb, hogget or mutton) and milk. and continue to be important for wool and meat today. They are also occasionally raised for pelts, as dairy animals, or as model animals for science. Sheep husbandry is practiced throughout the majority of the inhabited world and has been fundamental to many civilizations (Sheep (2012).

Numbering a little over 1 billion worldwide, sheep are the most numerous species in their genus, Ovis. The population of sheep in the world is estimated at 1.3 billion flocks, Africa has 20 million flocks, about 3.4 million of which was estimated to be found in Nigeria (Geooff, et al., 2005; RIM 1992). According to FAOSTAT, 2012, the population of sheep in Nigeria is currently estimated at 33.9 million making up 3.1% of the world's total.

The Balami sheep is the largest native sheep in Nigeria and is favoured as a stall-fed breed by Muslims throughout the Nigerian Middle Belt. It is white and hairy with pendulous ears, a bulbous nose and a long thin tail: rams have a throat ruff and are homed but ewes are normally polled (Blench, 1995).

Reproductive performance is economically important in small ruminant because of its effect on the number of offspring produced per year (Greyling, 2000). To maintain a good reproductive performance a clear idea about the reproductive organs of small ruminant is necessary. The biometry of genital tracts of the female reveals the overall wellbeing of the animals. The knowledge of biometrical status of female genital tract is essential to perform artificial insemination, pregnancy diagnosis and dealing with infertility problems (Kunbhar et al., 2003) and its treatment (Kumar et al., 2004). The information on biometry of the reproductive tract of the pregnant Balami goat is rare in literatures. This present study aims at documenting information on the progressive biometrical changes in the ovaries and uterus during pregnancy in this breed of sheep.

II. Materials and Methods

These studies were carried out on uteri and ovaries of thirty pregnant and ten non-pregnant adult Balami sheep at the Maiduguri Metropolitan abattoir. The animals were apparently healthy, sexually matured and of varying ages (2 - 3½ years and above) and weights. The organs were collected immediately after slaughter.Scalpels and blades were used to incise, excise, separate and dissect the organs. Tanning coat and a superior tailoring rule (Butterfly Brand) were used to measure length and widths of uteri and ovaries. The ovarian thickness was measured using micrometer screw gauge (Mitutoyo Brand). Lengths of uteri and

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ovaries were measured in centimeters. Ovarian weights were measured in grams using electronic precision balance (Metra brand).

The 20 weeks gestation period of the Balami sheep was divided into three stages (6-8weeks, 8-14weeks and 14-20weeks).The stages of gestation were established, after measurements of the dimensions of the ovaries and uteri, by determining the age of fetuses associated with each pregnancy. This was done through comparing their crown-rump length and body weight measurements with those of the tropical ovine fetuses from the Maiduguri abattoir, as reported by Sivachelvan et al. (1996).

The length of either ovary was the distance between its anterior and posterior ends. The breath was the distance between its attached and free borders and the thickness, the distance between its medial and lateral surfaces. The length of the uterine body was the distance from the point of bifurcation of the uterine horns to the tip of internal os of the cervix and the breath was the greatest distance of its right and left walls. The length of the uterine horn was the distance from the middle of the point of bifurcation of the uterus utero-oviductal junction and the breath the distance between its right and left walls. The length of the cervix was the distance from the tip of internal os to the tip of external os of the cervix, and the breath the greatest distance of its right and left walls.

The differences between the above dimensions along the three periods of gestation were tested using the ANOVA from the computer statistical software, Graph pad Instat®, version 3.06, 32 bit for Windows.

III. Results

In the adult non-pregnant Balami ewe studied the ovaries (Figure 4.1) were observed to be almond in shape. In the pregnant ewe however, the ovaries were characterized with copora lutea that altered their size and form. The copora lutea were firm in consistency along the stage of gestation.

In adult non-pregnant Balami ewe studied, the left ovary measured 1.63+0.61cm in length, 1.43+0.32cm in diameter, 1.04+0.24cm in thickness and 1.04+0.34g in weight, while the right ovary measured 1.15+0.55cm in length, 1.35+0.38cm in diameter, 0.76+0.44cm to thickness, 0.79+0.38g weight. . The measurements of the left ovary showed no significant increase along the stages of gestation while only length of the right ovary showed very significant ($p<0.01$) and extremely significant ($p<0.001$) increases respectively during the second and third stages of gestation (Table1).

In the adult non-pregnant Balami ewe, the left uterine horn (figure1) measured 11.78+1.86cm in length and 3.56+0.70cm in diameter, while the right uterine horn measured 11.76+2.03cm in length and 4.07+1.81cm in diameter. . The length and diameter of the left uterine horns showed levels of significant increases ($p<0.05$ – $p<0.001$) during the last two stages of gestation while those of the right uterine horn showed extremely significant increases ($p<0.001$) during the said periods (Table1).

In the non-pregnant Balami ewe, the uterine body (figure 1) measured 8.67+1.21cm in length and 6.33+1.73cm in diameter. The length of the uterine body showed extremely significant increase ($p<0.001$)while diameter showed levels of significant increases ($p<0.01$ – $p<0.001$) throughout pregnancy (Table 1).

In the non-pregnant Balami ewe, the cervix measured 6.69+2.61cm in length and 2.22+0.93cm in diameter. The cervical diameter showed extremely significant increase during the last stage of gestation (Table 1).

Figure 4.1 : Photograph of the reproductive system of a of non-pregnant Balami ewe showing the ovaries (O) uterine horns (UH), Uterine body (UB) and Cervix (C).

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Table1 : Pregnancy related biometrical changes in the ovaries and uterus of the Balami Sheep with good body condition scores in Maiduguri, Nigeria

Organs		Dimension (Mean ± SD)	Physiological state of ewe			
			Non-Pregnant	Pregnant		
				1st Stage	2nd Stage	3rd Stage
Ovary	Left	Length (cm)	1.63±0.61	1.63±0.34 ns	1.59±0.42 ns	2.04±0.62 ns
		Diameter (cm)	1.43±0.32	4.81 ±0.33 ns	1.22±0.46 ns	1.46±0.23 ns
		Thickness (cm)	1.04±0.24	1.11±0.34 ns	1.07±0.18 ns	0.59±0.63 ns
		Weight (g)	1.04±0.34	1.08±0.49 ns	0.74±0.23 ns	0.99±0.43 ns
	Right	Length (cm)	1.15±0.55	1.58±0.57 ns	1.85±0.29**	2.04±0.36***
		Diameter (cm)	1.35±0.38	1.39±0.52 ns	1.54±0.38 ns	1.51±0.29 ns
		Thickness (cm)	0.76±0.44	1.08±0.40 ns	0.81±0.40 ns	1.14±0.35 ns
		Weight (g)	0.79±0.38	0.94±0.33 ns	1.22±0.46 ns	1.04±0.37 ns
Uterine horn Left		Length (cm)	11.78±1.86	16.66±5.79 ns	23.59±0.45**	31.88±12.69***
		Diameter (cm)	3.56±0.70	8.43±3.54 ns	11.65±4.72*	19.22±6.83 ns
	Right	Length (cm)	11.76±2.03	15.42±5.02 ns	26.5±10.08***	26.65±6.82***
		Diameter (cm)	4.07±1.81	6.28±2.48 ns	13.8±2.93***	14.89±2.09***
Uterine body		Length (cm)	8.67±1.21	15.52±5.29 ns	23.5±8.73***	35.54±7.43***
		Diameter (cm)	6.33±1.73	14.36±4.67**	25.16±5.04***	30.94±5.46***
Cervix		Length (cm)	6.69±2.61	6.59±1.02 ns	5.5±1.31 ns	6.22±0.58 ns
		Diameter (cm)	2.22±0.93	2.42±0.45 ns	2.85±1.03 ns	4.69±0.91***

ns:Not Significant
*:Significant –P<0.05
**:Very Significant –P<0.01
***:Extremely Significant –P<0.001

IV. Discussion

In adult non-pregnant ewe studied, the ovaries were almond in shape. Ovaries of the pregnant ewe, were characterized with corpora lutea, which altered their size and form. The copra lutea were firm in consistency along the stages of gestation.The development and further increase in corpus luteum across the stages of gestation were associated with a significant increase in the overall size of ovaries in agreement with Smith (1986). The results of the length, diameter, of the present study of non-pregnant ewe show slight increase when compared with those of Hafez (1987). This may be due to breed related difference. Feeding of ewes on bush leaves, dry fodder or grasses with less supplemented feeds from two or three weeks of age have also shown to cause retarded growth and development of reproductive their tract (Obwolo, 1992). It can also be due to climatic effect of the first dry season when growth may be seriously retarded Oyeyemi et al (2001). The gravid and non-gravid right ovaries were larger in dimensions and heavier in weight as compared to left ones which confirms the fact of right ovary being more active than the left one, in agreement with Pineda (2003), as in doe (Gupta, 2011; Jaji et al. (2012) and cow (Pineda (2003). The left ovary is the most active in the camel (Jaji et al., 2010) and mare and sow (Pineda, 2003).

Just like in the doe (Jaji et al., 2012), the uterus of the ewe of the present study was observed to be of the bicornuate type. In the non-pregnant ewe, the length and diameter of the left and right uterine horn show a slight decrease when compared with those reported by Smith (1986). The increased length and diameter of the left uterine horns could be attributed to the increases in the fetal sizes and fluids associated with each stageof gestation as incamel (Jaji et al., 2010).There is no significant difference between dimensions of the left and night ovaries and uterine horns both in the pregnant and non-pregnant ewes. This could be attributed to twinning that is often associated with the ovine pregnancy, which engages ovary and horn of either side of the reproductive system of the ewe.

In non-pregnant ewe, the length and diameter of the non-gravid uterine bodyrecoded in this study were higher than the values recorded by Sisson and Grossman (1975). The discrepancy could be due to breed variation (Obwolo, 1992). The uterine body of the pregnant ewe, showed very significant increase in biometrical values during the three stage of gestation. These were attributed to the attendant increases in fetal

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sizes and fluids associated with each stage of gestation as in camel (Jaji et al., 2010).

The length and diameter of the cervix of non-pregnant ewe show a slight decrease when compared to those recorded by Smith (1986) in the doe.The anatomy of the sheep cervix is highly variable between animals and may explain the differing success of transcervical Artificial Insemination between individuals (Keshaw et al., 2005). Breed, age, parity and physiological state influence the length of the ovine cervix. Themean length of the cervical canal has been described as, 6.5, 5.5 and 6.7 cm (Fukui Y & Roberts, 1978; Halbert, 1990; More, 1984) respectively and the length ranges from 5.7 to 10 cm (Abusineina , 1969) illustrating the high variabilitybetween individuals.

The results of this study have established the baseline data for the dimensions of the two vital organs in the reproductive system of the Balami ewe. This information will make diagnosis of the various abnormalities of these organs easier. More of such work on other local (Udder and Yankasa) and internationalbreeds need to be carried out for better understanding of reproduction in this species. Further histological studies need to be undertaken to determine the sequential histological changes during pregnancy in this breed, towards a better understanding of its reproductive anatomy.

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Hematological and Serum Biochemical Alteration in Cattle and Buffaloes Suffering from Natural Infection of Black Quarter

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Abstract- Hematological and serum biochemical changes in cattle suffering from natural outbreak of Black quarter (BQ) in different areas of Punjab, Pakistan were studied. Blood samples from infected cattle were subjected to TLC, TEC, DLC, hemoglobin and PCV while serum samples for estimation of Cpk, ALT and AST (n=50). It was found that mean erythrocyte count decreased significantly ($P < 0.05$) while mean leukocyte count increased significantly ($P < 0.05$) in diseased animals. On the other hand mean Hb, platelets count and PCV in diseased animal did not differ significantly ($P > 0.05$) as compared to healthy animals.Average DLC values were found varying to great extent. It was found that mean neutrophils and lymphocytes (%) increased significantly ($P < 0.05$) while those of monocytes and basophils (%) increased non-significantly ($P > 0.05$) in diseased animals.

Keywords: black quarter, cattle, buffalo, serum chemistry, hematology.

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HEMATOLOGICALANDSERUMBIOCHEMICALALTERATIONINCATTLEANDBUFFALOESSUFFERINGFROMNATURALINFECTIONOFBLACKQUARTER

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Hematological and Serum Biochemical Alteration in Cattle and Buffaloes Suffering from Natural Infection of Black Quarter

A. Idrees [α], Z. I. Chaudhary [σ] & M. Younus [ρ]

Abstract- Hematological and serum biochemical changes in cattle suffering from natural outbreak of Black quarter (BQ) in different areas of Punjab, Pakistan were studied. Blood samples from infected cattle were subjected to TLC, TEC, DLC, hemoglobin and PCV while serum samples for estimation of Cpk, ALT and AST (n=50). It was found that mean erythrocyte count decreased significantly ($P < 0.05$) while mean leukocyte count increased significantly ($P < 0.05$) in diseased animals. On the other hand mean Hb, platelets count and PCV in diseased animal did not differ significantly ($P > 0.05$) as compared to healthy animals.Average DLC values were found varying to great extent. It was found that mean neutrophils and lymphocytes (%) increased significantly ($P < 0.05$) while those of monocytes and basophils (%) increased non-significantly ($P > 0.05$) in diseased animals. On the other hand serum biochemical analysis revealed that there was a significant increase ($P > 0.05$) in CpK, ALT and AST levels in diseased as compared to healthy animals.

Keywords: black quarter, cattle, buffalo, serum chemistry, hematology.

I. Introduction

Black quarter is important disease of cattle and buffaloes causing significant mortality in Pakistan. Cattle and buffaloes are domesticated ruminants facing lot of challenges in Pakistan like production, management, nutrition and health care problems. Clostridia are commonly found in environment as well as in the intestinal tract of humans and of many animals. Several Clostridium species are pathogenic to humans, domestic animals and/or wildlife and are responsible for well knownclostridial diseases such as gas gangrene, botulism, pseudo-membranous colitis and food borne illness (Hatheway, 1990).

Clostridiumchauvoei is a gram-positive, spore-forming anaerobe that has strong hemolytic activity. Within the space of few days disease occurs and within a herd it is more likely to be affected a number of animals. The disease is enzootic in particular areas, especially when they are subject to flooding; such an area may vary in size from a group of farms to an individual field. The case fatality rate in blackleg approaches 100% (Radostitset al., 2006). It causes serious toxemia and high mortality in cattle, sheep and many other ruminants associated with spore contaminated soil. It is considered the most important Clostridium producing economic losses in livestock (Smith and Williams, 1984).

Knowledge and understanding of the epidemiological profile of contagious diseases is quite necessary in order to devise strategies to eradicate diseases of sporadic nature. Seasonal prevalence of Black Quarter in different areas of Punjab can be proved as a useful tool to understand the pattern and mode of transmission of disease. The parameters like geographical and seasonal distribution of contagious diseases were recorded and analyzed during scanning surveillance (Khan, 2010).

This disease caused huge economical losses in the form of mortality of cattle and buffaloes. Lack of research on this pathogen in Pakistan created many difficulties in the control, prevention and management. Hence the present study was planned to find suitable solution for the problem. The objective of this study was to study hematology and serum biochemistry of infected animals in different districts of the Punjab, Pakistan.

II. Materials and Methods

A survey of prevalence of black quarter was conducted in different districts of the Punjab province and samples were collected from suspected and infected animals to explore the hematology and serum chemistry.

Approximately 2 ml blood sample was collected from each of the suspected animals with the help of sterile needle and poured in ethylenediaminetetra-acetic acid (EDTA) mixed vaccutainers. haematological parameters studied were total erythrocyte count (TEC), haemoglobin (Hb), haematocrit, thrombocyte count, total leukocyte count (TLC) and differential leukocyte count (DLC). All these parameters were simultaneously performed in an automated hematology analyzer (Beckhim and Coleman, USA). The results were obtained in printable form with the help of printer attached with the instrument.

Animal were divided in four experimental groups and three groups were infected artificially with Clostridium cha obtained from field. Serum samples were collected from animals in all groups at scheduled

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(0, 10, 20, 30) hours post infection for three days consecutively. Blood glucose was measured by taking a drop of blood from each animal on code free strip and reading was noted by Glucometer (code free, China).

a) Creatinine Phospho-Kinase (CpK)

Creatinine kinase was assayed from serum samples using Fortress Diagnostics kit BXC0452 – CK-MB. The readings of each sample were recorded using spectrophotometer.

III. Results

Hematological data revealed a significant decrease ($P < 0.05$) in leukocyte count on second and third day of sampling and is non-significant ($P > 0.05$) at 1stday of sampling (Fig. 2). Mean values of total erythrocyte count were significantly decreased ($P < 0.05$) while there was no significant difference ($P > 0.05$) observed on 1stof sampling (Fig. 1). There was significant decrease ($P < 0.05$) of hemoglobin level in all treatment groups as compared to healthy animals (Fig. 3). Mean platelets count was significantly increased ($P < 0.05$) on 3rd day of sampling but it was non-significant ($P > 0.05$) on 1st and 2nd day of sampling (Table 1). Data were analyzed through ANOVA and DMRT was used for the comparison of means of different treatment groups using SAS (SAS Int. Cary, North Carolina, V 9.1).

Table 1 : Mean Comparative Hematology of infected and non-infected cattle (n=50)

Days of Infection	White Blood Cells / µl	Red Blood Cells 10^6/ µl	Hemoglobin g/dl	Packed Cell Volume %	Platelets 10^3/ µl
1st sampling	8130 ±558.52 [a]	6.647 ± 0.095 [b]	9.57±0.175 [bc]	28.16±0.56 [b]	375±11.18 [ab]
2nd Sampling	6965±461.93 [b]	7.55 ±0.40 [a]	9.09±0.26 [bc]	28.16±0.56 [b]	375±11.18 [b]
3rd sampling	5514±534.13 [b]	5.56 ±0.29 [c]	10.09 ±0.33 [b]	28.16±0.56 [b]	388.75±19.40 [a]
Normal	8140 ±258.47 [a]	7.47±0.16 [a]	11.21 ±0.18 [a]	28.1±0.81 [a]	347.75 ±7.60 [b]

*Means with different superscripts in columns are significantly different at ($P < 0.05$)

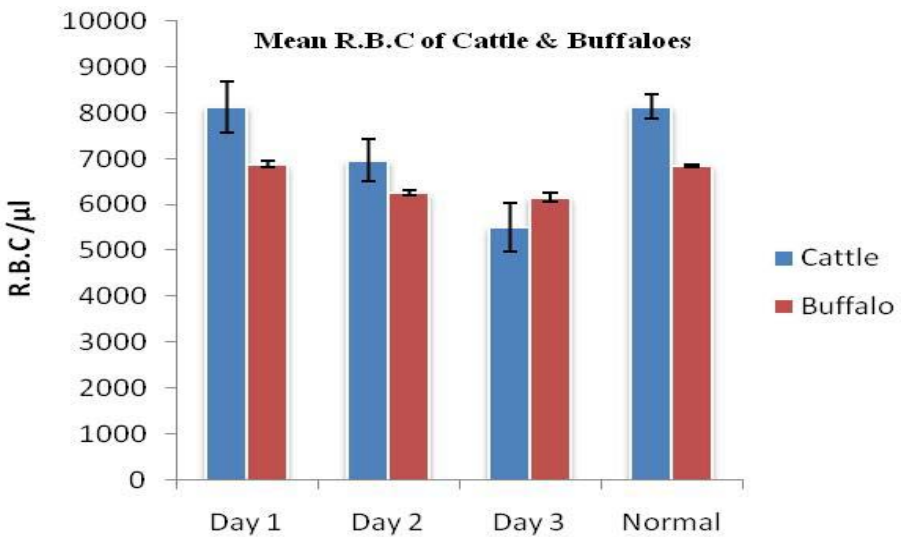

Figure 1 : Mean RBC count for cattle and buffaloes (n=50)

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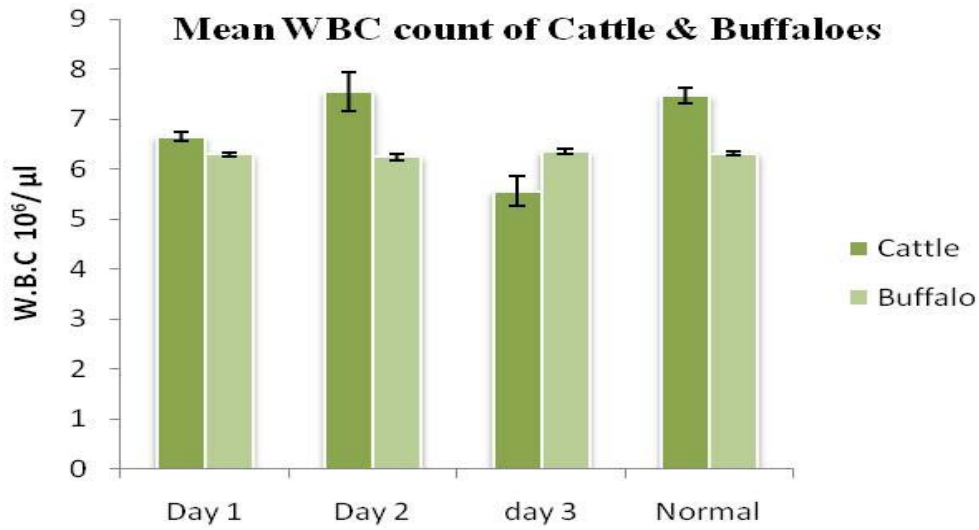

Figure 2 : Mean WBC count for cattle and buffaloes (n=50)

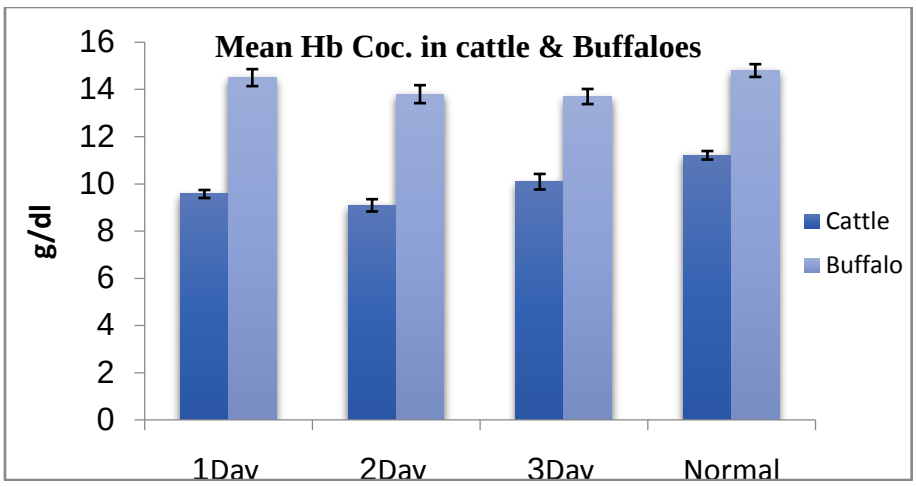

Figure 3 : Mean Hbconcentration for cattle and buffaloes (n=50)

Means with different superscripts in columns are statistically significant different (P>0.05). Results summarized in Table 2 indicate there was a significant increase in neutrophils count (P >0.05) noted as compared to healthy buffaloes at each sampling day but they were not found significantly different (P<0.05) among each other. Similarly Lymphocyte count was significantly decreased as compared to normal cattle while not significant (P<0.05) with other treatment groups (Fig. 3).

