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In *Vitro* Cytotoxic Screening of Combination of Honey Bee Venom and Ethanol Extract of Zingiberaceae and *in Vivo* Anticancer Evaluation of them against DLA and EAC Bearing Mice

By Dr. Somayeh Afsah Vakili, Ajay George & Syed Fayazuddin
Visveswarapura Institute of Pharmaceutical Sciences

Abstract- In spite of the fact that substantial advancement have been made in the remedy and control of cancer progression, remarkable inadequacy for improvement remain. Natural therapy can diminish adverse effect of chemotherapy. Currently over 60% of the drugs are derived in one or other way from natural source including plant, marine organism and micro-organism. The present investigation was concerned with pharmacological potential of honey bee venom and ethanol extract of Zingiber officinale Roscoe towards anticancer activity. The cytotoxic potency of combination of honey bee venom and ethanol extract of Zingiber officinale Roscoe was evaluated on cultured cancer cells Hep-2 by sulphoradamine B assay (IC_{50}=53 µg/ml) and moreover, it was invested by brine shrimp lethality assay (IC_{50}=48.31 µg/ml), tryphan blue exclusion assay (IC_{50}=37.49 µg/ml), and MTT assay. MTT assay exhibited that combination of honey bee venom and ethanol extract of Zingiber officinale Roscoe has more cytotoxicity potency towards human breast adenocarcinoma cells (MCF-7) than normal Chinese hamster lung fibroblast cells (V79).

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Cytotoxic Screening and Anticancer Activity of Honey Bee Venom and Ethanol Extract of *Zingiber Officinale Roscoe*

Dr. Somayeh Afsah Vakili [α], Ajay George [σ] & Syed Fayazuddin [ρ]

Abstract- In spite of the fact that substantial advancement have been made in the remedy and control of cancer progression, remarkable inadequacy for improvement remain. Natural therapy can diminish adverse effect of chemotherapy. Currently over 60% of the drugs are derived in one or other way from natural source including plant, marine organism and micro-organism. The present investigation was concerned with pharmacological potential of honey bee venom and ethanol extract of *Zingiber officinale Roscoe* towards anticancer activity. The cytotoxic potency of combination of honey bee venom and ethanol extract of *Zingiber officinale Roscoe* was evaluated on cultured cancer cells Hep-2 by sulphoradamine B assay (IC_{50}=53 µg/ml) and moreover, it was invested by brine shrimp lethality assay (IC_{50}=48.31 µg/ml), tryphan blue exclusion assay (IC_{50}=37.49 µg/ml), and MTT assay. MTT assay exhibited that combination of honey bee venom and ethanol extract of *Zingiber officinale Roscoe* has more cytotoxicity potency towards human breast adenocarcinoma cells (MCF-7) than normal Chinese hamster lung fibroblast cells (V79). In EAC ascites model significantly ascending of life span with restoring of haematological parameters and additionally, in DLA solid tumour model crucial decline in the tumour weight and tumour volume was observed as compared to control.

Keywords: honey bee venom, zingiber officinale roscoe, cytotoxic potency, human breast adenocarcinoma cells.

I. Introducton

One of the governing causes of global mortality is cancer (Lopez et al., 2006). World Health Organization (WHO) reported that there were 8.2 million deaths in 2012 and it is estimated up to 13.1 million deaths in 2030 (Ferlay et al., 2008). In the United States, one in four deaths is ascribed to cancer (Jemal et al., 2007). Cancer cells are susceptible to chemotherapy by reason of losing normal function and uncontrolled proliferating of cells. Nevertheless, some of the anticancer drugs have carcinogenicity themselves such as alkylating agents and antracycline antibiotics (Sharma and Sharma, 2007). Natural products have tremendous potential to issue newest medicines since their natural chemicals may supply chemoprotective potential versus cancer. Bee venom contains major components that include histamine catecholamines, polyamines, melittin, and phospholipase A2. Melittin represents about 50-70% of all antimicrobial peptides present in bee venom. Some antimicrobial peptides isolated from insects display a wide range of biological activities including melittin, cecropin related peptides and the magainins which have been shown to exhibit antitumor activity for cells derived from mammalian and human tumours. It is also one of the most potent inhibitors of calmodulin activity and a potent inhibitor of cell growth and clonogenicity (Orsolic et al., 2009). *Zingiber officinale Roscoe* belongs to family *Zingiberaceae* commonly called as ginger (Radhakrishnan, 2014). Ginger contains the volatile compounds such as alpha-zingiberene, beta-sesquiphellandrene, alpha-farnesene, beta-bisabolene, alpha-curcumene, which are mostly consisted of sesquiterpene hydrocarbons and the non-volatile pungent compounds are mainly oleoresin (gingerol, shogaol), phenol (zingerone, gingeol). Ginger also possesses anthelmintic, anti-bacterial and anti-viral activities. Moreover, ginger was found to be active against inflammatory, allergic, degenerative, cardiovascular and metabolic disorders and anticancer activity (Poltronieri et al., 2014). The current investigation was undertaken to anticipate the anticancer potential of combination of honey bee venom and ethanol extract of *Zingiber officinale Roscoe*.

II. Materials and Methods

a) Plant material and Preparation of extract

The rhizomes of *Zingiber officinale Roscoe* (*Zingiberaceae*) were collected from Mysore District, Karnataka State, India and authenticated by Green

Author α ρ: Department of pharmacology, Visveswarapura Institute of Pharmaceutical Sciences, Bangalore-560070, Karnataka, India.
e-mails: Somayehafsah@yahoo.com, syedfayazuddin.f1@gmail.com
Author σ: Department of Pharmacology, St. Johns Pharmacy college, Bangalore-560104, Karnataka, India. e-mail: ajay.jorge@yahoo.com

Chem of India, Bangalore, Karnataka, India; a voucher specimen (MZO-GR-101) was conserved for future references. The rhizomes were dried and converted into fine powder using an electrical blender. Fine powder (100 g) was homogenized in ethanol (95%; 500 mL) and left in a conical flask at room temperature for 3 days. The mixture was filtered through a fine muslin cloth and a filter paper (Whatman No. 1). The extract became concentrated by using the Eyela rotary evaporator. The percentage yield of ethanol extract of *Zingiber officinale Roscoe* was 12%.

b) Bee venom

Lyophilized whole bee venom was purchased from New Technique Laboratory Ltd (Georgia). Bee venom was reconstituted in distilled water to obtain the desirable concentrations for *invitro* and *invivo* study and centrifuged at 12,000 rpm for 10 minutes to remove insoluble materials.

c) Chemicals

3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), Fetal bovine serum (FBS), Sulphoradamine B (SRB), Minimum essential medium (MEM) and Trypsin were purchased from Sigma-Aldrich Comany, Bangalore, India; 96 well plates, T flasks (T-25 cm^2), were purchased from Tarsons, Kolkata, India. All other chemicals were analytical grade.

d) Cell lines

Human breast adenocarcinoma cells (MCF-7), Normal V79 cells (Chinese hamster lung fibroblast) and HEp-2 (Human epithelial carcinoma) cells procured from National centre for cell sciences, NCCS Pune, India. The cell lines were grown in 25 cm^2 tissue culture flasks containing Minimum essential media (MEM media) with 10% fetal Bovine Serum (FBS), 1% L- glutamine and 50 μg/ml gentamycin sulphate at 37°C in CO_2 incubator in an atmosphere of humidified 5% CO_2 and 95% air. The cells were maintained by routine sub culturing in 25cm^2 tissue culture flasks twice a week. Ehrlich ascites carcinoma cells (EAC) and Dalton's ascites lymphoma cells (DLA) were obtained by Amala Cancer Research Center, Thrissur, Kerala, India and were maintained by weekly intraperitonial (i.p) inoculation of 10^6 cells/mouse in the laboratory. Both the cell lines maintained in the peritoneal cavity of Swiss albino mice were collected from an animal having 7 days old ascitic tumour by aspirating the ascetic fluid in sterile isotonic saline. The viable EAC/DLA cells were counted (Trypan blue indicator) under microscope. A fixed number of viable cells 10^6 cells were inoculated into the peritoneal cavity of each recipient mouse.

e) Animals

The experiments were done on 8-10 weeks old Swiss albino mice of either sex weighing 25-35 gm were procured from a registered breeder in Viveswarapura Institute of Pharmaceutical Sciences. Animals were maintained under controlled conditions of temperature (25 ± 3°C) and humidity (50 ± 5 %) and were caged in sterile polypropylene cages containing sterile paddy husk. The study protocol was authorized by Institutional Animal Ethics Committee (IAEC), Visveswarapura Institute of Pharmaceutical Sciences, Bangalore. (Registration No: 152/1999, renewed in 2012).

f) Cytotoxic screening

i. *Sulforhodamine B colorimetric assay for cytotoxic screening*

The monolayer cell culture of HEp-2 (Human epithelial carcinoma) was trypsinized and the cell count adjusted to 1.0 x 10^5 cell/ml using medium (MEM) supplemented with 10% FBS. To each well of the 96 well microplate, 1x10^4 cells in a volume of 0.1ml was added and incubated for 24 h in CO_2 incubator for cell adherence. After 24 h, cells were treated with combination of honey bee venom (5.7 µg/ml) and ethanol extract of *Zingiber officinale Roscoe* (100 µg/ml) in a volume of 100 µl. The plates were then incubated at 37°C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations recorded every 24 h. After 72 h incubation, cell monolayers were fixed with 10% (w/v) trichloroacetic acid and stained for 30 min, after which the excess dye was removed by washing repeatedly with 1% (v/v) acetic acid. The air-dried plates were stained with 100 µl of 0.4% Sulforhodamine B solution (SRB) (0.4 g of SRB was dissolved in 100ml of 10 mM Tris base solution) for 30 min. The unbound dye was then removed by rapidly washing four times with 1% acetic acid. The plates were shaken vigorously for 5 min. The absorbance was measured using micro plate reader at a wavelength of 510 nm (Orellana and Kasinski, 2016).