Table 2 : Differential Leukocyte count of cattle infected with BQ (n= 50)

Days of Infection	Neutrophils %	Lymphocytes %	Eosinophils %	Monocytes %
1st Sampling	64.55 ± 2.28[a]	21.30± 1.46[b]	9.15± 0.90[b]	5.35± 0.6[a]
2nd Sampling	66.85± 1.42[a]	22.1± 1.45[b]	7.65± 0.34[c]	3.40± 0.30[a]
3rd Sampling	68.1± 1.13[a]	20.90± 1.21[b]	7.75± 0.35[c]	3.25± 0.29[a]
Normal	32.80 ± 4.76[b]	55 ± 8.1[a]	3.70± 1.30[a]	8.50 ± 2.49[b]

*Means with different superscripts in columns are significantly different at (P < 0.05)

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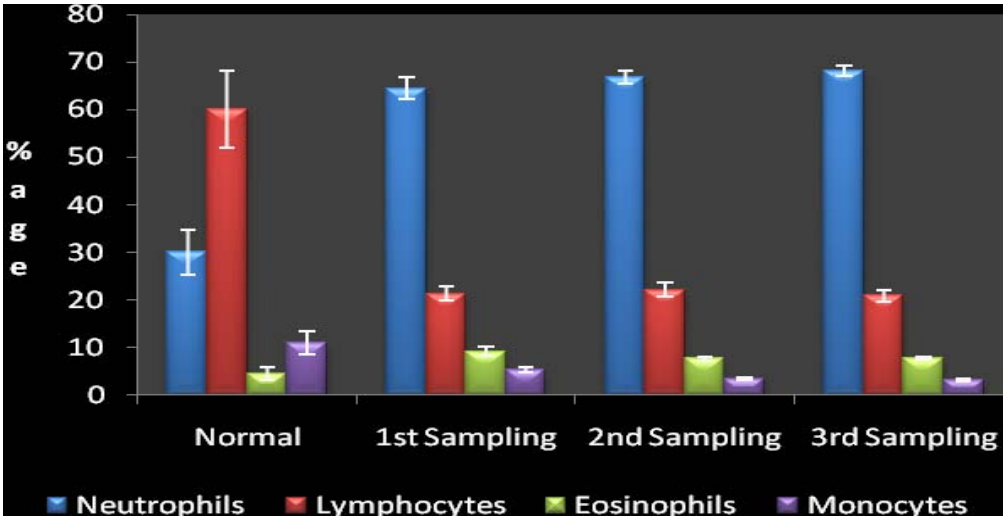

Figure 4 : Differential leukocyte Count of Cattle infected with BQ (n=50)

Differential leukocyte count for buffaloes is presented in Table 3 andthe results show that there was significant increase in neutrophils count in all treatment groups (P < 0.05) as compared to normal while there opposite trend i.e. decrease in lymphocyte count. Monocytes count was also showing increase as compared to normal groups (Fig. 4).

Table 3 : Differential Leukocyte count of buffaloes infected with BQ (n= 50)

Days of Infection	Neutrophils %	Lymphocytes %	Eosinophils %	Monocytes %
1st Sampling	58.15 ± 1.77[a]	24.90± 1.04[b]	7.45± 0.61[a]	9.65± 0.56[a]
2nd Sampling	61.3± 1.56[a]	22.15± 1.24[b]	8.0± 0.53[a]	8.55± 0.45[a]
3rd Sampling	62.05± 1.45[a]	19.88± 1.21[b]	7.88± 0.49[a]	8.41 ± 0.49[a]
Normal	34.0 ± 1.17[b]	54.45 ± 0.81[a]	6.80± 0.61[a]	4.75 ± 0.41[b]

Means with different superscripts in columns are significantly different at ((P < 0.05)

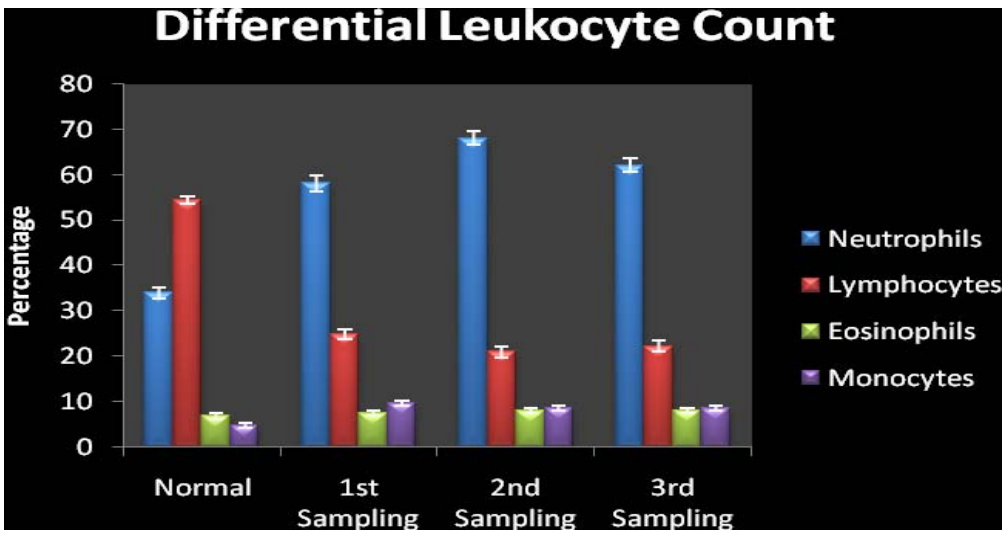

Figure 5 : Differential leukocyte Count of Buffaloes infected with B.Q (n=50)

IV. Serum Biochemical Tests

Results of biochemical test are given in Table 4. Means with same superscripts in a column are not significantly different (P <0.05) as shown in Table 4. It can be inferred from the results that the mean values of Blood glucose were increased significantly (P <0.05) on 1st day of sampling and then decreased gradually and were non-significant among each other during 2nd and

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3rd day of sampling (Fig. 6). Mean values of CPK also increased much significantly (P >0.05) in infected cattle as compared to normal during 1st day of sampling and then showed a gradual decrease in the subsequent sampling days having non-significant difference among each other (Fig. 7).Mean ALT conc. Was also found significantly higher (P >0.05) in all treatment groups as compared to healthy animals (Fig. 8), while interestingly AST levels were significantly higher at 1st sampling day (P >0.05), insignificant at 2nd sampling (P <0.05) and significantly lower than normal at 3rd Sampling day (Fig. 9).

Table 4 : Serum Biochemical Values for Cattle Infected with BQ at different days of Disease (n=50)

Days of Infection	Blood Glucose m.mol/ l	CPk IU/ l	ALT IU/l	AST IU/l
1st Sampling	8.85 ± 0.56[a]	702.19±116.77[a]	43.85 ± 0.48[a]	55.0 ± 9.52[a]
2nd Sampling	7.80 ± 0.42[ab]	555.75 ± 87.79[ab]	42.87 ± 0.48[a]	33.26 ± 5.28[b]
3rd Sampling	7.07 ± 0.21[bc]	325.17 ±55.68[bc]	43.10 ± 0.67[a]	20.00 ± 1.99[bc]
Normal	6.19 ± 0.82[c]	114.2 ±16.25[c]	18.85 ± 2.53[b]	38.60 ± 3.86[c]

*Means with different superscripts in columns are significantly different at (P < 0.05)

Table 5 : Serum Biochemical Values for Buffaloes Infected with BQ at different days of Disease (n=50)

Days of Infection	Blood Glucose m.mol/ l	CPk IU/ l	ALT IU/l	AST IU/l
1st Sampling	3.87 ± 0.19[a]	149.80 ± 6.0[a]	39.93 ± 2.37[a]	139.60 ± 7.34[a]
2nd Sampling	4.03 ± 0.22[ab]	111.08 ± 6.73[ab]	38.35 ± 2.37[a]	105.34 ± 4.87[b]
3rd Sampling	3.58 ± 0.22[ab]	134.63 ± 9.88[ab]	36.61 ± 1.97[a]	94.80 ± 4.06[b]
Normal	3.2 ± 0.87[b]	67.7 ± 3.68[b]	36.2 ± 3.80[a]	105 ± 5.56[b]

*Means with different superscripts in columns are significantly different at (P < 0.05)

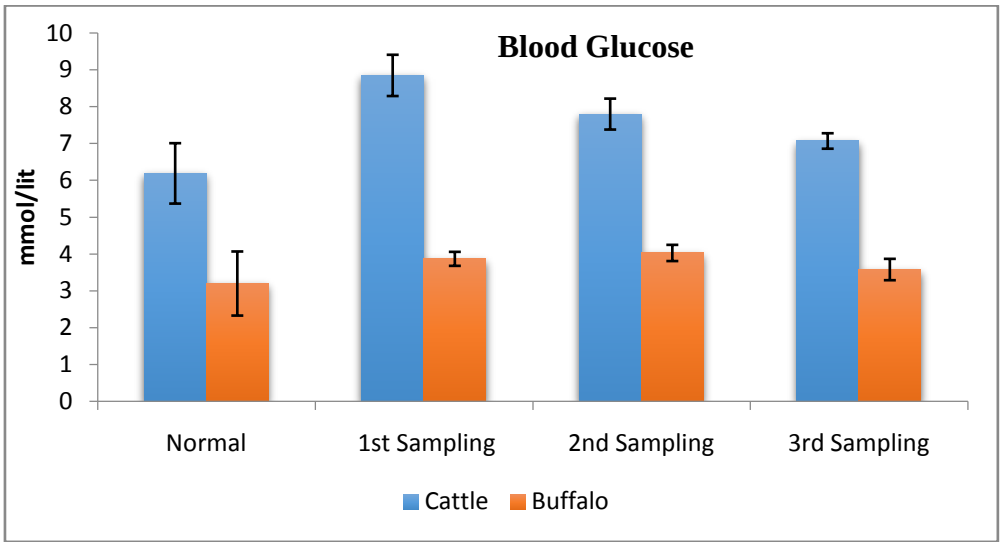

Figure 6 : Comparative blood glucose levels in cattle and buffaloes (n=50)

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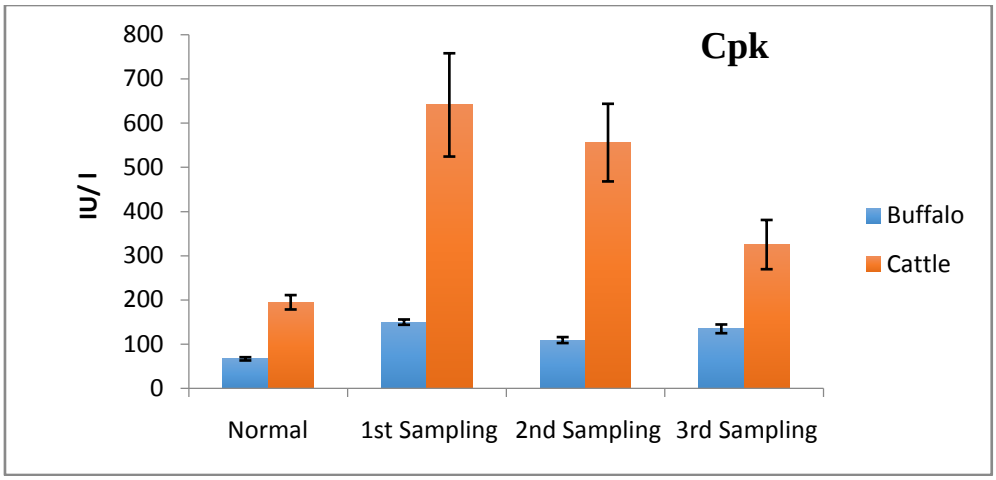

Figure 7 : Comparative CpK levels in cattle and buffaloes (n=50)

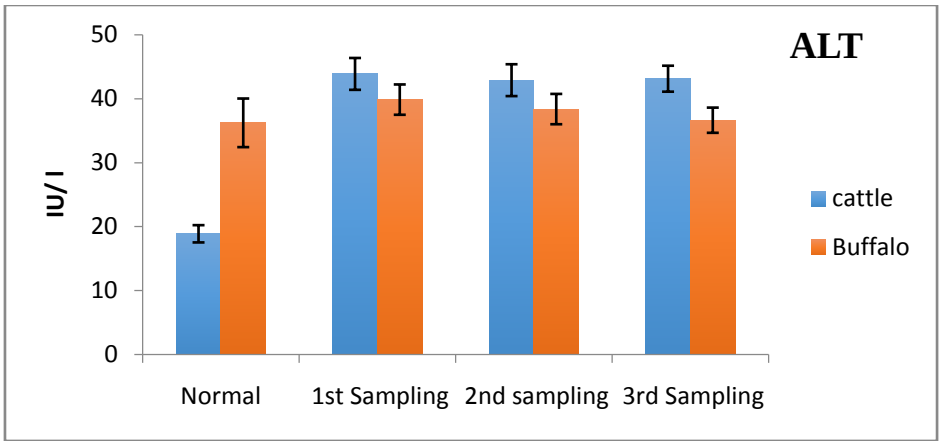

Figure 8 : Comparative ALT levels in cattle and buffaloes (n=50)

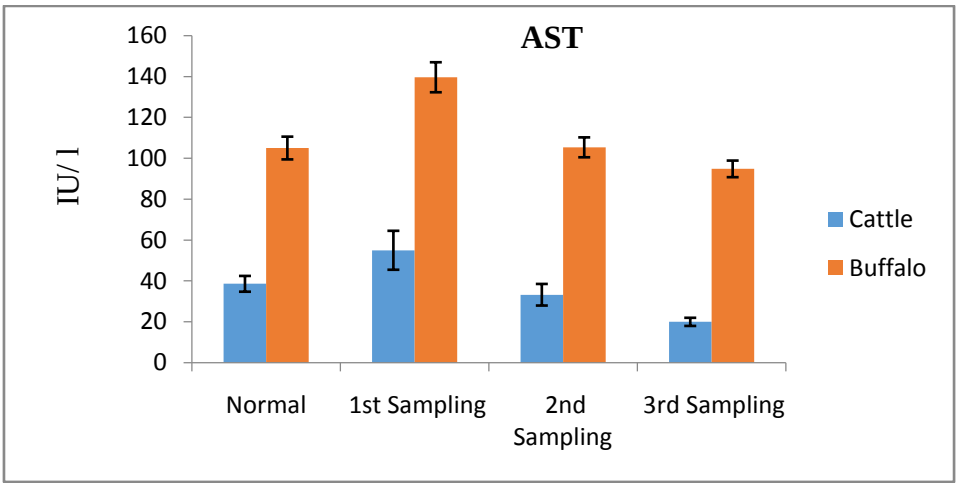

Figure 9 : Comparative AST levels in cattle and buffaloes (n=50)

Data regarding serum enzyme level estimation for healthy and infected buffalo is shown in Table 5. A significantly higher level of CPK and ALT ($P < 0.05$) was observed in 1st sampling group subsequently decreasing in next treatment groups while the values of AST also increased in 1st sampling group significantly while it continued to decrease in 2nd and in 3rd group it was significantly decreased than normal (Fig. 7, 8, 9).

V. Discussion

Although previously reported literature emphasized on the fact that hematological values are not much significant to tell about the course of infection and disease pathogenesis. Yet some parameters have some clinical importance. In the present study, significant leukopenia was noted, however, decreasing

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tendency was observed with the passage of time. Singh et al. (1991) reported somewhat similar pattern of leukopenia in experimentally infected hill bulls. The results of present studies are also in agreement with the findings of Usehet al. (2008). The studies conducted in our experiment reveal that during day 1 and 3 mean WBC's count was significantly decreased while during day 2 it was found non-significant with the healthy animals. The possible reason behind leucopenia may be attributed to the production of neuraminidase by Clostridiumchauvoei. The outcome of this neuraminidase is to deacylate leukocytes, leading to their decrease concentration in peripheral blood (Esievo and Saror, 1983).

Observations regarding Erythrocyte count revealed a significant decreased tendency during 1st and 3rd day of sampling while it was not significantly different from normal during 2nd day of sampling. These results are in contrary with the findings of Singh et al. (1993), who reported an increase in TEC in the all the infected groups of cattle. The reason for this variability may be the course and nature of disease occurring naturally versus the experimental inoculation of the infectious agent. The decrease in erythrocyte count may also be the consequence of hemorrhages and hemolysis due to the effect of toxins produced by the bacteria (El-sawiet al., 1989).

Mean Hb Concentration and PCV values were found significantly increased as compared to normal healthy cattle attributing to the effect of loss of Plasma volume as a result of dehydration (El-Sawiet al., 1989). These results were corroborated by the studies reported by Usehet al. (2008). Another possible reason for increase in PCV may be due to the activity of neuraminidase consequential to enhance vascular permeability leading to edema, hypovolemia, hemoconcentration and ultimately leading to decreased PCV in infected animal (Useh, 2002; Usehet al., 2006). The significance of present findings were strengthened by the results of Singh et al. (1993) which also concluded his studies reporting a significant increase in PCV and Hb. Concentration in the experimentally infected bulls.

Platelets count in all the sampling days was found to be increased ($P < 0.05$) as compared to normal group. The results of this finding about this particular parameter are in contrast to the results of studies of some other researchers (El-sawiet al., 1989; Singh et al., 1993) have shown thrombocytopenia in their respective studies. One of the hypotheses behind this discrepancy may be the nature of infection and healing tendency of edema found more pronounced in the present studies relating to an increased production of platelets.

Differential leukocyte count was observed with variable trend in all infected groups when compared to normal healthy group. Neutrophils count was significantly much higher ($P > 0.05$) in all sampling days while the values of lymphocytes and monocytes were observed gradually increasing throughout from day 1 to day 3 of infection. On the other hand values of eosinophil count were significantly higher on day 1 decreasing gradually subsequently. The results of present study were in somewhat agreement with the findings of (Singh et al., 1993; Usehet al., 2008). The conclusion of their findings was based on the fact that there was lymphopenia and neutropenia (El-sawiet al., 1989), lymhopenia (Singh et al., 1993),Eosinopeniaand monocytopenia (Usehet al., 2008). These findings are as a possible result of migration of leukocytes towards the site of infection resulting in their decrease in peripheral circulation (Rodostitiset al., 2006).

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Knowledge and Epidemiological Risk Factors of Japanese Encephalitis in Community Members of Rupandehi District, Nepal

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Abstract- To study knowledge, attitude and risk factors of Japanese Encephalitis (JE), a research was conducted from May to November 2012 in Rupandehi district Nepal including household (HH) survey, pig survey and swine sero survey. Questionnaire survey on One hundred HH (50 pig raisers and 50 pig non raisers) to compare JE risk factors; 100 pig farmers to study roles of pig as risk factor for JE in human was conducted. Altogether 54% of respondents heard about JE with 60% (30/50) in pig raisers and 48% (24/50) in pig non raisers, which was not significantly different ($p > 0.05$). The media like Radio, TV, were found the most important source of information. The knowledge of JE was found significantly higher ($p<0.01$) in younger people (16-40 yr). The important predictors for knowledge of JE were education ($\beta 1 = 0.195$), access to media ($\beta 1 = 0.357$), and age ($\beta 1 = 0.165$) of respondents. In next 100 pig farmer's survey, 84.5% of pig farmers had seen mosquitoes in pig shed and 52% had seen mosquitoes biting pigs.

Keywords: knowledge, risk factors, vaccination, je, rupandehi.

GJMR-G Classification : NLMC Code: QW 800, W 20

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Knowledge and Epidemiological Risk Factors of Japanese Encephalitis in Community Members of Rupandehi District, Nepal

Khanal T. [α], Pandey K. [σ], Dhakal I. P. [ρ] & Datt D. D. [ω]

Abstract- To study knowledge, attitude and risk factors of Japanese Encephalitis (JE), a research was conducted from May to November 2012 in Rupandehi district Nepal including household (HH) survey, pig survey and swine sero survey. Questionnaire survey on One hundred HH (50 pig raisers and 50 pig non raisers) to compare JE risk factors; 100 pig farmers to study roles of pig as risk factor for JE in human was conducted. Altogether 54% of respondents heard about JE with 60% (30/50) in pig raisers and 48% (24/50) in pig non raisers, which was not significantly different ($p > 0.05$). The media like Radio, TV, were found the most important source of information. The knowledge of JE was found significantly higher ($p<0.01$) in younger people (16-40 yr). The important predictors for knowledge of JE were education ($\beta 1 = 0.195$), access to media ($\beta 1 = 0.357$), and age ($\beta 1 = 0.165$) of respondents. In next 100 pig farmer's survey, 84.5% of pig farmers had seen mosquitoes in pig shed and 52% had seen mosquitoes biting pigs. Most farmers (68%) saw mosquitoes biting pigs everyday and major biting time was dusk (49%) and night (39%). There was significant association between knowledge on JE and their practices to avoid mosquitoes in pig shed ($p < 0.01$). The swine sero prevalence of JE was found to be 67.3% (37/55). Although the community members were at risk of JE but none of them had vaccinated against JE. Swine farmers vaccinated their pigs against infectious diseases like Foot and Mouth Disease and Swine Fever (44%) but none of them had vaccinated their pigs against JE. The pig and human vaccination, human awareness programs and provision of insecticide treated nets (ITNs) can significantly reduce JE in human.

Keywords: knowledge, risk factors, vaccination, je, rupandehi.

I. Introduction

Japanese Encephalitis (JE) was first clinically identified in 1871 in Japan and known as "summer encephalitis" (Mechenzie et al., 2007). In 1933, the virus responsible for Japanese Encephalitis B (JEB) was re-isolated and ultimately characterized in 1934, when it was experimentally inoculated into monkey brain and successfully reproduced the disease (Jani, 2009). JE appeared endemic within the Indochinese Peninsula including Cambodia, Laos, Thailand and Vietnam, and further on to Malaysia, Burma, Singapore (rare cases), Brunei (Erlanger et al., 2009). Then, within the following four decades, JE occupied subsequently most of the Asian continent from Pakistan to Sri Lanka on the east of its range (Solomon et al., 2000) and then Bangladesh, Nepal. Ardeid wading birds are the primary maintenance hosts, pigs are the main amplifying hosts, and Culex mosquitoes are the primary mosquito vectors (Igarashi, 2002). The disease was first recorded in Nepal in 1978 as an epidemic in Rupandehi & Morang District. The major objective of this research was to study knowledge, attitude of community members towards JE, to assess its risk factors which includes the Swine sero prevalence of JE.

II. Materials and Methods

Rupandehi was chosen a study site because it is an endemic district for JE (DHS, 2007), many community members have frequent mobility to India (DDC, 2010) and live pigs are imported from Indian endemic region to Nepal (DLS, 2010). Two study communities named Charange and Majuwa were selected as per the information on risk factors relating to JE from District Livestock Stock Office, District Hospital and Zonal hospital. The Charange and Majuwa were dense pig populated area of Rupandehi (DLSO, Rupandehi, 2011). To study knowledge, attitude and risk factors of JE, a research was conducted from May to November 2012 including HH survey, pig survey, sera collection and rapid kit for JE antibody. Using purposive random sampling, questionnaire survey was conducted on hundred households (50 pig raisers and 50 pig non raisers) to compare knowledge on JE risk factors; 100 pig farmers to study roles of pig as risk factor for JE in human. A total of 55 pig sera samples were taken from two research sites for JE surveillance.

III. Result

Fifty four percent (54/100, 95% CI: 44.2 to 66.6%) of the respondents heard about JE which was 60% (30/50, 95% CI: 46 to 72.8%) in pig raisers and 48% (24/50, 95% CI: 34.5 to 61.8%) in pig non raisers indicating a non significant difference among two respondent types. The media (television and radio) were

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found to be mostly used information source (56%) for JE and other vector borne diseases (VBDs) followed by health personnel (26%) and formal academic study (18%). Only 50.9% (28/54) of the respondents who heard of JE knew about mosquito as the vector, 50.9% (28/54) knew about its transmission cycle, and 49.1% (27/54) knew that JE could be treated. It was found that 50% of the community members were known about basic symptoms of JE (high fever, severe headache, neck rigidity and vomiting). The pig raisers were found to be less careful on the practices to avoid mosquito bite. The knowledge on JE was associated significantly with age ($\chi^2 = 3.931$; $p = 0.047$, Table 1).

Table 1 : Association of respondent characteristics to knowledge of JE

Respondent type	Knew about JE	Didn't know about JE	χ^2 (P value)
Adult	27 (50%)	14 (30.4%)	
Older	27(50%)	32 (69.6%)	3.931 (0.047)
Access to TV, radio	52 (96.3%)	36 (78.3%)	
No access to TV, radio	2 (3.7%)	10 (21.7%)	7.651 (0.006)

The access of HH to the source of information like Radio, Television was the best predictor of knowledge on JE. The explanatory variables selected for the knowledge of JE in community members as dependent variable were: access to information source (Radio, Television), Education status of respondents, and age factors of the respondents & the curve fitting of the model is adequate (F value= 5.112 & p= 0.002). The prediction model was $Y= \beta0+ \beta1X1+ \beta2X2+ \beta3X3 +e$ Where $\beta0$ is constant and $\beta1$, $\beta2$, $\beta3$ are partial correlation coefficient for explanatory variables X1 (Education status), X2 (Access to media) and X3 (Age of respondent), Y Knowledge on JE) being the response. Thus the fitted regression equation was: Y= 0.031+ 0.195 Education status + 0.337 Access to media + 0.165 Age factor.

In next 100 pig farmer's survey, 84.5% of pig farmers had seen mosquitoes in pig shed and 52% had seen mosquitoes biting pigs. Most farmers (68%) saw mosquitoes biting pigs everyday and major biting time was dusk (49%) and night (39%). Similarly, one third (33%) of pig raisers applied practices like disinfection, fumigation outside building, and removing stagnant water to avoid mosquitoes while the remaining 67% had done nothing. Half of farmers (50/100) reported being bitten by mosquito while working in the pig farms, 15% were in doubt, but 35% didn't suffer from mosquito bite. Only 44 (44%) of them had vaccinated their piglets against few infectious diseases like Swine Fever, FMD but none of them had vaccinated against JE. There was a significant association between knowledge on JE and their practices to avoid mosquitoes in pig shed ($\chi^2 = 10.684$; $p = 0.001$, Table 2).

Table 2 : Association of mosquito avoidance practices in pig shed to knowledge of JE

Pig farmer practice	Knew about JE	Didn't know about JE	χ^2 (P value)
Avoid mosquito	20(52.6%)	13 (21%)	
Don't avoid mosquito	18(47.4%)	49 (79%)	10.684 (0.001)
Total pig farmers	38	62	

A total of 55 sera samples were collected aseptically from pigs puncturing ear vein for rapid antibody detection of JE. The prevalence of JE was 67.3% (37/55) (Table 3).

Table 3 : Sero-prevalence of JE in pigs of community members in Rupandehi

District	Site	Farms	Total pigs	Avg. pig/farm	Pigs >6 months	Sera samples	Rapid kit test	
							+ve	-ve
Rupandehi	Majuwa	9	151	16.77	67	25	17	8
	Charange	15	308	20.53	97	30	20	10
Total		24	661	26.70	164	55	37	18

IV. Discussion

a) *Risk factor of JE among community members*

The community members ranked mosquito bite as major cause of fever both in Rupandehi and Kapilvastu. In community member at Rupandehi, 54% (54/100) and in Kapilvastu, only 12 % (12/100) of respondents knew about JE which was found different from that of Morang (USAID, 2010) where 32 % of respondent were aware of JE. As per the research of Pandit (2010), in Mandya district of Karnataka, about 42% of respondents had knowledge of JE and in Koppal district, 19.85% of the heads of household had the knowledge of JE. Similarly, 38% of the respondent pig farmers in Rupandehi had known about JE which supports the knowledge in pig farmers of Kathmandu (42%) (Dhakal et al., 2012) and contrast among in pig farmers (10%) of mountain districts (Thakur et al., 2012)

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of Nepal. This variation of knowledge might have influenced by the socioeconomic and education status of respondents (USAID, 2003). The lower level of knowledge in Kapilvastu might have been due to low economic and education status. The major source of information regarding VBDs were found to be media like Radio, Television. Similar research was supported by USAID (2003) as they had found that knowledge and awareness of VBDs increased with radio ownership. The younger age, high literacy rate and access to the media were found important predictors for Knowledge on JE. According to CDC (2011) 40% of respondent (20/50) get bite while working in the field and 60% got bite while in house at different time in house which is similar to our finding. In similar research of USAID (2003), 85% use bed nets among those aware of JE compared to 68% among those not aware but it avoidance practices were not significantly associated with the knowledge.

b) Swine sero prevalence of JE

The prevalence of JE was found was different in different places. Our research is contrast with that of Thakur et al., (2012) their results showed that 16.7% (17/102), 4% (4/100), 6.6% (10/151) and 44.6% (45/101) of pigs had anti-JEV antibodies in Sindhupalchowk, Dolakha, Solukhumbu and Kavrepalanchowk districts respectively. The higher prevalence of JE in pigs in our research site could be because of higher prevalence of culine mosquitoes but is was similar to that of Kathmandu (Pant, 2006). Sero prevalence of JE in pigs varies considerably across geographic locations, and the result of this study is slightly larger than estimates of sero positivity from other Asian countries: 49% in Bali, 6% in Java, Indonesia (Yamanaka et al., 2010); 4.5% in Ishigaki Island, Japan (Nidaira et al., 2009); and 33.3% in Tibet (Li et al., 2011). The higher sero prevalence could be high vector prevalence and their breeding in nearby rice field of the southern belt of Nepal. The expansion of the JE virus-endemic area depends on irrigated rice farming and pig rearing (Oya and Kurane, 2007). High densities of JE vector were reported in rice fields after the rainy season when there is plenty of water and temperatures are high, facilitating larval grow in large numbers (Sunish and Reuben 2001). Conlan (2012) identified proximity to rice fields (OR 2.93, 95% CI 1.57–5.45), pig ownership (OR 2.24, 95% CI 1.17–4.26), and older age (OR 1.21, 95% CI 1.09–1.33) as being independently associated with the risk of JE. A research in laos showed that peak JEV transmission coincides with the start of the monsoonal wet season and poses the greatest risk for human infection. Many of the ecological, environmental, climatic and human behavioral factors are involved in the JE virus spread (Solomon, 2006). The practice of paddy cultivation, proximity of houses to water bodies and suitable climatic factors were the most important environmental factors associated with several recent JE outbreaks in Northeast India (Phukan et al., 2004).

V. Conclusion

The community members in Rupandehi were found at high risk of JE as the amplifying hosts harvest large prevalence of JE virus and agro-ecological scenario favors transmission of JEV from maintenance hosts to amplifying host. Many pig farmers were illiterate so the training regarding the piggery management along with the measures to be prevented from vector borne diseases like JE should be provided through informal teaching learning process like farmer's trainings and demonstration. This is an important public health disease governed by many environmental, social, climatic and ecological factors. Thus, stakeholders are required to address the problems remaining inside the umbrella of One Health Strategy.

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Is Eating Fish Related-Psychiatric Disorders? A Case Report of Female Child with Schizopherinia Related Fish Ingestion

By Abd Elrazek Mohammad Ali Abd Elrazek, Aldoushy Mahdy & Khaled Youssef AbouelFadl

Abstract- Many studies worldwide reported about patients developed gastrointestinal symptoms generally within three to six hours of eating the fish. Neurologic symptoms, including circumoral paresthesias and weakness in the lower extremities, can occur later; occasionally a reversal of hot-cold taste sensation is reported. Neurologic symptoms often persist for several weeks, but in some cases can last for years.

We have reported a case study of female child presenting with A Schizophrenia like attack just after fish ingestion, following the patients up for 30 months, the disease symptoms disappeared when patient was adherent to medications, relapsed when eating fish in a surprising character. In such an interesting case report, we shed light on the food born-neurological and psychiatric illness produced by eating fish.

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ISEATINGFISHRELATED-PSYCHIATRICDISORDERS?ACASEREPORTOFFEMALECHILDWITHSCHIZOPHERINIARELATEDFISHINGESTION

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Is Eating Fish Related-Psychiatric Disorders? A Case Report of Female Child with Schizopherinia Related Fish Ingestion

Abd Elrazek Mohammad Ali Abd Elrazek [α], Aldoushy Mahdy [σ] & Khaled Youssef AbouelFadl [ρ]

Abstract- Many studies worldwide reported about patients developed gastrointestinal symptoms generally within three to six hours of eating the fish. Neurologic symptoms, including circumoral paresthesias and weakness in the lower extremities, can occur later; occasionally a reversal of hot-cold taste sensation is reported. Neurologic symptoms often persist for several weeks, but in some cases can last for years.

We have reported a case study of female child presenting with A Schizophrenia like attack just after fish ingestion, following the patients up for 30 months, the disease symptoms disappeared when patient was adherent to medications, relapsed when eating fish in a surprising character. In such an interesting case report, we shed light on the food born-neurological and psychiatric illness produced by eating fish.

I. Introduction

Seafood is a frequent source of food-borne illness. Such illness can arise from consumption of fish, especially shellfish, contaminated by viruses such as Norwalk or Hepatitis A; ingestion of a toxin such as scombroid, found in certain finfish; or poisoning from fish or shellfish contaminated with toxins from algae and other marine life forms, such as dinoflagellates that produce toxins causing ciguatera fish poisoning and paralytic shellfish poisoning, or Gymnodinium breve, which causes serious disorders such as what is known as red tide [1,2].

One of the famous reported fish poisoning outbreaks; Ciguatera poisoning which is the most common form of seafood poisoning and occurs after ingestion of certain species of local reef fish, which vary from location to location. Between 1998 and 2002, 84 outbreaks resulting in 315 cases were reported to the CDC; several outbreaks have been described in Australia. The fish appear and taste normal; cooking does not destroy the toxin and thus, avoidance of these fish is the only means of prevention, most resort areas in the Caribbean area [3, 4]. Many questions have arisen related - Scombroid fish. Scombroid Fish poisoning occurs after the ingestion of finfish, notably tuna, mahi-mahi, and bluefish. One of the largest reported outbreaks of scombroid fish poisoning in the United States was associated with neurological disorders, till now the mechanism of Poisoning-related neurological /Psychiatric disorders is not fully understood [5].

II. Case Report

2 years ago, female child 10 years old, presented with increased activity beyond her developmental age, together with visual hallucinations in the form of demons and angles, she also showed lack of need of sleep even if she tired. There was no family history of any psychiatric diseases.

Parents claimed the condition presentation and progression just after eating fish, which did not concerned as important history by pediatrician, psychologist or psychiatrist.

The child's chronological age, intellectual capabilities, and environmental factors were evaluated into account for assessing the level of functional impairment or improvement in early and follow up course.

24 months following cognitive behavior therapy, psychotherapy and pharmacotherapy, patient improved with complete disappearance of schizophrenic symptoms and signs, patient showed significant progressive improvement in her intellectual activities together with social abilities.

Gradual pharmacotherapy withdrawal was done by expert pediatric psychiatrist with intimate follow up, surprisingly just the patient has eaten fish with her family, she showed aggressive relapsing of hallucination both audible and visual with significant hyperactivity syndrome, for the second time her parents claimed the condition for the child related- eating fish, they mentioned no similar condition for them or siblings when eating fish also. Accordingly we minded the condition seriously, following the patient up for another 6 months, patient showed some social and psychiatric improvement, when we asked parents allowing the child to eat fish (any kind) she showed the same previous relapsing attack, which cannot be explained.

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III. Discussion

Paralytic shellfish poisoning (PSP) is the food borne illness associated with the consumption of seafood products contaminated with the neurotoxins known collectively as saxitoxins (STXs). This family of neurotoxins binds to voltage-gated sodium channels, thereby attenuating action potentials by preventing the passage of sodium ions across the membrane. Symptoms include tingling, numbness, headaches, weakness and difficulty breathing [6, 7].

Schizophrenia is one of the top ten illnesses contributing to the global burden of disease ranked by the World Health Organization, whatever the disease is much serious in childhood, affecting the Psycho-motor System. Antipsychotic medications were prescribed to the patient as first-line treatment for schizophrenia. Medications were very effective in reducing symptoms and behaviors associated with the disorder [8]. The concept that certain food ingestion may lead to diseases or interaction diseases related disorders have been reported in many studies which could estimate the usefulness versus the harmless of certain food. Other American-marine studies reported that fish eating may lead to certain neurologic manifestations which may take several weeks to years. One of the largest reported outbreaks of scombroid fish poisoning in the United States was associated with neurological disorders, till now the mechanism of Poisoning-related neurological /Psychiatric disorders is not fully understood [9-10].

One of our Explanation regarding Psychiatric/ Neurological disorders-related Fish ingestion, the idea that Histidine; presenting with high concentration in certain sea food breaking down or converting to Histamine , in a cumulative matter with pre-coding gene stimulator like effect, when eating fish containing high amount of Histidine, it may lead to neurological and Psychiatric diseases.

In organic disorder it will be much Histamine related allergies; in a case antihistaminic drugs could be helpful Whatever eating fish may help sleeping and sometimes relaxation in many institutions. Accordingly we have no idea regarding the mechanism of action. There were 118 outbreaks of scombroid from 1998 to 2002, resulting in 163 cases; One Pennsylvania outbreak was associated with consumption of a tuna-and-spinach salad in a restaurant. Flushing, nausea, sweating, diarrhea, and headache occurred from five minutes to two hours after ingestion and resolved within hours. Histamine levels were found to be elevated in the fish, which had been caught in the Gulf of Mexico by a long-line method that kept the fish suspended for 12 to 24 hours on the line in the relatively warm water prior to harvesting.

The illness is caused by histamine and other products from bacteria that propagate on the fish in warm water or when they are inadequately refrigerated. Prompt harvesting and refrigeration until the fish is cooked are the best means of preventing this poisoning [11-13]. In our current case study we have no scientific explanation on the moment; ultimately we have to research and understand more about these neuropsychiatric disorders related eating fish.

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Effect of Emblica Officinalis on Stress Induced Biochemical and Psychological Changes in Mice

By C. Pradeep Kumar, Swathi Rawath. S, D. Yashwanth Kumar & K. S. S. N. Neelima

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Abstract- Emblica officinalis Gaertn. (Euphorbiaceae) [EO] fruit is traditionally used as a general health tonic in Ayurveda. In the present study the effect of alcoholic extract of EO fruits was evaluated on acute stress induced biochemical and psychological changes in mice. Acute stress was induced by restraint stress method and the effect of EO at the doses of 100, 250 and 500mg/kg was evaluated on levels of plasma glucose and corticosterone. The level of anxiety induced by acute stress was measured by subjecting the mice to elevated plus maze. The extract at the doses of 250mg/kg and 500mg/kg significantly countered the stress induced elevated plasma glucose and cortisol levels. Likewise, the extract at the doses of 250 and 500mg/kg increased the number of entries and time spent in the open arm in elevated plus maze. In conclusion the Emblica officinalis fruit extract significantly reduces the adverse effects of stress on physical and mental health.

Keywords: stress, anxiety, emblica officinalis.

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Effect of Emblica Officinalis on Stress Induced Biochemical and Psychological Changes in Mice

C. Pradeep Kumar [α], Swathi Rawath. S [σ], D. Yashwanth Kumar [ρ] & K. S. S. N. Neelima [ω]

Abstract- Emblica officinalis Gaertn. (Euphorbiaceae) [EO] fruit is traditionally used as a general health tonic in Ayurveda. In the present study the effect of alcoholic extract of EO fruits was evaluated on acute stress induced biochemical and psychological changes in mice. Acute stress was induced by restraint stress method and the effect of EO at the doses of 100, 250 and 500mg/kg was evaluated on levels of plasma glucose and corticosterone. The level of anxiety induced by acute stress was measured by subjecting the mice to elevated plus maze. The extract at the doses of 250mg/kg and 500mg/kg significantly countered the stress induced elevated plasma glucose and cortisol levels. Likewise, the extract at the doses of 250 and 500mg/kg increased the number of entries and time spent in the open arm in elevated plus maze. In conclusion the Emblica officinalis fruit extract significantly reduces the adverse effects of stress on physical and mental health.

Keywords: stress, anxiety, emblica officinalis.

I. Introduction

The present day life style has increased the physical and psychological demands resulting in an up rise in various stress- related disorders like anxiety and depression. This necessitates an urgent need to develop agents to overcome these stress associated psychopathological conditions (Bhattacharya et al., 2000). Traditional medicines are rich in non-specific anti-stress agents which can be used clinically in the treatment of stress related maladies.

Emblica officinalis (EO) belonging to family Euphorbiaceae, is an important ingredient of Ayurvedic system of medicine. It is also named as Amla, Phyllanthus Emblica or Indian gooseberry. The fruits of the plant are categorised as rasayanas, a group of plant-derived drugs that are reputed to promote health and longevity by augmenting defence against disease, arresting the aging process, revitalising the body in debilitated conditions, increasing the capability of the individual to resist adverse environmental factors and creating a sense of mental well-being (Weiner and Weiner, 1994). It has its beneficial role in cancer, diabetis, liver treatment, heart trouble, ulcer, anemia and various other diseases. Similarly, it has application as antioxidant, immunomodulatory, antipyretic, analgesic, cytoprotective, antitussive and gastroprotective. Additionally, it is useful in memory enhancing, ophthalmic disorders and lowering cholesterol level (Khan et al., 2009).