The percentage growth inhibition was calculated using the formula below:

$$\% \text{ Growth inhibition} = 100 \times (T - T_0) / (C - T_0)$$

T is the OD after exposure to certain concentration of drugs, T_0 is the OD at the start of drug exposure and C is the OD of untreated group which served as control.

ii. *Trypan blue dye exclusion method*

The combination of honey bee venom (20 µg) and ethanol extract of *Zingiber officinale Roscoe* (500 µg) was used for the preparation of the stock solution (520 µg/ml) in Phosphate buffered saline. Serial dilutions (25µg/ml, 50µg/ml, 75µg/ml, 100µg/ml of sample solution) were prepared in PBS. The amount of 200µl of sample solutions were poured in tubes and made up to 800 µl with PBS (Phosphate buffered saline). 100µl of EAC with a concentration of 10^6 cells/ml of Phosphate buffered Saline was added to the tubes. Solvent alone was served as control. 100 µl of trypan blue was added to all test tubes after 3 hours incubation. Ascetic tumour cells were counted by Cell Counting machine (Cedex,

Roche). The percentage of cytotoxicity (% dead cells) was calculated using the formula (Saluja et al., 2011): % Cytotoxicity= (Total cells counted- total viable cells) / Total cells counted×100

iii. *Brine shrimp lethality (BSL) bioassay*

The brine shrimp (Artemia salina) eggs were supplied from Brine Shrimp Direct, Ogaden, UT, USA. The tested chamber was partitioned into two equal parts with aeration supply. One part was lighted up with a bulb (60 W), while the other was darkened. Brine shrimp eggs were placed in the dark side and incubated at room temperature for 48h. The nauplii were moved towards the illuminated side after hatching, where they were collected by a Pasteur pipette. The tested sample were prepared by dissolving the combination of honey bee venom (0.26 mg) and ethanol extract of *Zingiber officinale Roscoe* (4.74 mg) in 5ml of DMSO to obtain 500 ppm stock solution and then diluted with sea water to get the requisite concentration (20, 50, 75, 100, 200 and 300 μg/ml). 5- Flourouracil (5-FU) was used as standard (10, 25, 50 and 75 μg/ml). Ten shrimps were transferred in each vial and made up of volume to 5ml with sea water. A drop of dry yeast suspension (3mg in 5ml sea water) was poured to each vial as food. Control vials were provided by adding equal volumes of distilled water. The vials were sustained under illumination. Survivors were counted by using 3 × magnifying glasses after 24h and the percentage of deaths (% Mortality) and IC_{50} value were calculated by using Finney Computer program (Meyer et al., 1982).

iv. *MTT assay*

Cells were subcultured in 96-well plates at a density of 10^3 cells per well with combination of honey bee venom (5.7 μg/ml) and ethanol extract of *Zingiber officinale Roscoe* extract (100 μg/ml) and cisplatin as standard (2.5 μg/ml) for 48 h in a final volume of 100 μl of media. Then, the medium was removed and 10 μL of MTT (5 mg/mL in PBS) was added to the fresh medium. After 4 h incubation at 37 °C, 100 μL DMSO was added to each well and plates were agitated for 1 min. The optical density (OD) was read using a conventional ELISA plate reader at 570nm. The percentage of viability was calculated as the following formula (Lai et al., 2012): (Viable cells)%=(OD of drug-treated sample/OD of untreated sample)×100

g) Treatment designed

For EAC/ DLA evaluation, Healthy, adult Swiss albino mice were divided into 6 groups consisting of 6 animals in each group. In EAC study, all the animals in each group, excluding group 1 received 10^6 EAC cells/mouse i.p. Group 1 considered as normal and group 2 was EAC control. Group 3 was administrated by standard drug cisplatin 3.5 mg/kg b.w, i.p, group 4, 5 and 6 were administered, orally with formulation of F_1 (honey bee venom (10 mg/kg) and ethanol extract of *Zingiber officinale Roscoe* extract (100 mg/kg), F_2 (honey bee venom (25 mg/kg) and ethanol extract of *Zingiber officinale Roscoe* extract (150 mg/kg) and F_3 (honey bee venom (50 mg/kg) and ethanol extract of *Zingiber officinale Roscoe* extract (200 mg/kg), respectively for 10 consecutive days. For DLA investigation, Group 1 served as normal control, group 2 was DLA control. Group 3 received standard drug cisplatin 3.5 mg/kg b.w, i.p, group 4, 5 and 6 were administered, orally with formulation of F_1, F_2 and F_3 respectively for 10 days.

h) Determination of survival time

At termination surviving animals of EAC/DLA tumour bearing mice were counted and the Mean survival time (MST) and the % increase in life span (% ILS) were calculated by the formula (Durairaj et al., 2009).

$$\% \text{ ILS} = \left(\frac{\text{MST of treated group}}{\text{MST of the control group}} - 1\right) \times 100$$

i) Body Weight Analysis

All mice were weighed on the day of tumour inoculation and the weekly intervals. Average gain in body weight and % increase in body weight was calculated by the formula 1 for EAC tumour bearing mice by and % reduction in body weight was calculated by formula 2 for DLA tumour bearing mice (Durairaj et al., 2009).

Formula 1: [% increase in body weight = (animal bw on resp. day/animal bw on day 0)-1 x 100]

Formula 2: [% reduction in body weight= (Gain in bw of control-gain in bw in treated gp/ gain in bw of control) x 100]

j) Hematological parameters

At the end of evaluation, on day 11, mice were anaesthetized by is oflurane. blood was collected from retro-orbital of mice for reckoning of white blood cell (WBC) count, red blood cells (RBC) count and the hemoglobin (Hb) content by standard procedures (Jain, 2005).

k) Statistical analysis

The data were manifested as mean ± S.E.M. The results were statistically analyzed by means analysis of variance (ANOVA) followed by Dunnett's post hoc test where the difference was contemplated significant if $p < 0.05$.

III. Results

a) In vitro cytotoxic screening

In SRB assay, combination of honey bee venom (5.7 μg/ml) and ethanol extract of *Zingiber officinale Roscoe* (100 μg/ml) displayed good cell growth inhibition with IC_{50} value of 53 μg/ml. In trypan blue dye exclusion assay, the mentioned formulation caused mortalities effectively with IC_{50} value of 37.49 μg/ml. In

BSL assay, the combination of honey bee venom (0.26 mg) and ethanol extract of *Zingiber officinale Roscoe* (4.74 mg) exhibited mortality of cells with IC_{50} value of 48.31 μg/ml. The combination of honey bee venom (5.7 μg/ml) and ethanol extract of *Zingiber officinale Roscoe* extract (100 μg/ml) was examined on normal Chinese hamster lung fibroblast cells (V79) and human breast adenocarcinoma cells (MCF-7) by MTT assay to assess its selectivity towards normal and cancer cells. On normal cells, V79, the IC_{50} of formulation and cisplatin as standard was found to be 89.61 μg/ml and 6.43 μg/ml respectively with 77% of cell survival for formulation. On MCF-7, the IC_{50} of formulation and cisplatin as standard was found to be 66.52 μg/ml and 1.91 μg/ml respectively with 61% of cell survival for formulation. So it means the combination of honey bee venom (5.7 μg/ml) and ethanol extract of *Zingiber officinale Roscoe* extract (100μg/ml) exhibited more cytotoxic activity towards cancer cells.

b) In vivo anticancer study

i. *Effect of combination of honey bee venom and ethanol extract of Zingiber officinale Roscoe on body weight in DLA/ EAC tumour bearing mice*

Figure 1 exhibits the percentage reduction in the body weight after treatment with of combination of honey bee venom and ethanol extract of *Zingiber officinale Roscoe* on body weight in DLA tumour bearing mice. The percentage reduction in body weight of DLA tumour bearing mice was found to be 65.38 % in the group treated with cisplatin. Maximum percentage reduction in body weight was found to be 55.21% in the group treated with F_3 formulation. Figure 2 displays the percentage increase in the body weight after treatment with of combination of honey bee venom and ethanol extract of *Zingiber officinale Roscoe* on body weight in EAC tumour bearing mice. Substantial rise in body weight was perceived in EAC control mice with a maximum gain (20.85 ± 0.41 %). Standard cisplatin and all herbal formulation treatment significantly declined the elevated body weight, hence the percentage increase in body weight was found to 3.68% and4.61% in group treated with cisplatin and F_3 formulation respectively.

ii. *Effect of combination of honey bee venom and ethanol extract of Zingiber officinale Roscoe on tumour volume in DLA/ EAC tumour bearing mice*

Figure 3 shows the effect of combination of honey bee venom and ethanol extract of *Zingiber officinale Roscoe* on tumour volume in DLA tumour bearing mice. The DLA inoculation increased the tumour volume (0.82 cm^3) in mice. The cisplatin treated group significantly ($a < 0.05$) exhibited maximum reduction in tumour volume (0.22 cm^3) in mice. F_3 formulation treated group has shown very effective in diminishing tumour volume to 0.29 cm^3 when compared with DLA control. Table 1 manifests the effect of combination of honey bee venom and ethanol extract of *Zingiber officinale Roscoe* on tumour volume in EAC tumour bearing mice. F_3 formulation treated group significantly ($r < 0.001$) decreased the tumour volume as compared to the EAC control group.