Stress has been postulated to be involved in the etiopathogenesis of a diverse variety of diseases, ranging from psychiatric disorders such as depression and anxiety, immunosuppression, endocrine disorders including diabetes mellitus, male sexual dysfunction, cognitive dysfunctions, peptic ulcer, hypertension and ulcerative colitis (Elliott and Eisdorfer, 1982). The benzodiazepine anxiolytics, despite having significant antistress activity have not proved effective against stress induced adverse effects on immunity, behavior, cognition, peptic ulcer and hypertension (Elliott and Eisdorfer, 1982). Furthermore, these drugs have adverse effects on the fetus during pregnancy and on the neonate during lactation (Trevor and Way, 2001).

Therefore, to find a solution to an effective antistress agent with a broad spectrum of activity and overcoming the adverse effects of benzodiazepines, the present study evaluates the effect of Emblica officinalis fruit extract on acute stress induced biochemical and psychological changes in mice.

II. Materials and Methods

Shade dried fruits of EO were purchased from the local market for the whole batch of experiments. The fruits were authenticated by matching with the reference specimen no. 2156 at the Botany Department, Government Science College, Durg, India.

a) Preparation of extract

The powdered fruits, (250 g) were loaded in a soxhlet extractor and were defatted with petroleum ether (60–80). The marc was dried and further extracted with 70% ethanol by maceration (Riebling and Walker, 1975). The extract was concentrated on rotary flash evaporator and vacuum dried over anhydrous sodium sulphate. The dried material (48.9%) was stored under refrigeration at 4–8 ◦C until its use.

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b) Animals

Male, Swiss albino mice (20-30 g) were used for behavioral studies, whereas biochemical estimation was performed in male Wister albino rats. Each experimental group consisted of atleast six animals. The animals were housed for a minimum of five days prior to the pharmacological experiments, with free access to standard rodent pellet diet (Lipton India Ltd) and tap water, and maintained on a 12/12 h light-dark cycle.

All experiments were conducted in accordance with institutional Animal Ethics Committee guidelines. The experimental protocols were approved by the institutional animal ethics committee (IAEC) - SIP/CPCSEA/IAEC/2013/I/0. The minimum number of animals and duration of observations required to obtain consistent data were employed.

c) Extract and standard drug

The hydroalcoholic extract was formulated as suspension using 0.1% Sodium carboxymethyl cellulose (CMC). Ginseng 100mg/kg (Revital™) was used as reference drug. The extract was adjusted to give a fixed volume of 10 ml/ kg orally in doses of 100mg/kg, 250mg/kg and 500mg/kg.

d) Acute restraint stress model

In the present study stress was induced using acute restraint stress (Masood et al., 2003) model with minor modifications. The mice were divided into six groups of six animals each of either sex. Stress was induced by restraining the animals in PVC restrainers for a period of 4 hours. Group 1 animals served as normal control were administered 0.5% Sodium CMC in water and were not exposed to stress. Group 2 animals served as negative control as untreated stress induced; Group 3 animals were administered ginseng 100mg/kg orally. While, Group 4, 5 and 6 were administered EO extract orally at the doses of 100mg/kg, 250mg/kg and 500mg/kg respectively. The animals were pretreated with the extracts and the reference drug for a period of seven days before the induction of stress.

Following the induction of albino stress the male Swiss mice were evaluated for behavioral changes on the Elevated plus maze model. A different set of male Wistar rats, treated as above, were used for biochemical analysis. The animals were sacrificed post stress induction by cervical decapitation, the blood was withdrawn from the jugular vein and serum glucose and corticosterone levels were determined.

e) Elevated plus-maze test (EPM)

This test has been widely used to measure anxiety in rodents (Morra et al., 2006). The wooden apparatus, consisted of two open arms (50 cm×10 cm each), two enclosed arms (50 cm×10 cm×40 cm each) and a central platform (10 cm×10 cm), arranged in such a way that the two arms of each type were opposite to each other. The maze was elevated 70 cm above the floor. Immediately after the induction of stress, each animal was placed at the center of the maze, facing one of the enclosed arms. During the 5-min test period, the number of open and enclosed arms entries, plus the time spent in open arms, was recorded. Entry into an arm was defined as the point when the animal places all four paws onto the arm.

f) Blood Collection

A different set of male Wistar rats, treated likewise, was used for biochemical analysis. The animals were sacrificed immediately after acute stress induction. The blood was collected and separated in a refrigerated centrifuge at 4°C. The serum was stored at -80°C until further analysis of corticosterone and glucose.

g) Estimation of Corticosterone

Serum corticosterone levels were determined by fluorimetric method (Glick D et al 1964) with minor modifications. Briefly, 500 μL of serum was extracted with 2mL of chloroform. The chloroform was further extracted with 1ml of acid alcohol and the fluorescence was measured at 462 nm and 518 nm.

h) Estimation of serum glucose

The serum glucose level was determined using the (GOD–POD method) glucose oxidase–peroxidase–aminoantipyrine and phenol method (Glucose determiNATion kit, Merck) where the quinonemimine dye formed is estimated spectrophotometerically at 540 nm (Philip et al 1994).

i) Statistical analysis

The data was analyzed using Prism Graph Pad software and showed as mean±S.D. Comparison between control and drug treated groups were made by one-way analysis of variance (ANOVA) followed by Dunett's test, P values of less than 0.05 were considered to be significant.

III. Results

a) Elevated plus-maze model (EPM)

The ANOVA revealed a significant increase in the number of entries in the open arm in the normal, ginseng treated and EO (250 and 500mg/kg) treated animals as compared to the stress control ($P<0.05$). Further, EO treated animals significantly increased ($P<0.05$) the time spent in the open arm at the doses of 250 and 500mg/kg. The results are shown in table 1.

b) Effect of EO extract on serum glucose level

The induction of stress by restraining in mice was confirmed by measuring the serum glucose levels. The animals on exposure to acute restraint stress showed a significant increase in blood glucose levels as compared to the normal mice ($P< 0.05$). Further, treatment with EO extract at a dose of 250 and

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500mg/kg significantly countered this elevation in blood glucose level. The results are depicted in the table 2.

c) Effect of EO extract on serum corticosterone level

Exposure to acute restraint stress resulted in a significant elevation in serum corticosterone level. Further, treatment with EO at 250mg/kg and500mg/kg significantly reduced the elevated levels of serum corticosterone. The results are depicted in the table 2.

IV. Discussion

Severe stressful conditions are responsible for the etiopathogenesis of various psychosomatic disorders. Homeostasis which is maintained by the various neurotransmitters is challenged during stressful conditions. These alterations in neurotransmitter activity result in behavioral changes as well as a cascade of hormonal release from the hypothalamus–pituitary–adrenal (HPA) axis. The imbalance of these monoamines due to prolonged stressful conditions has been associated with a wide range of central and peripheral disorders like anxiety, depression, obsessive compulsive disorder, eating and sleeping disorders, hyperglycemia and decreased immune response (Kalia, 2005; Rashid et al 2008).

The elevated plus maze (EPM) is considered to be an etiologically valid animal model of anxiety which uses natural stimuli like fear of a novel open space and fear of balancing on a relatively narrow, raised platform that can induce anxiety in mice (Dawson and Tricklebank, 1995). However it was observed after measurement of anxiety states post acute restraint stress induction that the animals showed further pronounced anxious behavior in EPM. EO fruits have shown significant pharmacological effects like enhancement in swimming performance of rats in forced swimming test (Sudhakar et al., 2009) and are used in Ayurveda as a general tonic (Deole et al., 2009), which further proposes evaluating its effects on stress induced neuropsychological conditions. The present study investigated the effects of hydroalcoholic extract of EO fruits on the acute stress induced anxiety in mice.

Typically a stress response is characterized by the activation of HPA axis resulting in an increase in blood corticosterone levels which in turn lead to an increase in serum triglycerides levels and hyperglycemia. The study indicated that administration of EO extract significantly countered altered blood glucose and corticosterone levels in animals exposed to acute restraint stress.

Further, administration of EO extracts and evaluation of these stress induced animals in models of anxiety revealed a significant lowering of anxiety response such as a significant increase in the number of crossings in the EPM and time spent in the open arm.

V. Conclusion

Although this study does not suggest anything about the mechanism of antistress potential yet it proves to be a potential lead in this class of drugs and further relates with the works reported by others on its adaptogenic effect which needs to be further evaluated and optimized.

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Table 1 : Effect of EO on Elevated plus maze behavior following acute restraint stress in mice

Groups	Time spent in the open arm (seconds)	Number of entries in the open arm
Normal	268.97±10.34*	11.24±0.60*
Stress control	37.88±2.14	2.14±0.36
Ginseng 100mg/kg	260.13±14.80*	10.34±0.90*
EO 100 mg/kg	48.77±3.47	3.60±1.50
EO250 mg/kg	160.7±9.74*	6.78±1.10*
EO500 mg/kg	255.36±20.10*	9.77±0.67*

*, P< 0.05; in comparison to stress induced animals (n = 6 animals in each group)

Table 2 : Effect of EO on serum glucose and corticosterone level following acute restraint stress in mice

Groups	Serum glucose levels (mg/dl)	Serum corticosterone levels (mg/100ml)
Normal	90.28± 8.59 **	126.9±20.31
Stress control	150.46 ± 9.64	311.20±47.26
Ginseng 100mg/kg	101.51 ±6.47 **	157.3±17.88***
EO 100 mg/kg	121.56±11.23	284.32±30.33
EO250 mg/kg	117.86 ±12.45 *	207±26.56*
EO500 mg/kg	103.21±8.12**	161±18.77**

*, P< 0.05; in comparison to stress induced animals (n = 6 animals in each group)

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Autochthonous Cases of Dirofilaria in Dogs from Timiş County (Western Part) Romania

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Abstract- Background: Taking into consideration that dirofilariosis spreading in Romania, in this study we intended to determine the prevalence of Dirofilaria spp. infection in dogs from Timiş County.

Methods: The epidemiological research was conducted between February 2008 and October 2010, on a total of 457 dogs.

The modified Knott's method was used to identify the Dirofilaria spp. microfilariae. Blood samples positive for microfilariae were examined using the polymerase chain reaction (PCR), at the "Szent István" University, Faculty of Veterinary Science in Budapest, Hungary in two periods 2009 and 2010.

Results: Using the modified Knott's method, 33 dogs were positive for microfilariae (7.2%, 33/457) and using molecular technique, the following species were identified: D. repens (18/28) and D. immitis (1/28). Five samples (5/33) positive for microfilariae, not examined by PCR were examined by the Diro Speed® Test / Heartworm (Bio Veto Test, France).

Keywords: dirofilaria immitis, dirofilaria repens, dog, timiş county, romania.

GJMR-GClassification : NLMC Code: QW 70, WC 900

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Conclusions: This is the first report of canine dirofilariosis in the Timiş County, Romania. This survey shows that there is potential environmental condition for the development of intermediate host mosquito species and the spread of D. repens and D. immitis at the Western part of Romania, too.

Keywords: dirofilaria immitis, dirofilaria repens, dog, timiş county, romania.

I. Background

Dirofilaria immitis and Dirofilaria repens represent the most important filarioid species in Europe because of their pathogenicity on dog's health and because of their zoonotic potential [11]. D. immitis is commonly found in the pulmonary arteries and the right ventricle of dogs and causes canine heartworm disease, but it also occurs in cats and humans [18].

D. repens is the less pathogenic form, infesting subcutaneous tissues [14], occasionally, dermal swelling and subcutaneous nodules containing adult worms may be observed [22].

In the past decade the infections caused by filarioid nematodes in dogs and cats are apparently spreading in different geographic areas [10].

The distribution of Dirofilaria spp. in different European countries has been attributed to several factors including the introduction of new species of mosquitoes like Aedes albopictus [5], and consequence of climate changes together with increased pet travel [23].

Cutan dirofilariosis is considered an emerging zoonosis in Europe, though most cases of D. repens infections are benign in humans because the adult nematode is localized mainly invisible way in subcutaneous tissue. Occasionally this parasite can cause subcutaneous nodules or ocular lesions sporadically it can reach deeper tissues. In lesions the immature or adult nematode can be formed nodules, which can mimic a tumor [15, 17]. Human D. repens infection cases have been described from northeastern countries such as Hungary [19], Slovak Republic [16], Poland [24] and Russia [12]. Recent reports are from Croatia [4], Romania [13], Russia [9] and France [15].

II. Materials and Methods

a) Study Area

Timiş County is located at the Western Romanian border with Hungary and Serbia, and has an area of 8.696 km2. The Timiş landscape is predominantly flat and is crossed from East to South-West by the Bega and Timiş rivers. The climate is classified as moderate continental, with Mediterranean influences. The average annual temperature is 10.7 °C.

b) Blood Sampling

The epidemiological study was carried out from February 2008 to October 2010, with 457 asymptomatic dogs, of different breeds, aged between 6 months and 15 years. In this group of dogs 183 were females and 274 males. Dogs examined were from 42 localities in Timiş County. Some of them belonged to different owners and some dogs were from shelters. Blood samples were collected into vacutainers with EDTA anticoagulant.

c) Modified Knott's test

For this test, 1 ml of blood was used on the EDTA, adding approximately 9 ml of 2% formaldehyde.

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The liquid was centrifuged, and the sediment was mixed with equal parts of 1:1000 methylene blue dyes. The colored sediment was spread on a slide, and covered with coverslip, and was examined under the microscope, using the 10x and 40x light microscope objective.

d) Molecular Techniques

Positive blood samples with microfilariae were examined using molecular techniques, at the Department of Parasitology and Zoology of Faculty of Veterinary Science "Szent István" University in Budapest, Hungary, in 2009 and 2010. The DNA extraction was performed in 100 µl of blood collected on EDTA, for each positive sample. We used the Blood and Tissue Dneasy kit (QIAGEN). The general primers and the thermal profile described by Casiraghi et al., 2006 [6] were used for the PCR, with species specificity for detecting D. repens and D. immitis presence. The migration of amplicons was performed by electrophoresis in 1.5% agarose gel. The ethidium bromide dye was used for preparing the agarose gel. After the migration of PCR products through the agarose gel, the gel image with the migrated DNA fragments was photographed using a Kodak EDAS 290 Polaroid system. The PCR-products were sequenced by Biomi Ltd. (Gödöllő, Hungary) directly using the ABI technology, in order to verify the specificity of PCR reactions.

e) Diro Speed® Test / Heartworm (Bio Veto Test, France)

Five samples (5/33) positive for microfilariae, not examined by PCR, were investigated to detect D. immitis antigen. The examination of the samples was processed according to the manufacturers description.

III. Results

As a result of the examination of blood samples, using the modified Knott's method, the microfilariae were identified in 33 of the 457 dogs examined (Table 1). The prevalence of Dirofilaria spp. in dogs, according to the modified Knott's method was 7.2%. The microfilariae examined using the Olympus microscope with video extension, and 400 x objectives, showed the morphology of D. repens microfilariae. This means the absence of the cephalic hook, the front end having a slightly tapered shape, and the filiform caudal end presented an "umbrella handle" shape (Figure 1,2,3). The length of the microfilariae observed was between 330-380 µm.

The dogs infected with Dirofilaria spp. belonged to 16 breeds and 17 of them being males and 16 females aged between 2 and 13 years (Table 2, 3).

The size of the fragments amplified for the 12SrDNA in 18 samples (18/28) were ~ 390 base pairs and suggested that the samples isolated from dogs with microfilaremia belonged to the D. repens species, and ~ 450 base pairs at a sample of 28, that suggested an infection with D. immitis. An example is shown in Figure 4.

Only one dog tested positive for infection with D. immitis, a Rottweiler breed female, aged 11 years, from Timişoara. The result of the infection with D. immitis, obtained by PCR, is questionable. At this patient further investigation were not possible. The number of microfilariae (mf) / ml of blood in dogs with dirofilariosis ranged from <30 to 5000 mf / ml of blood. This molecular biology study, performed for the first time in Romania, shows that the dogs diagnosed with microfilaremia were infected with D. repens and D. immitis. Tested with the Speed®Diro / Heartworm (Bio Veto Test, France), all 5 dogs (5/33) examined were negative for antigens of D. immitis.

IV. Discussions

Most dog of diagnosed with microfilariae of Dirofilaria spp. came from localities crossed by a river, such as Timiş, Albina, Bega Veche, Behela rivers and the Bega Channel (Figure 5). The rivers, the lakes and the ponds are considered as preferred habitats for mosquito's larval development. So far, there are no statistics available concerning the species of mosquitoes identified in Timiş County. The increasingly high temperatures in summer, the mild winters, the large number of vectors, and numerous stray dog and the dog imports, has led to dirofilariosis spread in the Western part of Romania.

The dogs with microfilaremia were considered local cases because they have not left our country and have not been imported from Europe. Taking into consideration the results of this research we concluded that the infection with D. repens is endemic in the Western part of Romania.

The number of dogs carried subcutaneous dirofilariosis is growing, and this is why this study should be continued. The D. immitis parasitism was not identified so far in the Western part of Romania. The case tested positive by PCR in this survey, is the first one found.

Between 2008 and 2010, several necropsies in dogs, were achieved at the Necropsy Pathology Laboratory in Timişoara, for "finding" D. immitis. Special attention was given to the thoracic cavity and heart. Using this technique, only adult D. repens were found, located in the subcutaneous tissue in certain body regions. An interesting case was represented by a dog, which presented a D. repens nematode in the scrotum after orchidectomy. This surgical procedure was performed in a veterinary clinic EUROPET-FV in the city.
In Hunedoara County (central-western part of the country) 92 dogs were tested at the shelter. Two dogs were diagnosed with D. repens (2.2%, 2/92) [7]. In 2009

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– 2010, in Arad, 30 dogs were tested for microfilariae. A single dog was diagnosed with subcutaneous dirofilariosis (3.3%, 1/30) (Ciocan, unpublished observations).

In Bucharest (Southeast part of the country) the infection with D. immitis and D. repens of dogs had been confirmed long time ago. Coman et al. (2007) from the "Spiru Haret" University of Veterinary in Bucharest confirms the presence of D. immitis in 12 dogs examined (23.1%, 12/52) [8]. Tudor et al. (2008) reported a prevalence of 29.3% (34/116) for the D. immitis infection in dogs in the Bucharest area [21]. A year later, in Bucharest the diagnosis of D. immitis was confirmed again, after the parasitological examination of 35 dogs [20].

In Iaşi (Northeastern part of Romania) in March 2009 was reported the first case of infestation with D. immitis in dogs, and afterwards 27 new cases were diagnosed in dogs raised by owners and 41 cases in dogs from shelters. A total number of 303 dogs were examined and 68 of them tested positive for microfilariae (Knott's method), with a prevalence of 22.4% (D. immitis). Using the Snap Heartworm rapid test, 16 dogs tested positive for D. immitis (5.3%). The first case of D. repens infestation was diagnosed in February 2009 [1, 2]. Acatrinei et al. (2008) confirms the presence of D. immitis in four dogs from the southeast part of the country (Tulcea) [3].

V. Conclusions

Following the research, we concluded that the prevalence of D. repens infection is high in Timiş region. The Western Region meets all the conditions for developing of Culicidae larvae and therefore canine dirofilariosis may increase in the future.

The presence of stray dogs in many places represents a reservoir in the spread of both dirofilarioses in other animals and humans. It is pointed out that Timiş County bordered with Hungary and Serbia where the subcutaneous dirofilariosis cases in dogs are very frequent. A special attention should be given on imported dogs. Veterinarians and dog owners should be informed of the presence of D. immitis and D. repens in this part of Romania and therefore, it is very important to inform the dog owners about the available prevention possibilities.

VI. Competing Interests

The author(s) declare that they have no competing interests.

VII. Acknowledgements

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Figure 1 : D. repens microfilaria in a dog, modified Knott's method (400x objectives)

Figure 2 : D. repens microfilaria in a dog, modified Knott's method (400x objectives)

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Figure 3 : D. repens microfilaria in a dog, modified Knott's method (400x objectives)

Table 1 : The *D. repens* infection of dogs (n=457) correlated with breed, age and genderNo 33/457

Breed	No. Pos	Age (years) 1-5	Age (years) 6-10	Age (years) ≥ 11	Sex	
					Male	Female
Half breed	8/33	6	1	1	4	4
Caucasian Shepherd	3/33	1	2	0	2	1
Bernese Shepherd	1/33	0	1	0	1	0
Amstaff	2/33	0	2	0	2	0
Bordeaux Dog	1/33	1	0	0	0	1
American Cocker	1/33	1	0	0	0	1
Cocker Spaniel	1/33	0	1	0	1	0
Doberman	2/33	0	2	0	1	1
German Wirehaired Pointer	1/33	1	0	0	1	0
Poodle	1/33	0	0	1	0	1
Rottweiler	2/33	0	1	1	0	2
German Shepherd	6/33	3	3	0	1	5
Mioritic Sheepdog	1/33	1	0	0	1	0
Labrador	1/33	1	0	0	1	0
Cane Corso	1/33	1	0	0	1	0
Dalmatian	1/33	0	1	0	1	0
Total	**33**	**16**	**14**	**3**	**17**	**16**

Legend: n: number of examined dogs; No. Pos: Number of positives dogs

Table 2 : The prevalence of *D. repens* in dogs correlated with age

Age	n	Knott's test positive	Prevalence (%)
1-5	279	16/279	5.7%
6-10	133	14/133	10.5%
≥11	45	3/45	6.6%
Total	**457**	**33/457**	**7.2%**

Legend: n: number of examined dogs

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Table 3 : The prevalence of *D. repens* in dogs correlated with gender

Sex	n	Knott's test positive	Prevalence (%)
Males	274	17/274	6.2%
Females	183	16/183	8.7%
Total	**457**	**33/457**	**7.2%**

Legend: n: number of examined dogs

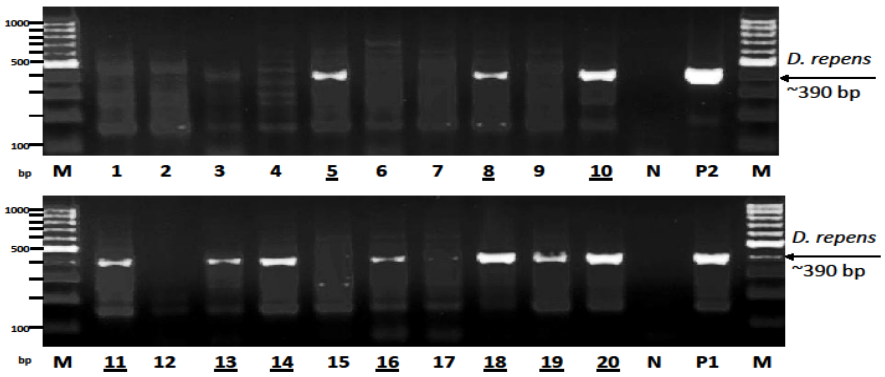

Figure 4 : The DNA products amplified with the specific *D. repens* primers show ten positive samples (underlined numbers)

Legend: M: molecular marker 100-1000 bp; bp: base pairs; *D. repens*: *Dirofilaria repens*; N: negative controls; P1, P2: positive controls.