Table 1: The effect of combination of honey bee venom and ethanol extract of *Zingiber officinale Roscoe* on tumour volume in EAC tumour bearing mice

Treatment groups	Tumour volume (mL)
EAC	8.29± 0.16
Cisplatin	1.13 ± 0.31^{r}
F_1	3.77 ± 0.35^{r,z}
F_2	3.46 ± 0.46^{r,z}
F_3	3.15 ± 0.75^{r,z}

Values are mean ± S.E.M. (n=6). P values: $r < 0.001$, as compared with EAC control. $z < 0.001$, as compared to cisplatin as standard (by one way ANOVA followed by Dunnett's multiple comparison test)

iii. *Effect of combination of honey bee venom and ethanol extract of Zingiber officinale Roscoe on mean survival time and percentage increase in life span in DLA/ EAC tumour bearing mice*

Table 2 indicates the Effect of combination of honey bee venom and ethanol extract of *Zingiber officinale Roscoe* on mean survival time and percentage increase in life span in DLA tumour bearing mice. In DLA control group, the mean survival time was 20 days and it rose significantly up to 32 and 28 days with remedy by standard cisplatin group and F_3 formulation group respectively. The % ILS was observed to be 55.64% and 39.89% in DLA induced mice treated with standard cisplatin group and F_3 formulation group respectively. Table 3 revealed the Effect of combination of honey bee venom and ethanol extract of *Zingiber officinale Roscoe* on mean survival time and percentage increase in life span in DLA tumour bearing mice. The mean survival time in cisplatin treated mice found to be 25 days ($a< 0.05$). As compared to the EAC control group, F_3 formulation treated group has shown significant ($a < 0.05$) increase in the life span than the rest of the groups.

Table 2: Effect of combination of honey bee venom and ethanol extract of *Zingiber officinale Roscoe* on mean survival time and percentage increase in life span in DLA tumour bearing mice.

Treatment groups	MST (Days)	%ILS
DLA	20.63 ± 0.41	-
Cisplatin	32.11 ± 0.68[a]	55.64
F_1	23.17 ± 0.40 [b]	12.31
F_2	25.23 ± 0.45[a,b]	22.29
F_3	28.86 ± 0.70[a,b]	39.89

Values are mean ± S.E.M. (n=6). p values: a< 0.05, as compared with EAC control. b < 0.05, as compared to cisplatin as standard (by one way ANOVA followed by Dunnett's multiple comparison test)

Table 3: Effect of combination of honey bee venom and ethanol extract of *Zingiber officinale Roscoe* on mean survival time and percentage increase in life span in EAC tumour bearing mice.

Treatment groups	MST (Days)	%ILS
EAC	15.20 ± 0.51	-
Cisplatin	25.34 ± 0.38[a]	66.71
F_1	19.65 ± 0.71 [b]	29.27
F_2	22.93 ± 0.35[a,b]	50.85
F_3	23.77 ± 0.40[a,b]	56.38

Values are mean ± S.E.M. (n=6). P values: a< 0.05, as compared with EAC control, b < 0.05, as compared to cisplatin as standard (by one way ANOVA followed by Dunnett's multiple comparison test)

iv. *Effect of combination of honey bee venom and ethanol extract of Zingiber officinale Roscoe on hematological parameters in DLA/ EAC tumour bearing mice*

Table 4 presents the Effect of combination of honey bee venom and ethanol extract of *Zingiber officinale Roscoe* on hematological parameters in DLA tumour bearing mice. The total WBC count found significantly increased in DLA control group when compared with the normal group (c< 0.001). F_1, F_2 and F_3 formulations exhibited activity at per with cisplatin as standard and these differences were statistically non-significant for F_3 formulations treated group and y< 0.01, z< 0.001 for F_2 and F_1 formulations treated group respectively. RBC count and Hb count in DLA groups were significantly (c< 0.001) diminished as compared to normal group. Treatment with F_3 formulations revealed superior ascend in RBC count and Hb count when compared with DLA control group and restored these values towards normal. Treatment with the all three formulation significantly rose the RBC level and Hb content when compared to the EAC control [Figure 4,5]. F_3 formulation indicated better effect than other combinations as compared with EAC control (b < 0.05). The WBC count has been reduced significantly when compared with the EAC bearing mice and restored more towards the normal level with remedying by all three formulations. F_3 formulation displayed better activity when compared to the rest of the evaluated formulation and the cisplatin (c < 0.05) [Figure 6].

Table 4: Effect of combination of honey bee venom and ethanol extract of *Zingiber officinale Roscoe* on hematological parameters in DLA tumour bearing mice

Treatment groups	RBC count (x 10^9/mL)	WBC count(x10^4/mm^3)	Hb (g%)
NORMAL	4.81± 0.13	8.09± 0.10	14.78± 0.39
DLA	3.11± 0.6 [c]	19.78± 0.55 [c]	10.06± 0.42 [c]
Cisplatin	3.75±0.25 [r]	9.13± 0.23[r]	13.87± 0.45 [br]
F_1	3.27± 0.20[cq]	17.06± 0.52 [crz]	11.88± 0.17 [cx]
F_2	3.49± 0.52[cq]	15.58± 0.69 [bry]	12.53± 0.01 [c]
F_3	3.66± 0.30[cq]	11.52± 0.73 [r]	13.01± 4.10 [cq]

n = 6, Values are mean ±S.E.M, one way ANOVA followed by Dunnet's multiple comparison test. p values: b< 0.01, c< 0.001, compared to the normal group; q< 0.01, r<0.001, as compared with EAC control; x< 0.05, y< 0.01, z< 0.001, as compared cisplatin treated group.

IV. Discussion

The use of natural products to control or seize the carcinogenic activity issues an alternative to the use of typical allopathic remedy for therapy of the ailment (Balachandran and Govindarajan, 2005). Natural sources have been investigated in clinical researches and are being evaluated to comprehend their cancericidal properties against varied cancers (Balachandran and Govindarajan, 2005). Inevitably, this topical investigation was endeavoured to predict anticancer potential of combination of honey bee venom and ethanol extract of *Zingiber officinale Roscoe* against different cell lines. The cytotoxic screening of combination of honey bee venom and ethanol extract of *Zingiber officinale Roscoe* revealed the beneficial effect against cancer cell lines for *invitro* studies. Cytotoxic of anticancer drugs towards the normal cells are crucial problems in cancer remedy and engender the risk of promoting secondary malignancy (Shi et al., 2008). There has been a concerted research in the current years for the discovery of novel selective anticancer agents which show more cytotoxic activity towards cancer cells than normal cells. Therefore, in the present investigation of the cytotoxicity of combination of honey bee venom and ethanol extract of *Zingiber officinale Roscoe* on cancer (MCF-7) and normal (V79) cells was determined to check their selectivity that indicated more cytotoxic activity towards cancer cells with 61% of cell survival for formulation. In ascites /solid tumour models, a considerable rise in body weight of the animals was perceived in EAC/DLA control mice due to progressive accumulation of ascites tumour cells and rapid solid tumour growth respectively. The trustworthy criterion for determining the potential of any anticancer drugs is the extension of life span of animal (Dai and Mumper, 2010). The present study showed F_3 combination of honey bee venom and ethanol extract of *Zingiber officinale Roscoe* significantly ascended the life span in both EAC/DLA models. Additionally, the diminished volume of tumor and extended survival time of mice recommend the retarding effect of formula on cell division (Ames et al., 1993). Pivotal erythrocytopenia and anemia in cancer patients is mostly due to myelosuppression during chemotherapy (Mondal et al., 2014). Results acquired from investigation evinced F_3 combination of honey bee venom and ethanol extract of *Zingiber officinale Roscoe* reestablished the hematological parameters so it can alleviate erythrocytopenia and anemia in cancer patients. The prior phytochemical evaluation of honey bee venom has disclosed the presence of melittin which has antitumour activity (Orsolic et al., 2009). It is also one of the most potent inhibitors of calmodulin activity and a potent inhibitor of cell proliferation and clonogenicity. Calmodulin is vital for numerous processes that are crucial for normal cellular function, including the assembly and disassembly of microtubules, calcium extrusion from cells by a calcium-magnesium, ATPase, and the activation of many intracellular enzymes, such as phosphatases, protein kinases and cyclic nucleotide phosphodiesterase. Through interfering with any of these known functions, calmodulin inhibitors would be potentially toxic to cells. The evidence advocates that calmodulin inhibitors are cytotoxic to malignant cells (in both *invitro* and *invivo* investigation) which can postulated by either of following mechanisms: 1- Interfering with cell cycle by and block the movement of chromosome during metaphase that leads to inhibit the DNA synthesis, 2- Apoptosis and lysis of tumour cells (Orsolic et al., 2009). The previous phytochemical analysis has divulged the presence of [6] gingerol as the non-volatile pungent compounds in *Zingiber officinale Roscoe* (Poltronieri et al., 2014). [6] gingerol demonstrated antioxidant activity by modifying the redox status through inhibition of RNS (particularly peroxynitrite) (Radhakrishnan et al., 2014) and also inhibited the COX-2 expression by reason of blocking of P38 MAP kinase and NF- Kappa B (NF_kB) signalling pathway (Kim et al., 2005). There is ample evidence that COX-2 is overexpressed in about 85% of cancers. COX-2 is prostaglandin-endoperoxide synthase enzyme which catalyses the conversion of arachidonic acid to prostaglandin such as PGE2. PGE2 cause to increase the level of VEGF (Vascular endothelial growth factor) which lead to angiogenesis of cancer cells. It increases the level of AKt which enhances transcription factor of anti apoptosis in nucleus, additionally, it ascends the level of Bcl-2 which is an anti-apoptotic agent and hence it prevents the apoptosis process in cancer cells which causes to mortality of them. It means COX-2 inhibitor can induce apoptosis as well as anti-angiogenesis (Sharma and Sharma, 2007). Zingerone is another the non-volatile pungent compounds with phenolic nature in *Zingiber officinale Roscoe* (Poltronieri et al., 2014) .The data obtained from literature revealed that zingerone also has antioxidant activity against peroxynitrite and superoxide anion (Radhakrishnan et al., 2014).