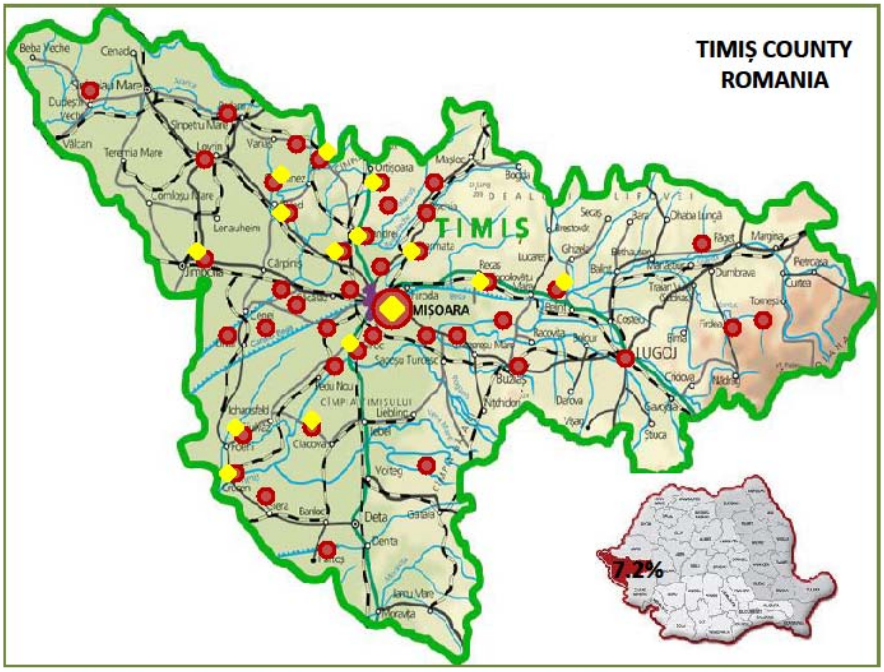

Figure 5 : The regional distribution of dogs examined is demonstrated by red circles, and origin of the positive dogs is indicated by yellow rhombus

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Evaluation of Anti Depressant Activity of Murraya Koenigii Leaf Extract in Mice

By Krishna Prasad. D, S. N.Sri Harsha, D. Yashwanth kumar & K. S. S. N. Neelima
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Abstract- Murraya koenigii Spreng (Rutaceae), a medicinally important herb of Indian origin, has been Used for centuries in the Ayurvedic System of Medicine. Aqueous extract of the leaves of Murraya koenigii possesses alexeteric, antihelmintic, analgesic, dysentry, purgative and blood disorders. Also they are reported to be useful in inflammation, healing of wounds, injuries, antioxidative activity. In folklore practice, the decoction of M. koenigii leaves has been reported to be useful in diarrhoea. In the present study the effect of hydroalcoholic extract of seeds was evaluated for antidepressant activity in mice. The models selected for the study were tail suspension test and despair swimming test. The extract at the doses of 100mg/kg, 250mg/kg and 500mg/kg significantly reduced the duration of immobility of mice in tail suspension test and despair swimming test as compared to the untreated group.

Keywords: murraya koenigii (mk), anti depressant activity, aqueous extract, mice.

GJMR-G Classification : NLMC Code: WD 730, WL 108

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Evaluation of Anti Depressant Activity of Murraya Koenigii Leaf Extract in Mice

Krishna Prasad. D [α], S. N.Sri Harsha [σ], D. Yashwanth kumar [ρ] & K. S. S. N. Neelima [ω]

Abstract- Murraya koenigii Spreng (Rutaceae), a medicinally important herb of Indian origin, has been Used for centuries in the Ayurvedic System of Medicine. Aqueous extract of the leaves of Murraya koenigii possesses alexeteric, antihelmintic, analgesic, dysentry, purgative and blood disorders. Also they are reported to be useful in inflammation, healing of wounds, injuries, antioxidative activity. In folklore practice, the decoction of M. koenigii leaves has been reported to be useful in diarrhoea. In the present study the effect of hydroalcoholic extract of seeds was evaluated for antidepressant activity in mice. The models selected for the study were tail suspension test and despair swimming test. The extract at the doses of 100mg/kg, 250mg/kg and 500mg/kg significantly reduced the duration of immobility of mice in tail suspension test and despair swimming test as compared to the untreated group. Further, it potentiated the amphetamine induced stereotypic behaviour and enhanced the compulsive gnawing behaviour in mice. In conclusion, our results suggest that Murraya koenigii exerts antidepressant-like effects comparable to those of imipramine in experimental animal models.

Keywords: murraya koenigii (mk), anti depressant activity, aqueous extract, mice.

I. Introduction

Depression is considered as an affective disorder characterized by change in mood, lack of interest in the surroundings, psychomotor retardation and melancholia. The prevalence of depression in general population is estimated to be around 5%. At present 121 million people are estimated to suffer from depression. An estimated 5.8% of men and 9.5% of women experience a depressive episode in their lifetime with suicide being one of the most common outcome of depression (WHO 1998, Stahl SM, et.al., 1998, Richelson E, et.al., 2001). Despite the development of new molecules for pharmacotherapy of depression, it is unfortunate that this disorder goes undiagnosed and untreated in many patients. Although the currently prescribed molecules provide some improvement in the clinical condition of patients, it is at a cost of having to bear the burden of their adverse effects (Tripathi KD 2008, Hardman JG, et.al., 2007). Furthermore, it is difficult to predict which patient will respond to any given treatment. It has been reported in earlier studies that only two out of three patients responds to any given antidepressant treatment, and of these, one would probably have responded to placebo alone (Walker R, et.al., 1999). Along with the classical theory of decrease in the neurotransmitter levels in the brain leading to the pathogenesis of clinical depression, recent studies have also shown the involvement of oxidative stress in the phenomenon (Sarandol A, et.al., 2007, Ibrahim E, et.al., 2007). Ayurveda, the Indian traditional system of medicine, mentions a number of single and compound drug formulations of plant origin that are used in the treatment of psychiatric disorders (Tripathi KD, et.al., 2008, Sembulingam K, et.al., 1997). On one hand these agents have a less adverse.

Effect profile, and on the other hand they have been shown to be comparable in efficacy to their synthetic counterparts. As various parts of Murraya koenigii L.are used for the treatments of various diseases so this plant has its own identity identity in medicinal, pharmaceutical and chemical sciences, due to anti-oxidant, anti-microbial antidiarrhea, antidibetic ,antinflammatory, hepatoprotective, hypercholestero-lemic, antiviral , diuretic, carminative properties how promising results in the treatment of diarrhoea. Aqueous extract of the leaves of Murraya koenigii (M. koenigii) possesses alexeteric, antihelmintic, analgesic, dysentry, purgative and blood disorders. Also they are reported to be useful in inflammation, healing of wounds, injuries, antioxidative activity (Kirtikar KR, et.al., 2008, Swaroop VR, et.al., 2011).

Along with the above mentioned properties Murraya also posses the anti dipreseent activity. so, the present study is designed to evaluate the anti depressant activity in mice.

II. Materials and Methods

Dried powdered leaves of Murraya.

a) Procedure for Extraction of Murraya Koenigii (MK)Leaf

The dried and powdered leaves of MK were defatted with petroleum ether (60-80°C) and the following extracts were prepared:

1. Aqueous extract by decoction method
2. Methanolic extract by Soxhlet extraction method
3. Hydroalcoholic extract (70% ethanol) by Soxhlet extraction method

i. *Aqueous extract by decoction method*

This method is used for the extraction of the water soluble and heat stable constituents from crude

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drug by boiling it in water for 15 minutes, cooling, straining and passing sufficient cold water through the drug to produce the required volume 14.

ii. *Methanolic extract by Soxhlet extraction method*

Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. Required amount of plant material is extracted with methanol. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled 15.

iii. *Hydroalcoholic extract (70% ethanol) by Soxhlet extraction method*

The required leaf extract is obtained using soxhlation using 70% ethanol15.

The Etractive yield of Murraya leaf is mentioned in Table No: 1.

b) Phytochemical Evaluation of Murraya Leaf

The qualitative Phytochemical Screening of Various Extracts Obtained from Murraya koenigii Leaves are in Table No: 2

Conclusion: Phytochemical investigations revealed presence of phytosterols in pet ether extract, carbohydrates, glycosides, proteins, amino acids in aqueous extract and alkaloids and phenolic compounds in hydro-alcoholic and methanolic extract.

c) Evaluation of Anti depressant activity

Animals: Healthy Swiss albino mice (20-30 g) of either sex were used for the studies. Each experimental group consisted of at least six animals. The animals were housed for a minimum of five days prior to the pharmacological experiments, with free access to standard rodent pellet diet (Lipton India Ltd) and tap water, and maintained on a 12/12 h light-dark cycle. All experiments were conducted in accordance with international Animal Ethics Committee guidelines. The experimental protocols were approved by the institutional animal ethics committee (IAEC) - SIP/CPCSEA/IAEC/2012/I/09.

d) Extract and standard drug

The hydroalcoholic extract was formulated as suspension using 0.1% Sodium carboxymethyl cellulose (CMC). Imipramine (Cipla Ltd, Mumbai, India) was used as reference drug. The extract was adjusted to give a fixed volume of 10 ml/ kg orally in doses of 100mg/kg, 250mg/kg and 500mg/kg. Imipramine (Depsonil 25 mg Sarabhai Piramal Pharma Ltd, Vadodara): at a dose of 25 mg/kg was used as a positive standard. Reserpine and apomorphine was purchased from Boehringer Ingelheim BI. D-Amphetamine (Dexamphetamine IP) was purchased from Smith Kline and French, India.

e) Despair Swimming Test

Mice were made to swim individually in a polypropylene vessel (45×40×30 cm) with a water level of 20 cm. This ensured that the mouse's feet did not touch the floor of the vessel and that it could not climb out of it. Each mouse was allowed to swim for 10 min. Thereafter, during the next 10 min, the periods of total immobility, characterized by complete cessation of swimming with the head floating just above water level, was noted. This immobility period, after the initial frenzied attempts to escape, is postulated to represent behavioural despair as an experimental model of endogenous depression (S.K. Bhattacharya et al., 2003).

f) Tail Suspension Test

The method described by N.N. Jain et al. (2003) was used. Mice were divided in groups of six each. They were suspended by tying a thread to their tail from a height of 50 cm above the table top. Duration of immobility was recorded for 10 min. Mice were considered immobile only when they hung passively and remain motionless. Mice were treated with vehicle, MP (100, 250 and 500 mg/kg p.o) 60 min before the test and imipramine (25 mg/kg p.o) 30 min before the test.

g) Enhancement of Amphetamine -Induced Excitation

Imipramine like antidepressant drugs, enhance and prolong the behavioral effects of amphetamine (Turner, 1971). Groups of 6 male mice were treated orally with test drugs and were then challenged 90 minutes later with an i.p. dose of 3mg/kg of d-Amphetamine. Each animal was then scored every 30 minutes for 5 hr post amphetamine treatment according to the following scale: 0 = No activity, 1 = normal activity, 2 = increased motor activity, 3 = stereotyped head searching, 4 = continuous licking. The change of the cumulative total activity score for drug treated mice was calculated by comparison to a control group.

h) Compulsive Gnawing in Mice

Male mice with a body weight between 18 and 20 gm were injected with 10mg/kg apomorphine S.C, 30 minutes, prior to apomorphine injection the animals were treated with the test drug or the vehicle. Immediately after apomorphine injection, 6 mice were placed into a cage with wired lid. The bottom of the cage was covered with corrugated paper, the corrugation facing upwards. The mice started biting into paper causing fine holes or tearing the paper. The number of bites into the corrugated paper was evaluated by placing template upon paper. The template had 10 rectangle windows divided into 10 areas of the same size. In a total of 100 areas the number of bites was checked. In this way percentage of damaged paper was calculated. Percent gnawing of the test compound was compared with that of standard antidepressant drug imipramine, considering its value as 100% (Turner, 1971).

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i) Statistical analysis

The data was analyzed using Prism Graph Pad software and showed as mean±S.D. Comparison between control and drug treated groups were made by one-way analysis of variance (ANOVA) followed by Dunett's test, P values of less than 0.05 were considered to be significant.

III. Results

a) Despair Swimming Test

MP at the doses of 250 and 500mg/kg significantly reduced the immobility time as compared to the untreated animals ($P<0.05$). However, MP 100mg/kg did not show significant reduction in immobility time. The reduction in immobility time was comparable to imipramine 25mg/kg. The observations are given in Graph 1.

b) Tail Suspension Test

The total duration of immobility in vehicle-treated mice was 76.16 ± 6.8 s. Oral administration of MP (100, 250 and 500 mg/kg) significantly reduced the duration of immobility ($P<0.05$). Imipramine (25 mg/kg p.o) also reduced the duration of immobility. The observations are given in Graph 2.

c) Enhancement of Amphetamine -Induced Excitation

MP at the doses of 100, 250 and 500 mg/kg significantly increased amphetamine induced behaviour as evident by the average cumulative scores in mice, as compared to the vehicle treated animals($P<0.05$). The observations are given in Graph 3.

d) Compulsive Gnawing in Mice

MP at the doses of 500 mg/kg significantly increased the average number of bites induced by apomorphine administration as compared to untreated mice ($P<0.05$). The observations are given in Graph 4.

IV. Discussion and Conclusion

Depression is one of the most prevalent and disabling neuropsychiatric disease. The available antidepressant drugs are safe and effective but half of the patient exhibit partial, refractory or intolerant responses to treatment, thus emphasizing the need to discover new antidepressants. Murraya koenigii (MK) is reported to be an aphrodisiac and tonic. Therefore, the present work evaluated the effect of MK in mice models of depression.

The despair swimming test (DST) is the tool most widely used for assessing preclinical antidepressant activity. The widespread use of this model is largely a result of its ease of use, reliability across laboratories, and ability to detect a broad spectrum of antidepressant agents. Most clinically active antidepressants are effective in the DST, while neuroleptics and anxiolytics produce different effects (Porsolt et al., 1979). It is suggested that mice or rats, forced to swim in a restricted space from which they cannot escape are induced to a characteristic behaviour of immobility. This behavior reflects a state of despair, which can be reduced by antidepressants, which are therapeutically effective in human depression. In our study MK at the doses of 100, 250 and 500mg/kg significantly reduced the immobility time of mice in FST. Further, the MK extract was evaluated in tail suspension test. The "tail suspension test" has been described by Jain et al. (2003) as a facile means of evaluating potential antidepressants. The immobility displayed by rodents when subjected to an unavoidable and inescapable stress has been hypothesized to reflect behavioural despair, which in turn may reflect depressive disorders in humans. Clinically effective antidepressants reduce the immobility that mice display after active and unsuccessful attempts to escape when suspended by tail. Oral administration of MK extracts (100, 250 and 500 mg/kg) significantly reduced the duration of immobility ($P<0.05$). The results from the FST and tail suspension test prove the potential antidepressant action of MK hydroalcoholic extract.

MK extract significantly increased the stereotypic behaviour of amphetamine. Further, it also enhanced the apomorphine gnawing behaviour. The compulsive gnawing in mice is induced by apomorphine is due to dopaminergic stimulation. Based on these findings, it can be postulated that the antidepressant effect of MK is possibly due to increase in the neurotransmitters level at the synaptic cleft.

In conclusion, our results suggest that Murraya koenigii leaf extract exerts antidepressant-like effects comparable to those of imipramine in experimental animal models.

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Table No : 1

Plant Name	Extractive Yield Values (% w/v)		
	Aqueous Extract	Methanolic Extract	Hydro alcoholic extract
Murraya koeinigii	30.3	35.1	32.8

Table No : 2

Test/Reagent Used	Hydro alcoholic Extract (70% Ethanol)	Methanolic Extract	Aqueous Extract
Alkaloids			
Mayer's test	+	+	+
Dragendroff's test	+	+	+
Hager's test	+	+	+
Wagner's test	+	+	+
Carbohydrates and Glycosides			
Molisch's test	-	-	+
Fehling's Test	-	-	+
Barfoed's test	-	-	+
Benedicts test	-	-	+
Phytosterols			
Liebermann's Burchard's test	+	+	-
Fixed Oils and Fats			
Spot test	-	-	-
Saponification test	-	-	-
Saponins			

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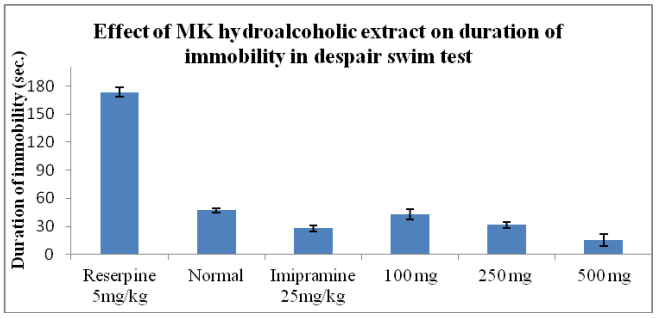
Foam test	-	-	+
Haemolysis test	-	-	+
Phenolic Compounds and Tannins			
Ferric chloride test	+	+	+
Gelatin test	+	+	+
Lead acetate test	+	+	+
Proteins and Amino Acids			
Biuret test	-	-	-
Ninhydrin test	-	+	+
Coumarins			
Fluorescence test	-	-	-

Graph No 1



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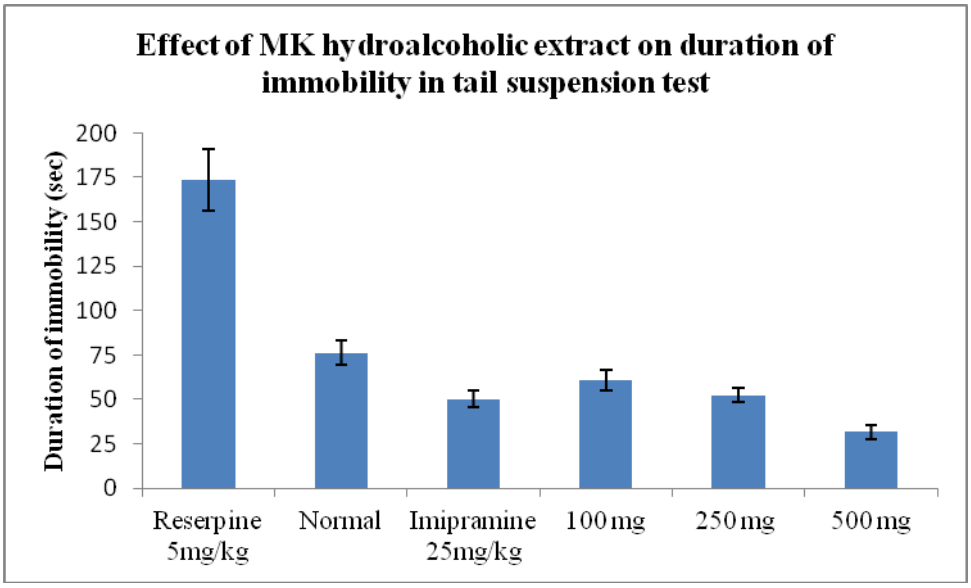

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Light-Trap Catch of the European Corn-Borer (Ostrinia Nubilalis Hübner) and Setaceous Hebrew Character (Xestia C-Nigrum L.) in Connection with the Height of Tropopause

By L. Nowinszky & J. Puskás
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Abstract- In present paper we examined the connection between height of tropopause and the light-trap catch two moth species.

The data of European Corn-borer come from the Hungarian national light-trap network between 1959 and 1973, and the Setaceous Hebrew Character come from the forestry light-trap network between 1961-1970.

Groups were made for data of the height of tropopause. The relative catch values of the examined species were categorised according to the characteristics of tropopause on each day, after it these values were summarised, averaged and depicted. We defined the parameters of the regression equations. We have found a close positive correlation between the height of the tropopause and relative catch of Setaceous Hebrew Character, but only the lowest and highest values of the tropopause reduce or rather increase of the light trap catch of the European Corn-borer.

Keywords: light trapping, tropopause, ostrinia nubilalis, xestia c-nigrum.

GJMR-GClassification : FOR Code: WC 900, WA 360

LIGHT-TRAPCATCHOFTHE EUROPEAN CORN-BORER OSTRINIA NUBILALISHUBNERANDSETACEOUSHEBREWCHARACTERXESTIAC-NIGRUMLINCONNECTIONWITHTHEHEIGHTOFTROPOPAUSE

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Keywords: light trapping, tropopause, ostrinia nubilalis, xestia c-nigrum.