V. Conclusion

Ergo, based on the current investigation, pharmacological potential of honey bee venom and ethanol extract of *Zingiber officinale Roscoe* towards anticancer activity was certified. Future evaluations can assess whether the combination of honey bee venom and ethanol extract of *Zingiber officinale Roscoe* may be more beneficial to impede the metastatic cancer or for remedy of established cancer.

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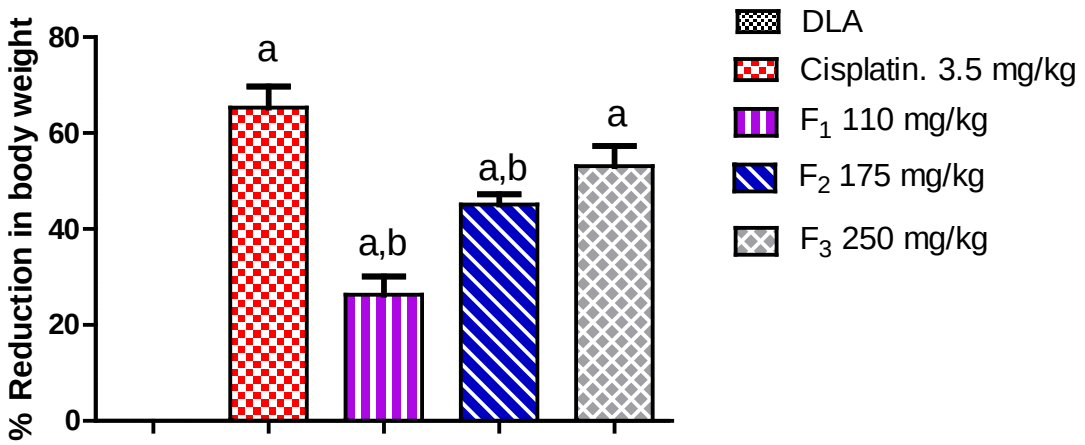

Figure 1: Effect of combination of honey bee venom and ethanol extract of *Zingiber officinale Roscoe* on body weight in DLA tumour bearing mice

n=6, values are mean ± SEM. Data were analysed by one way ANOVA followed by post hoc test. Where P values; a < 0.05 as compared to DLA control, b < 0.05 as compared to standard.

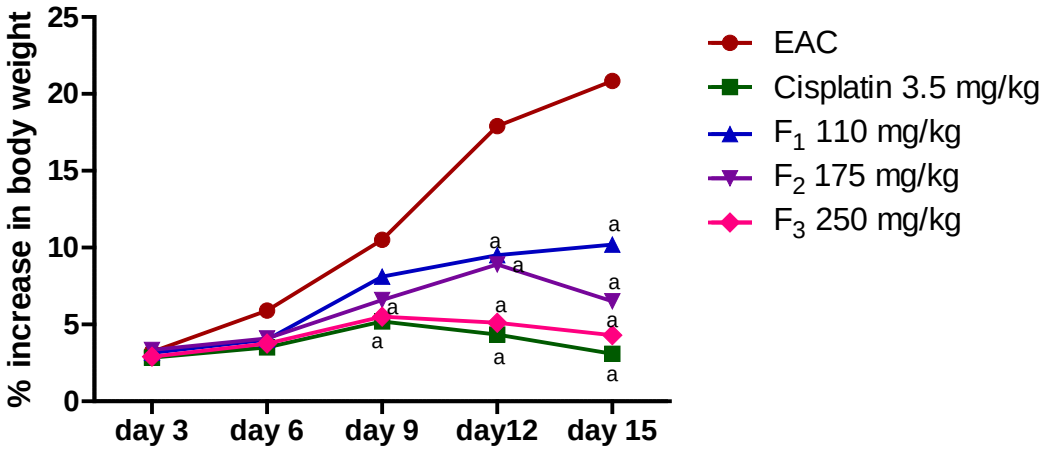

Figure 2: Effect of combination of honey bee venom and ethanol extract of *Zingiber officinale Roscoe* on body weight in EAC tumour bearing mice

n=6, values are mean ± SEM. Data were analysed by one way ANOVA followed by post hoc test. Where P values; a< 0.05 as compared to EAC control.