I. Introduction

The tropopause is a surface separating the lower layers of the atmosphere (troposphere) from the upper layers (stratosphere). It is of varying height. In the presence of very cold air masses from the Arctic it may be a mere 5 kilometres, while in the presence of sub-tropical air it may grow to 16 kilometres. Sometimes there are two or three tropopauses one above the other.

A low tropopause is related the presence of cold and high tropopause the presence of warm types of air, while insect activity is increased by warm and reduced by cold air. An over 13 km height of the tropopause often indicates a subtropical air stream at a great height. This has a strong biological influence. These results may lead us to assume that the electric factors in the atmosphere also have an important role to play, mainly when a stream of subtropical air arrives at great height. On such occasions the 3Hz aspheric impulse number shows a decrease, while cosmic radiation of the Sun will be on the increase [Örményi, 1984]. The preponderance of negative ions in polar air reduces activity, while the preponderance of positive ions in subtropical maritime air may spur flight activity [Örményi, 1967]. The warm air increases the activity of the insects; the cold reduces it on the other hand.

This fact will change the number of insects collected by light-trap. We published it already in the recent past the efficiency of his light-trap catch in connection with the height of the tropopause of the Heart and Dart (Agrotis exclamationis L.), the Common Cockchafer (Melolontha melolontha L.), the Turnip Moth (Agrotis segetum Den. et Schiff.) and Fall Webworm Moth (Hyphantria cunea Drury) [Puskás and Nowinszky, 2000], [Örményi et al., 1997] and Puskás and Nowinszky (2011). However we know of no other study besides our investigating the relationship between the height of tropopause and light trapping. In our present work we have examined the light-trap catch of European Corn-borer (Ostrinia nubilalis Hübner) and Setaceous Hebrew Character (Xestia c-nigrum L.) as a function of the height of the tropopause, too.

We found in our former studies that the light trapping efficiency of parallel increases if the tropopause height is about 13 kilometres. However, the tropopause is even higher values in the collection of different species can be seen continue to increase, but also decrease. Therefore, we refer to our earlier studies where the effects of air masses influencing the collection were investigated (Nowinszky et al, 1997; Örményi et al, 2003). In these studies the subtropical air masses were divided on the basis of their origin and the path as follows:

Sub-tropical air; Azores air moving from W and WSW; Continental sub-tropical air arriving from the Middle East from SE; Saharan air from the Middle East from SE (observing in the upper layers only); Saharan air from across the Mediterranean Sea; Saharan air from across the Black Sea and Warm air from the Black Sea.

It has been stated that the subtropical air masses, observed in the high altitudes, differently affect the efficiency of light-trap collection according to whether they come from that route over Hungary. The light-trap catch of Turnip Moth (Agrotis segetum Den. et

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Schiff.) and Heart & Dart (Agrotis exclamationis L.) is high during subtropical residence time of air masses, but during the Saharan air mass residence time it is low. It is just opposed the results to the Fall Webworm Moth (Hyphantria cunea Drury) light trapping catch.

II. Material

Data for Budapest on the height of the tropopause have been collected from the Annals of the Central Meteorological Institute of the Hungarian Meteorological Service. Because area of Hungary is 93 036 km2 only, so this data is valid for the entire territory of the country (Örményi et., 1997).

The development of light-trap network began in 1952 in Hungary. The traps were used in research institutes, for plant protection and forestry purpose. The three type light trap network works with uniformly Jermy trap which is still working.

The national light-trap network over the past decades, enormous and inestimable scientific worth of insect material is provided for entomological research and plant protection practice. We selected two moth species from this huge data from for the present work. They comprise:

- European Corn-borer (Ostrinia nubilalis Hübner 1769) (Lepidoptera: Pyraustinae) from the all collecting material of all light-traps between 1958 and 1973.

Setaceous Hebrew Character (Xestia c-nigrum Linnaeus 1758) from the materials of all forestry light-traps between 1961 and 1970. The examined species and their catching data can be seen in Table 1

Insert near here Table 1

The stations of light-traps, their geographical coordinates and the examined years can be seen in Table 2

Insert near here Table 2

III. Methods

Than the number of individuals of a given species in different places and different observation years is not the same. The collection efficiency of the modifying factors (temperature, wind, moonlight, etc.) are not the same at all locations and at the time of trapping, it is easy to see that the same number of items capture two different observers place or time of the test species mass is entirely different proportion. To solve this problem, the introduction of the concept of relative catch was used decades ago (Nowinszky, 2003).

The relative catch (RC) for a given sampling time unit (in our case, one night) and the average number individuals per unit time of sampling, the number of generations divided by the influence of individuals If The number of specimens taken from the average of the same, the relative value of catch: 1. The relative catch allows the processing of collecting aggregate data from different years and observation locations (Nowinszky, 2003).

From the collection data pertaining to European Corn-borer (Ostrinia nubilalis Hbn.) and Setaceous Hebrew Character (Xestia c-nigrum L.) we calculated relative catch values (RC) by light-trap stations and by swarming. Following we arranged the data on the height of the tropopause in classes.

Relative catch values were placed according to the features of the given day, then RC were summed up and averaged. The data are plotted for each species and regression equations were calculated for relative catch of examined species and tropopause data pairs.

IV. Results

The results are shown in the Figure 1 and Figure 2.

Insert near here Figure 1 and Figure 2

V. Discussion

In our above cited study (Puskás and Nowinszky, 2011), significant positive correlations were established at each of the three species' light-trap catch studied in contention with the height of tropopause (Common Cockchafer (Melolontha melolontha L.) and Heart and Dart (Agrotis exclamationis L.) specimens, but only the lowest and highest values of tropopause reduce or rather increase of the light trap of the Fall Webworm Moth (Hyphantria cunea Drury).

Our results show that the light-trap catch of European Corn-borer) (Ostrinia nubilalis Hbn), rising to 14.5 km tropopause height increases, but higher values have been greatly reduced. In contrast, the light-trap catch of Setaceous Hebrew Character (Xestia c-nigrum L.) after the initial modest rise 13 km from rising strongly as a whole in the tropopause height of 15 km. This result is contrary to the findings of earlier works (Nowinszky et al,, 1997) while the latter confirms. The reason of the contradiction can be explained, that the European Corn-borer (Ostrinia nubilalis Hbn.) in subtropical air masses residence at the time of very hot nights have reduced flight activity.

This hypothesis is based on the ability of a still unpublished result as the light-trap catch of species increased, however, measured to the 21 o'clock evening temperatures up to 25 °C, but at higher temperatures the catch decreased by nearly half value.

We do not know yet every detail of how effects the height of the tropopause the catch results. Further researches will hopefully lead to a clear answer.

VI. Acknowledgements

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Table 1 : Catching data of examined species

Species	Light-traps	Years	Number of Moths	Data	Nights
Ostrinia nubilalis Hbn.	49	15	70 203	11 573	1 648
Xestia c-nigrum L.	21	9	39 101	4 583	1 047

Table 2 : The stations of Plant protection light-traps, their catching years of and geographical coordinates

Light-trap stations	Years	Geographical coordinates Latitude	Longitude
Plant protecting light-traps			
Andorháza-Pacsa	1959–1971	46°43'N	17°00'E
Badacsony	1968	46°48'N	17°30'E
Balassagyarmat	1968-1973	46°43'N	17°00'E
Budapest-Rókushegy	1959-1968	47°28'N	19°09'E
Celldömölk	1968	47°15'N	17°09'E
Csopak	1959-1973	46°58'N	17° 55'E
Fácánkert	1959–1973	46°26'N	18°44'E
Gyöngyös	1959–1973	47°46'N	19° 55'E
Győr	1959–1964	47°41'N	17° 37'E
Hegyeshalom	1965–1973	47°54'N	17°09'E
Hódmezővásárhely	1959–1973	46°25'N	20°19'E
Kállósemjén	1959–1973	47°51'N	47°51'E
Kaposvár	1964–1973	46°21'N	17°47'E
Kenderes	1960–1973	47°13'N	20°43'E
Mikepércs	1959–1973	47°26'N	21°38'E
Miskolc	1959–1973	48°06'N	20°47'E
Mohora	1959–1973	47°59'N	19°20'E
Nagytétény	1959–1973	47°23'N	18°58'E
Pápa	1968–1973	47°19'N	47°19'E
Szederkény	1959–1973	45°59'N	18°27'E
Tanakajd	1959–1973	47°1'N	16°44'E
Tarhos	1959–1973	46°48'N	21°12'E
Tass	1959–1973	47°00'N	19°01'E
Toponár	1959–1962	46°21'N	17°47'E
Vasvár	1968	47°03'N	16°48'E
Velence	1959 –1973	47°14'N	18°39'E
Zalaegerszeg	1972–1973	46°50'N	16°50'E

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Table 3 : The stations of Forestry and Research Institute light-traps, their catching years of and geographical coordinates

Light-trap stations	Years	Geographical coordinates	
		Latitude	Longitude
Forestry light-traps			
Bakóca	1969-1970	46°12'N	17°59'E
Budakeszi	1961-1970	47°30'N	18°56'E
Erdősmecske	1969-1970	46°10'N	18°30'E
Felsőtárkány	1961-1970	47°58'N	20°25'E
Gerla	1967-1970	46°40'N	21°05'E
Gyulaj	1969-1970	46°30'N	18°17'E
Kőkút	1969-1970	46°11'N	17°34'E
Kömörő	1969-1970	48°01'N	22°35'E
Makkoshotyka	1961-1970	48°21'N	21°31'E
Mátraháza	1961-1970	47°46'N	19°55'E
Répáshuta	1962-1970	48°02'N	20°31'E
Sopron	1962-1970	47°41'N	16°34'E
Szakonyfalu	1967-1970	46°51'N	16°13'E
Szentpéterfölde	1968-1970	46°37'N	16°45'E
Szombathely	1962-1970	47°14'N	16°37'E
Tolna	1961-1970	46°25'N	18°46'E
Tompa	1962-1970	46°12'N	19°32'E
Várgesztes	1962-1970	47°28'N	18°23'E
Zalaerdőd	1969-1970	47°03'N	17°08'E
Research Institute light-traps			
Badacsony	1968	46°48'N	17°30'E
Budatétény	1960-1970	47°24'N	19°09'E
Kecskemét	1961-1968	46°54'N	19°41'E
Keszthely	1960–1971	46°46'N	17°15'E
Kisvárda	1959-1968	48°13'N	22°04'E
Kompolt	1959–1968	47°44'N	20°14'E
Martonvásár	1961	47°19'N	18°47'E
Sopronhorpács	1959–1968	47°29'N	16°44'E
Tarcal	1964–1968	48°07'N	21°20'E

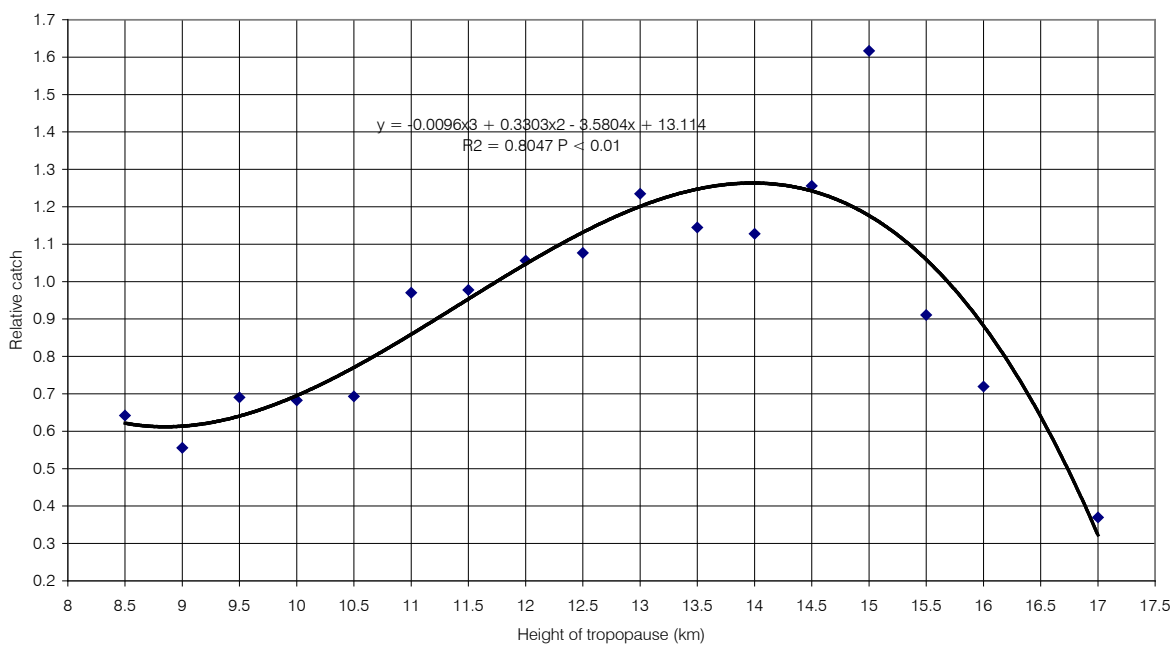

Figure 1 : Light-trap catch of European Corn-borer (Ostrinia nubilalis Hlon.) depending on the height of tropopause between 8.5 and 17 kilometres

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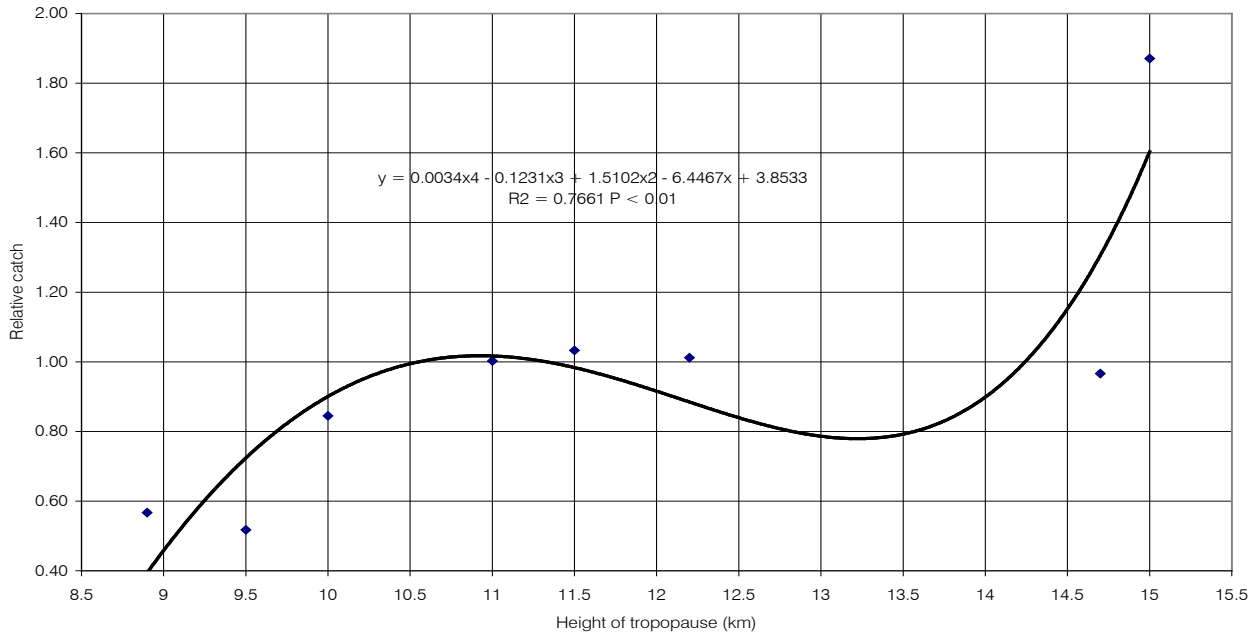

Figure 2 : Light-trap catch of the Setacoeus Hebrew Character (Xestia c-nigrum Linnaeus) depending on the height of tropopause between 1961 and 1970

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Increased TNF Serum Levels are Related to Highly Aggressive Behavior in Male Swiss Webster Mice

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Abstract- Since the 1960s, mouse behavior has been systematically studied in the laboratory environment; however, there is still no consensus regarding the causes of aggression in laboratory animals. The involvement of the immune response in aggressive animal behavior has not been well elucidated. Different studies have found that the levels of interferon alpha (IFN-**α**), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-**α**) are elevated in depressogenic/anxiogenic models. The aim of this study is to assess the correlations of serum cytokine (TNF, IL-6 and IL-10) levels with patterns of aggressive behavior (PBA). Our results suggest that mice exhibiting anxiety-like and highly aggressive behaviors have increased TNF serum levels and slightly decreased IL-10 levels. Additionally, a direct correlation was observed between high PBA scores and increased levels of TNF.

Keywords: aggressive behavior, laboratory mice, cytokine levels.

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Increased TNF Serum Levels are Related to Highly Aggressive Behavior in Male Swiss Webster Mice

FernanDa da Silva Oliveira[α], Cynthia Machado Cascabulho[σ], Kelly Cristina Demarque[ρ], Janaína Alves Rangel[ω], Frederico Villas Boas Rodrigues[¥], Wanderson Silva Batista[§], Lucas dos Santos Gameiro[χ] & Gabriel Melo de Oliveira[v]

Abstract- Since the 1960s, mouse behavior has been systematically studied in the laboratory environment; however, there is still no consensus regarding the causes of aggression in laboratory animals. The involvement of the immune response in aggressive animal behavior has not been well elucidated. Different studies have found that the levels of interferon alpha (IFN-α), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-α) are elevated in depressogenic/anxiogenic models. The aim of this study is to assess the correlations of serum cytokine (TNF, IL-6 and IL-10) levels with patterns of aggressive behavior (PBA). Our results suggest that mice exhibiting anxiety-like and highly aggressive behaviors have increased TNF serum levels and slightly decreased IL-10 levels. Additionally, a direct correlation was observed between high PBA scores and increased levels of TNF. In conclusion, TNF is an important cytokine related to highly aggressive behavior in male Swiss Webster mice.

Keywords: aggressive behavior, laboratory mice, cytokine levels.

I. Introduction

In the field of animal behavior, various ethological studies on aggression have related the ontogenetic characteristics and the phylogenetic adaptability of this type of behavior (Yerkes 1913; Blanchard et al. 1988; Pellis & Pellis 1988). Since the 1960s, the behavior of mice (and other animal models) has been studied systematically in the laboratory environment (McKinney 1989); however, there is still no consensus regarding the causes of aggression in laboratory animals. There is a complex network of factors involving genetics, biochemistry, physiology and neuroanatomy that contribute to the occurrence of aggressive episodes among individuals of a particular group (Edwards et al. 1993, Liebenauer & Slotnick 1996, Brodkin et al. 2003, Bray & Cotton 2003, Birger et al. 2003, Gleason et al. 2009).

Aggressive experimental models have been obtained through behavioral, pharmacological and genetic manipulations (Brodkin et al. 2002, Coccaro et al. 1997). However, such models are questioned for the following reasons: a) a small number of animals (often only two) is used, ignoring interactions with other individuals; b) aggression is induced directly, primarily by inducing aggressive territorial behavioral (e.g., test resident/intruder); c) only two hierarchical positions, the dominant (the aggressor) and the subordinate (the attacked), are determined; and d) the aggressive behavior is evaluated only for a short period of time (e.g., 5 minutes/test), thus preventing the assessment of the behavioral characteristics, such as those related to depression (depression-like) or anxiety (anxiety-like) disorders, inherent to each individual before the test, particularly during sexual maturation (Cryan & Holmes 2005).

The involvement of the immune response in aggressive animal behavior has not been well elucidated. There are interconnections between the genesis of psychiatric disorders and inflammation (Krishnadas & Cavanagh 2012). Most of this knowledge has arisen from an attempt to link specific illnesses (Major Depressive Disorder - MDD) with 'stress' biology, and the data raise the possibility of an 'initial common pathway' in which immune/inflammatory biomarkers and stress combine to cause changes in brain structure and function (Raison & Miller 2011). Consistent with the centrality of inflammation in this model system, in separate studies, elevated levels of interferon alpha (IFN-α), interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) have been shown to correlate with depressive symptoms (Prather et al 2009, Raison et al. 2010). In recent years, several groups have reported that a positive relationship exists between proinflammatory cytokines and aggression/hostility (Zalcman & Siegel 2006). For example, Suarez and colleagues showed in healthy male subjects that the scores on the Buss-Perry Aggression Questionnaire, which measures hostility, anger, and aggression, were associated with the increased production of TNF-α (Suarez et al. 2002). A recent study identified a potent role for this cytokine in the regulation of aggressive and anxiety-related behaviors, and the data suggest that

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TNF receptor (TNF-R1 and TNF-R2) signaling tonically modulates activity in specific brain regions (Patel et al. 2010).

The main objective of our study was to evaluate the production of pro- and anti-inflammatory cytokines in highly aggressive mice in the laboratory environment. We believe it is essential to monitor the behavioral characteristics of each individual mouse from the time of weaning. In this context, we grouped the animals before sexual maturity assessed the activity profile of each animal using the tail suspension test (TST). The mice were regrouped based on the activity categories in adulthood. Thus, we developed and analyzed a model of spontaneous aggressiveness (MSA). The serum levels of TNF, IL-6 and IL-10 were determined and were found to be correlated with depression-like and anxiety-like disorders and the pattern of aggressive behavior.

II. Material and Methods

a) *Mice*

Male albino Swiss mice (3 weeks old) were maintained in our animal facilities at the Division of Animal Experimentation of the Cell Biology Laboratory, Instituto Oswaldo Cruz (SEA/LBC - IOC). They were adapted to the environment for one week in ventilated racks, and the temperature, humidity and photoperiod were controlled according to the standard environmental regulations. The animals were maintained under stable conditions of temperature and light, with a 12-h light/dark cycle, and both food and water were available *ad libitum*. Routine cleaning was performed twice per week. The procedures were performed under license number LW-5/12 of the Ethics Committee for the Use of Animals (CEUA/FIOCRUZ).

b) *Model of spontaneous aggressiveness (MSA)*

The mice were separated into 10 groups (A1 to A10) of 10 mice each, and the mice in each group were individually identified (c1 to c10) (Scheme 1) (50 mice/assay). Three behavioral assessments—an ethological study, the TST and the Motor/Exploratory activity test (described below)—were used to assess all animals once per week between the 4th and 8th weeks of life. During the 10th week, the animals were regrouped into low, medium and high mobility groups, without directly induced aggression, based on the mobility profile defined by the TST. The same behavioral assessments were performed once per week between the 12th and 16th weeks of life.

c) *Behavioral Analysis*

i. *Ethological analysis*

We recorded (top view) each group (4th, 6th, 8th, 12th, 14th and 16th weeks) for 60 continuous minutes using a Canon Power Shot SX20 IS® (Lake Success, New York, USA). A total of 3600 minutes of video was recorded, and the most representative changes were recorded by photography. From these movies, we determined the variables to be evaluated in the ethogram: a) the pattern of aggressive behavior (PBA), including bites, wounds and injuries to an animal caused by fights among individuals in each group, and b) the qualitative and quantitative PBA intensities, determined by a scoring system. The scores were as follows: 0 (zero): the absence and/or presence of vocalizations and persecution, with no signs of bites or lesions on the animal's body; 1+: the presence of a low number of aggressive events, with or without sexual characteristics (attempted intercourse between individuals) and with the presence of small bites or injuries anywhere on the body; 2+: the presence of a low number of aggressive events without bites with sexual characteristics and discrete marks on the tail, back or scrotum; 3+: the presence of a moderate number of aggressive events and the observation of injuries and mild lesions on the tail, back and scrotum of the animals; and 4+: a high frequency (or intensity) of aggressive events (with or without specificity between individuals) and the presence of marked lesions and injuries on the tail, back and scrotum. In some cases, injuries of varying intensities were observed on other parts of the body, such as the chest, abdomen and forelegs (defensive injuries) (Oliveira 2012).

ii. *Tail suspension test (TST)*

Steru and collaborators (1985) introduced this methodology, which allows the determination of an animal's mobility in response to a stressful situation. This technique was developed and used for the preclinical evaluation of the efficacy of antidepressants and anxiolytics (Steru et al. 1985). In the TST, the animal is held vertically by the tail (final third) in a circular structure so that its muzzle is approximately twenty inches from the floor (Steru et al. 1985). We performed this test during the 4th, 6th and 8th weeks of life and after regrouping (12th, 14th and 16th weeks). We estimated the length of time that the animal remained motionless (immobile) during the five-minute test. Immobility was characterized by the absence of torsional movements, rotation and attempts to lift the body. We defined three categories based on the duration of immobility related to the animal's physical reaction to the stress challenge: a) hypoactive (Hypo) (low mobility or depression-like behavior), with a duration of 104 to 150 seconds, b) normal (Norm) (medium mobility), with a duration between 51 and 103 seconds, and c) hyperactive (Hyper) (high mobility or anxiety-like behavior), with a duration of 0 to 50 seconds (Oliveira 2012).

iii. *Measurement of cytokine levels*

TNF, IL-6 and IL-10 were detected in serum samples of mice using the BD CBA Mouse Soluble Protein Flex Set, according to the manufacturer instructions. The samples were acquired in a

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FACScalibur flow cytometer (Becton Dickinson, USA) and data analysis were performed using The CBA analysis FCAP software (Becton Dickinson, USA).

iv. *Statistical Analysis*

The Mann-Whitney non-parametric test was used to compare the two groups (SPSS software, version 8.0). Values correspond to the means ± standard deviations of three independent experiments.

III. Results

Our results clearly demonstrate the effectiveness of the MSA in the analyzing of aggressive behavior in Swiss Webster mice. The intensity of aggression was directly related to the profile for the TST (Fig. 1). During the weeks studied, we did not observe alterations in the individual results of the TST for the Hyper and Norm categories, but there were individual modifications in the Hypo group. The individual TST profile of this group consisted of 50% low, 40% high and 10% median mobility mice (Fig. 1A) during the 16th week of life. Regarding the ethogram results, regrouped animals with low mobility (Hypo) showed PBAs with 2+ relative scores (Fig. 1B). The animals with median mobility (Norm) had scores of 0/1+, with the lowest level of aggressive behavior (Fig. 1C). These data were the opposite of those for the high mobility (Hyper) group, which exhibited increased aggressive behavior, with relative PBA scores of 4+ (Fig. 1D). We also observed that the injuries from fights occurred in specific regions and had specific physical characteristics. Injuries to the front legs, chest and muzzle regions were the result of individual confrontations and defensive actions (Fig. 1E). It is important to note that during the grouping between the 4th and 8th weeks of life, no PBA was observed in any group studied (score = 0).

We measured the production of pro- and anti-inflammatory cytokines in various groups of animals (Fig. 2). Increases in the mean TNF serum levels (Fig. 2A) were observed in the Hypo (13.3 pg/ml) and Hyper (19.6 pg/ml) groups. Norm mice had very low levels of TNF (3.5 pg/ml). Furthermore, there was a greater difference in the values between individuals. The average deviations of the groups were as follows: Hypo: 13.3 ±11.0; Norm: 3.5 ±0.31; and Hyper: 19.6 ±13.8. The high value of the deviation was related to the individual PBA scores (Table 1). The occurrence of moderate or high-frequency (or intensity) aggressive events (with or without specificity between individuals) and the presence of marked lesions and injuries to the tail, back and scrotum were directly linked to the high levels of TNF (e.g., HypoC1: 34.2/4+; NormC7: 4.8/0; and HyperC3: 40.8/4+; TNF level/PBA score). In addition, the increase in the group's average value (Hypo - 13.3/2; Norm 3.5/0 and Hyper – 19.6/3, respectively; TNF level/PBA score) was directly related to the increase in the PBA score. The IL-6 level did not differ significantly between groups (Fig. 2B). However, there was a subtle trend of higher levels for the Hypo and Hyper groups: Hypo: 12.0±0.5; Norm: 11.5±0.5; and Hyper: 12.7±0.5 pg/ml. In addition, there were no significant differences in the IL-10 between the animals with different mobility profiles (Fig. 2C). However, there was a non-significant increase in the level of this cytokine in the Hypo group (6.3±0.1 pg/ml) relative to the others (Norm: 5.6±0.1 pg/ml and Hyper: 5.5±0.1).

IV. Discussion

We question the relevance of experimental models in which aggressive events are triggered by pharmacological or genetic manipulations for the study of aggression in mice (Steimer 2011, Oliveira 2012). Three relevant characteristics of the MSA are as follows: (i) individual/social behaviors are evaluated, and the same animal is monitored from the time of weaning through adulthood; ii) animals are separated into categories based on mobility (or activity), and the individuals most likely to be aggressive are selected; and iii) group formation, interactions between individuals and the hierarchy structure can be observed. In the standard methodologies, a pair of animals is used, in which case it is possible to identify only the aggressor/attacked relationship or the hierarchy defined as dominant/submissive at the time of the observation (5 minutes) (Ensminger & Crowley 2007).

Our results demonstrate that in the Norm and Hyper groups, the TST mobility profile remained constant. Furthermore, it was clearly observed that male Swiss Webster mice with anxiety-like behaviors are highly aggressive when regrouped in adulthood. Hypotheses regarding territorialism, disputes over females and access to food as the causes of such behavior do not satisfactorily explain the intensity of aggression (Benton et al. 1980, Kareen & Barnard 1986, Drickamer 2001, Weidt et al. 2008). The factors associated with the anxiety state impair the ability to establish a linear hierarchy (Oliveira 2012). Thus, the maintenance of leadership (or dominance) is imposed by increasing the number and severity of aggression behaviors. Furthermore, the severity of the lesions appears to be related to the individual's defensive ability. Animals with defensive behaviors had bites located on the front legs, chest and muzzle.

Regarding the cytokine production, our results suggest that mice with anxiety-like behaviors and highly aggressive mice have higher serum levels of TNF and a tendency to have lower levels of IL-10. We emphasize that in mice with depression-like behaviors, increases in the mean TNF and IL-6 levels were also observed in aggressive animals. Raison & Miller (2011) and Krishnadas & Cavanagh (2012) provided data that support two opposing explanations for this phenomenon: first, that individuals with increased

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inflammation comprise a physiologically discrete depressive/anxiety subtype, and second, that because inflammatory pathways are fully integrated into larger mind–body systems that have evolved to cope with environmental danger, it may be the case that even low/higher levels of inflammatory stimulation may be depressogenic/anxiogenic in individuals with vulnerability patterns in the nonimmune elements of these larger systems (Raison & Miller 2011, Krishnadas & Cavanagh 2012),.

Furthermore, we believe that the TNF/PBA correlation in animals is related to secondary infection by microorganisms after injury, resulting in increased levels of bacterial endotoxin and/or lipopolysaccharide (LPS), which is a potent hypothalamic–pituitary–adrenal (HPA) activator and elicits marked sickness behavior (Dantzer et al. 1998, Yirmiya 1996, Dantzer 2001). When LPS is systemically administered, Toll-like receptor-4 (TLR-4) on immune cells is activated, inducing the release of IL-1b along with a cascade of other cytokines, such as IL-6 and TNF (Laflamme & Rivest 2001, Kosnan et al. 2002, Rivest 2003). These cytokines are thought to influence central neuronal processes, thus promoting behavioral changes (Dantzer 2001, Kosnan et al. 2002, Kelley et al. 2003), including changes in the level of aggressiveness.

V. Conclusions

We believe that the cause of aggression is related to a complex and integrated network of genetic, behavioral, neurological and biochemical factors. In Swiss Webster mice, increased aggressive behavior is most likely related to maternal care disruptions, an anxiety-like profile and neuroendocrine deregulation. Our results suggest that TNF production is directly related to highly aggressive behavior in mice, especially in mice with the Hyper mobility profile. Moreover, due to the severity of the lesions promoted by bites and fights, there is a significant increase in the levels of cytokines, which clearly compromises the animal's welfare and the reliability of the obtained data.

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Legends to Figures

Scheme 1 : Structure and development of the MSA: Three-week-old male Swiss Webster mice were separated into 10 groups (A1 to A10) with 10 animals per group. During the 4th, 6th and 8th weeks, each animal was monitored by an ethological analysis and assessed using the TST. During the 10th week, the animals were regrouped (10 mice/cage) into the following categories based on the TST mobility profile: Hypo (low), Norm (medium) and Hyper (high mobility). The behavioral tests (ethological analysis and TST) were repeated in the 12th, 14th and 16th weeks. In the 16th week, the animals were euthanized, and blood samples were collected for the quantification of cytokines

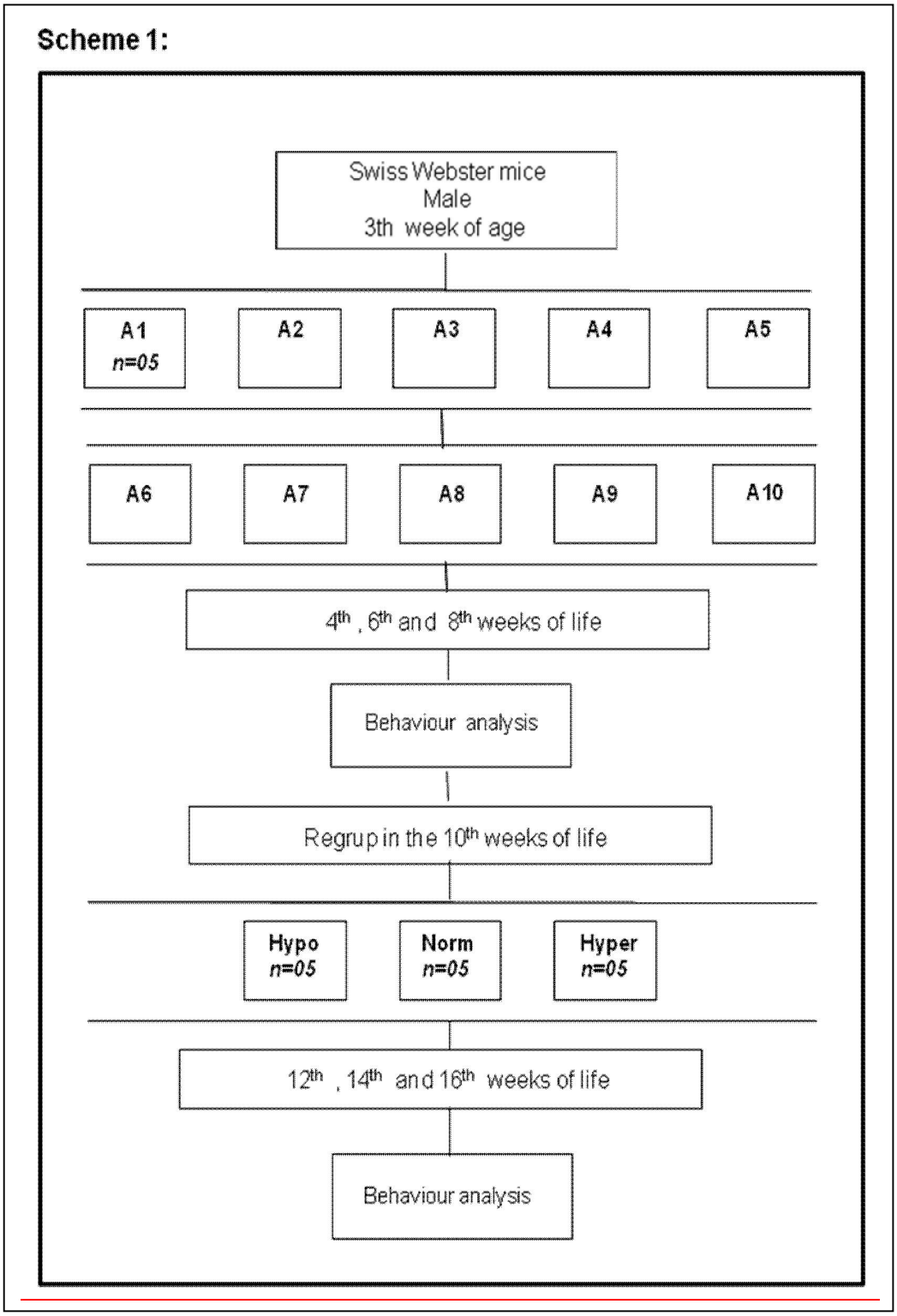

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Figure 1 : Categorization based on the TST results and the PBA score: (A) During the 10th week, the animals were regrouped based on the mobility profile defined by the TST: Low-*Hypo* (dark gray), Medium-*Norm* (gray), and High-*Hyper* (light brow). At the 16th week, the Hypo group consisted of 50% animals with low mobility, 40% animals with medium mobility and 10% animals with high mobility. The injuries associated with the different PBA scores are illustrated: (B) Hypo animal with a score of 2+; (C) Norm animal with a score of 1+ and (D) Hyper animal with a score of 4+ (D). Figure 3E shows injuries sustained during defense against aggression

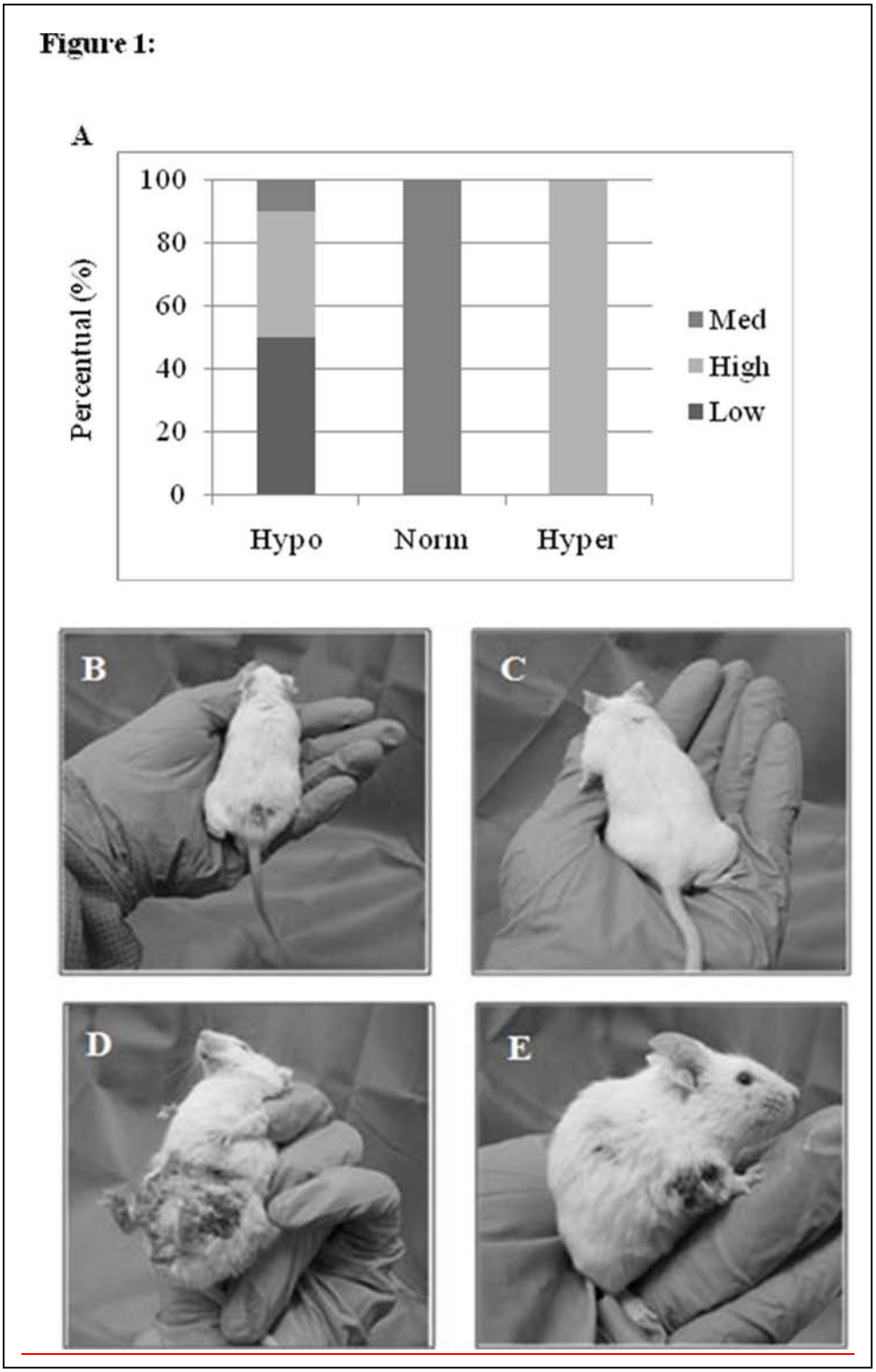

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Figure 2 : Cytokine production: When the mice were 16 weeks of age, the pro- and anti-inflammatory profiles of the humoral immune response were evaluated. (A) The TNF levels (pg/ml) in Hypo (circle), Norm (square) and Hyper (triangle) mice. The horizontal line indicates the average value for each group. The IL-6 (pg/ml) (B) and IL-10 (pg/ml) levels (C) were measured in the Hypo (gray bar), Norm (white bar) and Hyper (black bar) groups, and no significant differences were observed between the groups. Values correspond to the means ± standard deviations of three independent experiments

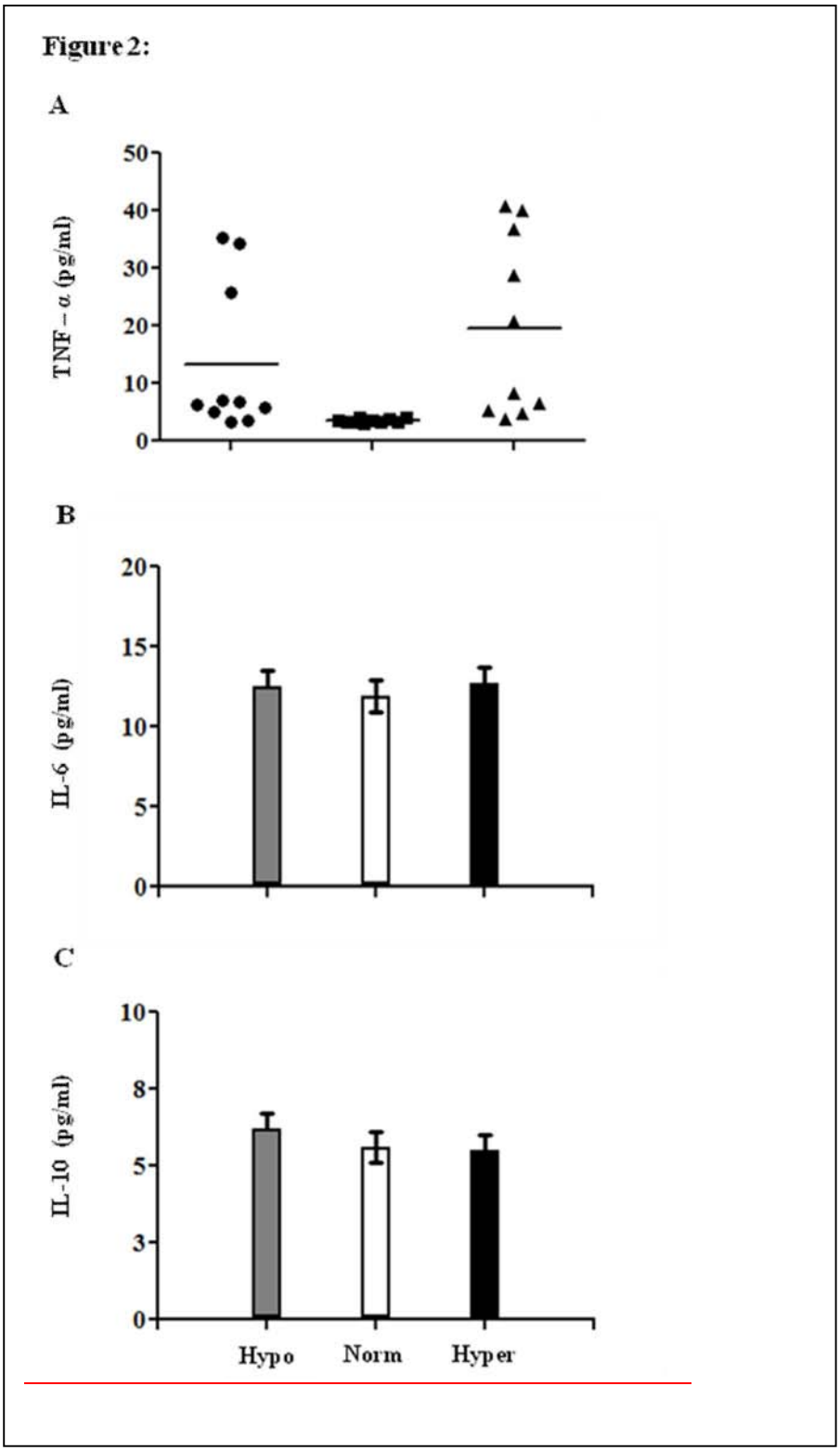

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Table 1 : Correlation between TNF levels and PBA score

Mice	TNF-α (pg/ml)	PBA (+)	Mice	TNF-α (pg/ml)	PBA (+)	Mice	TNF-α (pg/ml)	PBA (+)
HypoC1	34.2	4	NormC1	4.2	0	HyperC1	8.2	2
HypoC2	25.9	3	NormC2	4.5	1	HyperC2	36.9	3
HypoC3	35.3	3	NormC3	3.9	0	HyperC3	40.8	4
HypoC4	5.0	0	NormC4	4.0	0	HyperC4	20.9	3
HypoC5	6.9	1	NormC5	5.2	0	HyperC5	28.7	3
HypoC6	6.2	1	NormC6	6.2	1	HyperC6	40.1	4
HypoC7	5.9	1	NormC7	4.8	0	HyperC7	5.3	1
HypoC8	3.5	0	NormC8	5.9	0	HyperC8	6.6	2
HypoC9	3.3	0	NormC9	5.5	0	HyperC9	3.8	2
HypoC10	7.0	2	NormC10	4.0	0	HyperC10	4.7	0
AVG	13.3	2.0	AVG	3.5	0	AVG	19.6	3.0

AVG: Average number/group

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Prophylaxis of Imune Deficiencies and Neonatal Diarrhea Syndrome among Sucking Piglets by Administration of Organic Selenium (Sel-Plex)

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Abstract- In this study, it was investigated the action of the Sel- Plex product that was included in the combined forage for milking swine and sucker piglets. There were investigated 10 parturient swine and 101 piglets of 1-42 days.

It was concluded that the addition in basic ration of organic selenium has a benefic effect for swine growing and development that was expressed through:

• Mortality and neonatal diarrhea reduction, weight increase with 2.5 kg for the swine treated with Sel-Plex ($P<0,05$);

• Significant increase of the total number of T-lymphocytes ($P<0,001$), of the subpopulation of active T-lymphocytes ($P<0,01$) and of B-lymphocytes ($P<0,01$).

Keywords: milking swine, neonatal diarrhea, profilaxy, sucking piglets, sel-plex.

GJMR-G Classification : NLMC Code: WI 407, QW 815

PROPHYLAXISOFIMUNEDEFICIENCIESANDNEONATALDIARRHEASYNDROMEAMONGSUCKINGPIGLETSBYADMINISTRATIONOFORGANICSELENIUMSEL-PLEX

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It was concluded that the addition in basic ration of organic selenium has a benefic effect for swine growing and development that was expressed through:

- Mortality and neonatal diarrhea reduction, weight increase with 2.5 kg for the swine treated with Sel-Plex (P<0,05);
- Significant increase of the total number of T-lymphocytes (P<0,001), of the subpopulation of active T-lymphocytes (P<0,01) and of B-lymphocytes (P<0,01).

The postive action of the products is show at the level haemotopoiesis by increasing erythrocytes and hemoglobin (P<0,01) an dat the level of antioxidant system by reducing the level of DAM (Malonic dialdehyde) and increase of TAA (total antioxidant activity) in blood serum.

Keywords: milking swine, neonatal diarrhea, profilaxy, sucking piglets, sel-plex.

I. Introduction

Currently, studies on birds, pigs and calves show that in the first days of life, in the first months respectively, diarrhea is the most significant cause of illness and treatments performed [13,6]. Animal health and performance depend on many factors and it is considered more and more that diet plays an essential role in maintaining health and preventing illness.

The main role of the gastrointestinal tract is to extract nutrients from the diet ingested, after which nutrients are absorbed into the bloodstream. Therefore, the gastrointestinal tract is principal interphase of the internal hostile environment and host organism metabolism.

Research conducted at the swine complexes and traditional farms in the Republic of Moldova have clearly shown that infant animals are in great danger of gastrointestinal tract illness following exposure to a variety of risks from pathogen germs invasion to toxins in alimentation, dangerous metabolites produced by normal intestinal microflora or due to the lack of a complex microflora and a functional system at full capacity [14,4].

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In recent decades studies have shown that obtaining animal uncontaminated products with nitrates (nitrates) and nitrogen (nitrites), or presents of antibiotics residues represent one of the most important challenges in European Union countries.

Today it was necessary to find solutions to improve animal health by increasing their natural resistance to disease avoiding the use of antibiotics. Previous research conducted by us in industrial swine breeding conditions have certainly demonstrated that resulting from the increased bioavailability and biological activity of organic selenium (Sel-Plex) than inorganic forms (sodium selenite), it is applied in practice Veterinary Medical our country [2,12,3].

It was found that supplementation of basic ration with organic selenium to pregnant sows and during piglet growth, besides improving animal performance, have a positive influence on their health.

In specialized literature we often find statements that affirm that the only way to combat antibiotic biopersistence is elaboration new products, more active than those previously elaborated. But the rush for an "ideal product" that would combat "supermicrobes" can not remove the "reality". So instead of "defeating" or reducing resistance to antibiotics it is necessary only to decrease the number of recommendations for antibiotics [10].

Currently, in the new European context, remarkable efforts are made to substitute antibiotics with natural growth promoters, such as acids, prebiotics, probiotics, feed enzymes. Their major effect is to correct and maintain healthy intestinal environment through which to potentiate the use of digestive food.

This paper is aimed at studying the action of Sel-Plex product included in the mixed fodder for milking swines and sucking piglets on clinical and paraclinical indicators of milking swines and sucking piglets in 1-42 days post-partum.

II. Materials and Methods

The research was conducted on 10 multiparous milking swines with similar weights in 10-12 days before parturition and 101 piglets aged 1-42 days, belonging to tri-racial commercial hybrid (Large White, Landras, Diuroc), which were divided into two groups: control and

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experimental. Both groups of animals were kept inside the same technology equipped compartments entirely respecting the microclimate conditions, feeding, watering and free spaces.

Experimental and control groups were fed with mixed fodder for each animal category: milking swines in lactation period, sucking piglets, respecting the average structure and basic parameters recommended by the specialized literature. Basic ration of milking swines in the amount of 100 kg per-total included (in kg): corn - 32, barley – 19, grain - 19, sunflower meal - 5.5, soybean meal - 6.5, wheat bran - 13, calcium carbonate - 1.1, sodium chloride - 0.4, zoofort - 0.5. Recipe for mixed fodder used to feed sucking piglets in the amount of 100 kg per total included (in kg): corn - 41, wheat - 20, barley - 16, soybean meal - 12, vegetable fat - 1.9, calcium carbonate - 0.5, phosphate calcium - 0.9, sodium chloride - 0.2. zoofort - 1, bone-meat meal - 6,5.

Milking swines in both groups received daily 5 kg of mixed fodder for two times for each animal. The difference between groups was that in the fodder for the milking swines from the experimental group Sel-Plex was added at a rate of 1 kg / tone. The introduction of mixed fodder in sucking piglets forage was performed after the first week of life, which in the experimental group was supplemented with Sel-Plex in proportion of 1 g to 1 kg of mixed fodder.

The action of Sel-Plex on milking swines and sucking piglets was tested by assessment of the clinical state (body temperature, pulse, respiration, general condition, morbidity and mortality, feed consumption, daily surplus) and hematologic data (total number of leukocytes, leukocyte formula and classes of T and B lymphocytes, malonic dialdehyde content (DAM) and AAT (total antioxidant activity) in plasma and erythrocytes. Blood samples were taken in the 7th, 21st and 32nd day postpartum.

III. Results and Discussions

Milking swines in both groups during the investigation were fed and watered, according to the established schedule. 10-12 days before parturition and during the 42 days postpartum, the basic daily ration of the animals from experimental group was supplemented with Sel-Plex (1 kg per tonne of mixed fodder), which corresponded to 5.76 kg of the product, which is an average consumption (table 1). Piglets in the experimental group after the first week of life together with mixed fodder daily received Sel-Plex in proportion 1g - 1kg of feed. Thus, milking swines in the experimental group from 10 to 12 days before parturition and piglets in this group after the first week of life received mixed fodder supplemented with Sel-Plex.

Table 1 : Consumption of mixed fodder

Group	Milking swines daily consumption (kg/unit)	Piglets daily consumption (kg/unit)	Piglets total fodder consumption (10-42 days)
I (control)	5,64	0,365	11,68
II (experimental)	5,76	0,378	12,096

Data from Table 1 represent the consumption of combined fodder during the research. Both groups were healthy: food and water were consumed, animals were active and showed no deviations in behavior. However, in the experimental group an average daily consumption of fodder was with 0.12 kg more than in the control group.

Differences between the two groups of piglets were not significant, the total fodder consumption being by only 0.416 kg more than in the experimental group.

Table 2 presents the evolution of body weight of piglets from the control and experimental groups.

Table 2 : Evolution of piglets body weight

Group	n	Statistic data	Body weight				
			Days 1-2	Day 13	Day 21	Day 32	Day 42
I (control)	50	M±m	1,22±0,04	2,8±0,076	5,1±0,16	6,75±0,25	10,3±0,91
		Lim	1,1-1,3	2,33-3,0	4,7-5,8	5,8-7,5	9,4-12,5
II (experimental)	51	M±m	1,23±0,10	3,013±0,162	5.3±0.08	7.6±0.957	12.8±1.04*
		Lim	1,2-1,25	2.41-3.75	5.3-7.37	6.25-9.5	10.8±15.2

* $P_{1,2}<0,05$

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In the first day of life the piglets from both groups on average weighed 1.22 kg. At the age of 13 days piglets body weight consisted 2,8 ± 0,07 and 3,01 ±0,16 in the control group and experimental group accordingly. At the end of the third week piglets from the experimental which consumed Sel-Plex, had a daily surplus higher body mass daily, equal to 0.2 kg (P> 0.05). The trend of better growth was maintained during the 32-42 days that ended with a growth increase of 520 g / day in experimental group and 325 g / day in control group.

Average weight per capita was 10.3 ± 0.91 kg in control group and 12.8 ± 1.04 kg in the experimental group, the difference of 2.5 kg (24.2%) between the two groups being significant (P <0.05).

The positive effect of supplementation with organic selenium on growth and development of piglets is confirmed by leukocyte indices and in particular lymphocytes. Obtained indices are shown in Table 3.

The number of leukocytes in the first research (21 days after birth), consisted 7.95 ± 0.54 for animals in the control group and 9.28 ± I .33 for experimental group (P1, 2> 0.05). By the day 32 there was registered a significant increase (P1, 2 <0.05) of the total number of leukocytes that we consider as a positive effect of the refill of basic ration with Sel-Plex. Simultaneously, the dynamics of T-lymphocytes, which are key cells in the immune system expression, on the first survey (21 days postpartum) of piglets in the experimental group was expressed by a significant increase (P1, 2 <0.02). Also there was a fast growth to 25.4 ± 1.67% on the 21 day and to 36.0 ± 0.70% on the 32 day (P1, 2 <0.001).

According to data, received by Ştefan Ţurcanu and col. (2003) cellular immune status of piglets in ontogenesis is formed at a certain stage of physiological maturity of the body. As to the T-lymphocytes, a considerable increase up to 41.6% was observed by day 7 and 27.6% at the age of 3 weeks. An unessential increase of number of these cellules the authors observed by day 42 after birth, which consisted 13.4%.

Table 3 : Dynamics of T and B lymphocytes in piglets blood

Group	Days	Lymphocytes (%)	T-total (%)	T-active (%)	T-helper (%)	T-supres. (%)	B-lymphocytes (%)
		M±m	M±m	M±m	M±m	M±m	M±m
I (control)	21	25,4±1,67	50,6±1,34	25,8±1,48	34,2±1,92	16,4±0,89	25,4±1,34
II (experim.)	21	31,8±2,77*	53,6±2,07	29,6±1,14 *	37,6±0,54	16,0±2,34	31,5±3,96*
I (control)	32	28,6±0,89	50,6±0,89	27,6±0,89	31,0±1,22	19,6±1,51	24,4±0,54
II (experim.)	32	36±0,70***	55,8±1,64	32,8±1,09**	39,8±1,3***	16,0±1,01	37,0±5,6**

* $P_{1,2}$<0,05; ** $P_{1,2}$<0,01; *** $P_{1,2}$<0,001

In Table 3 it can be noted that active T-lymphocytes subpopulation has essentialy increased, with a high degree of authenticity P1, 2 <0.05 in the first research and P1, 2 <0.01 in the second

research (32 days after birth) for the piglets in the experimental group. The first survey (day 21) and the second one of the piglets in the experimental group showed that T-lymphocytes helpers subpopulation had a percentage of 37.6 ± 0.54 and 39.8 ± 1.30%, accordingly. In the control group this index was 34.2 ± 1.92% on 21st day, and it was followed by a decrease of up to 31.0 ± 1.22% on the 32nd day after birth.

Regarding the dynamics of B-lymphocytes, during this study there was proved a significant increase (P1, 2 <0.05, day 21 and P1, 2 <0.01 on the 32nd day) at piglets from the experimental group.

In Table 4 there are presented data referring to the content of erythrocytes, quantity of hemoglobin and leukocytes at piglets in both groups.

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Table 4 : Hematological indices of piglets

INDICATORS	GROUP	STUDY					
		I Day 7			II Day 32i		
		n	M±n	P	n	M±n	P
Hemoglobin g/L	I – experimental	5	107,6±0,55	$P_{1,2}$>0,05	5	143,26±0,55	
	II - control	5	106,23±0,56		5	122,9±0,56	P<0,001
Erythrocytes (x 10^{12}/L)	I – experimental	5	4,55±0,56	$P_{1,2}$>0,05	5	7,08±0,55	
	II - control	5	4,42±0,49		5	5,41±0,56	P<0,05
Leukocytes (x 10^{9} / L)	I – experimental	5	7,76±0,49	$P_{1,2}$>0,05	5	6,98±0,56	
	II - control	5	20,72±0,56		5	10,59±0,56	P<0,001

The first research showed similar data which are statistically unreliable (P> 0.05). It is necessary to mention that the second research showed that the number of erythrocytes of the amount of hemoglobin has essentially increased at the piglets in the experimental group (P <0.05) and (P <0.001).

The amount of leucocytes at piglets in the experimental group was stable and consisted 7.76 + 0.49 x 109/L initially and 6.98 + 0.55 x 109 / L at the following assessment. The piglets in the control group suffered a decrease of number of leukocytes from 20,72 + 0,56 at the first assessment to 10,59 + 0,58 x 109/L at the second one.

Table 5 presents data on Malonic dialdehyde content (DAM) determined in plasma and erythrocytes.

Table 5 : Enzymatic components of antioxidant system of piglets following administration of Sel-Plex by milking swines

INDICATORS	GROUP	STUDY			
		I Day 7		II Day 32	
		n	M±n	n	M±n
DAM in plasma (nmol/L)	I – experimental	5	11,926±	5	9,57±
	II - control	5	14,205±	5	14,296±
DAM in erythrocytes (nmol/L)	I – experimental	5	0,87±0,01	5	0,75±0,016
	II - control	5	0,89±0,10	5	0,82±002
Antioxidative activity in plasma (%)	I – experimental	5	66,43±3,34	5	67,7±2,44
	II - control	5	63,43±3,21	5	47,29±3,12
AAT in erythrocytes (%)	I – experimental	5	134,85±12,4	5	126,09±9,31
	II - control	5	127,74±11,02	5	100,77±4,95

During lipid metabolic processes, particularly through oxidation, the body accumulates a number of intermediate compounds and final products of their peroxidation, among which is the DAM. Data show that in the blood of piglets in the experimental group there was marked a tendency of decreasing concentration of DAM in plasma (P <0.02) and erythrocytes (P <0.05) compared to piglets from the control group, which is characteristic in case of reduction of lipid peroxidation processes.

Plasma total antioxidant activity is an index that reflects the ability of blood plasma to inhibit induced oxidation process of a model system. AAT shows us therefore that the summary content of compounds in blood plasma with antioxidative properties. Table 3 shows that AAT in plasma of animals from the experimental group has a tendency to be maintained at the level of 66.43 + 3.34% at the first survey (the 7th day of piglets life) and 77.2 + 2 , 44% at the end of research (32nd day of the piglets life), while among the animals from the second control group, this index essentially decreased from 63,43 + 3,21% to 47,29 + 3,12%.

AAT in erythrocytes on the 7th day after birth in the control group initially was 127,74 + 11,02% which at the end of research (day 32) reached 100,77+ 4,95%;, in the experimental group it was 134,85 + 12,9% and decreased to 126.09%.

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Given that research has been conducted on young animals, i.e in the development, the dynamics of AAT in plasma can be interpreted as a consequence of age particularities.

The results clearly demonstrate a beneficial effect of organic selenium (Sel-Plex) on clinical indices, hematologics and antioxidant system.

The data presented in Table 6 represent the percentage of the morbidity of piglets in both groups during the period from birth until weaning.

Table 6 : Morbidity and mortality of sucking piglets

Group	n	Diarrhea suffering (%)	Died		Survived (%)
			due to diarrhea (%)	Due to other infections (%)	
I control	50	22	12	6	82
II experimental	51	9,8	3,92	3,92	92,2

It was found a higher percentage of morbidity among piglets from the control group and it constituted 22%, compared to 9.8% in the experimental group. Lethality percentage was 18% and 7.8% accordingly. At the age of 42 days there survived 82% of piglets from the control group and 92.2% in II-experimental group, the ration of which was supplemented with selenium of organic origin.

V. Cociu et.al (2005) communicates that in the R.of Moldova there are two essential trace elements - Iodine and Selenium are not included in the premixes composition. Here is meant that the deficiency of these minerals is usually manifested latently affecting animal productivity and achieved product quality. These indices, however, in the subsisting village farms are not always taken into account.

Deficit of a single element in the organism such as Selenium, which is necessary in insignificant amounts (0.5 to 0.7 ppm) causes a variety of morbid states in various animal species [1]. Rations containing large amounts of unsaturated fat which are lacking protein as well, particularly those in sulfur amino acids, are factors that cause disease [8].

P.F. Surai, 2007, communicates that the content of selenium in foddle depends on region of cultivation, soil and a number of other factors. N. Abraham (1992) states that the content of selenium in foddle depends to a large extent on the amount of selenium in the soil, there being a close soil-plant-animal relation.

In recent years several studies have been devoted to testing Sel-Plex product action in maintaining the antioxidant-prooxidant balance in the digestive tract, blood and in prevention of the decline of productive and reproductive performance of milking swines and their offspring (11.2.12).

Our results allow us to conclude that the inclusion in the basic ration of Sel-Plex product had a positive impact on the general condition of milking swines and their offspring on, being manifested by a lower morbidity and mortality, so that the percentage of viability consisted 92.2% for the experimental group and 82% for the control group.

IV. Conclusions

1. Sel-Plex administered with food in proportion of 1kg per ton has a positive effect on reproductive indices (total number of piglets born alive, the birth weight, sucking piglets viability).
2. Mortality losses in the experimental group from birth to weaning were lower (7.8% of actual) than in the control group (18%).
3. Body weight of 42 days old piglets constituted alcătuit 10,3±0,91 kg and 12,8±1,04 kg in experimental and control groups respectively, the difference between the two groups being significant (P1, 2 <0.05).
4. Sel-Plex has a positive impact on the immune system, which is manifested by a significant increase (P1, 2 <0.05) of the total number of leukocytes, of total T-lymphocytes (P1, 2 <0.001 of active lymphocyte subpopulations (P1, 2 <0.01). Dynamics of B-lymphocyte growth was manifested by authentic (P1, 2 <0.05) and day 21 (P1, 2 <0.01) in the 32-day piglets in the experimental group.
5. Sel-Plex exercises an antioxidant effect at enzyme and hematopoietic level on the body, which is manifested by the significant increase of the number of erythrocytes and amount of hemoglobin (P <0.05), the reduction of the DAM level (Dealdehid Malonic) in blood plasma, the significant increase (P <0.01) of AAT (total antioxidant activity in blood serum.
6. The results show that it is time to replace sources of organic and inorganic selenium with organic sources such as the product enriched with selenium, Sel-Plex.

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References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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