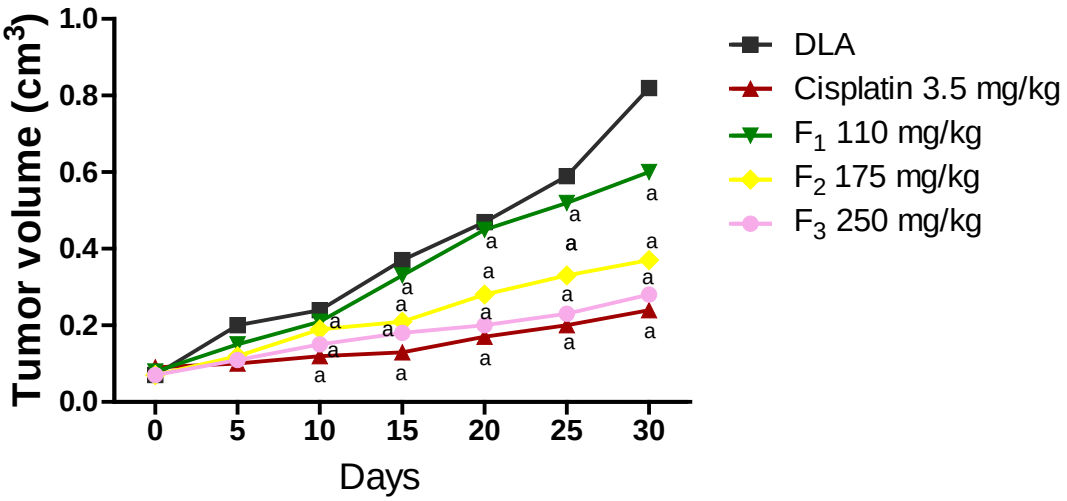

Figure 3: Effect of combination of honey bee venom and ethanol extract of *Zingiber officinale Roscoe* on tumor volume in DLA tumour bearing mice

n=6, values are mean ± SEM. Data were analysed by one way ANOVA followed by post hoc test. Where P values; a < 0.05 as compared to DLA control.

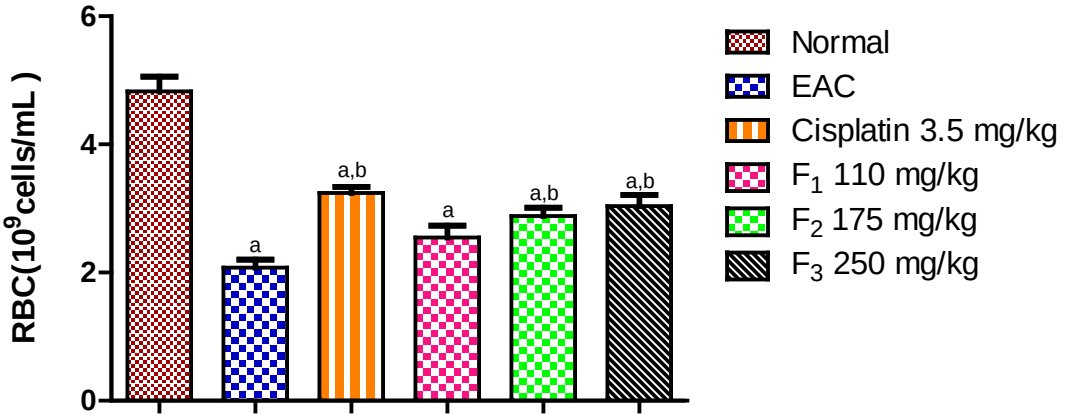

Figure 4: Effect of combination of honey bee venom and ethanol extract of *Zingiber officinale Roscoe* on RBC in EAC tumour bearing mice

n=6, values are mean ± SEM. Data were analysed by one way ANOVA followed by post hoc test. Where P values; a< 0.05 as compared to normal and b < 0.05 as compared to EAC control.

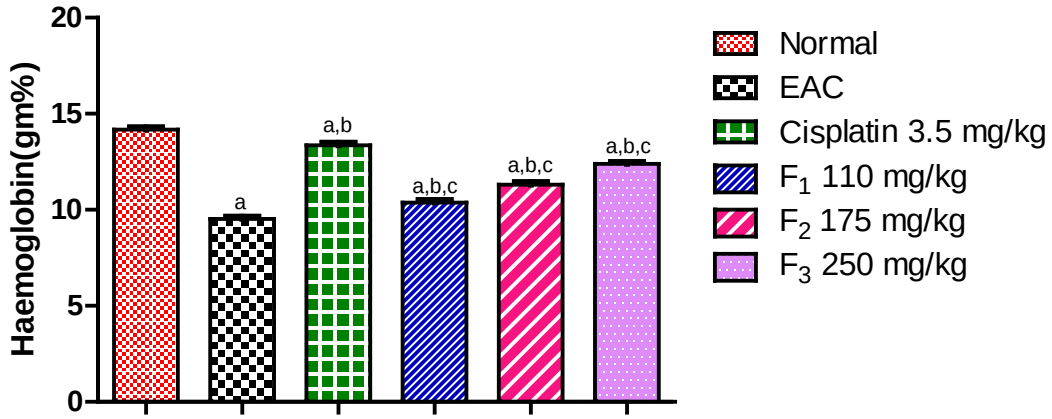

Figure 5: Effect of combination of honey bee venom and ethanol extract of *Zingiber officinale Roscoe*on Hb count in EAC tumour bearing mice

n=6, values are mean ± SEM. Data were analysed by one way ANOVA followed by post hoc test. Where P values; a< 0.05 as compared to normal, b < 0.05 as compared to EAC control and c< 0.05 as compared to cisplatin.

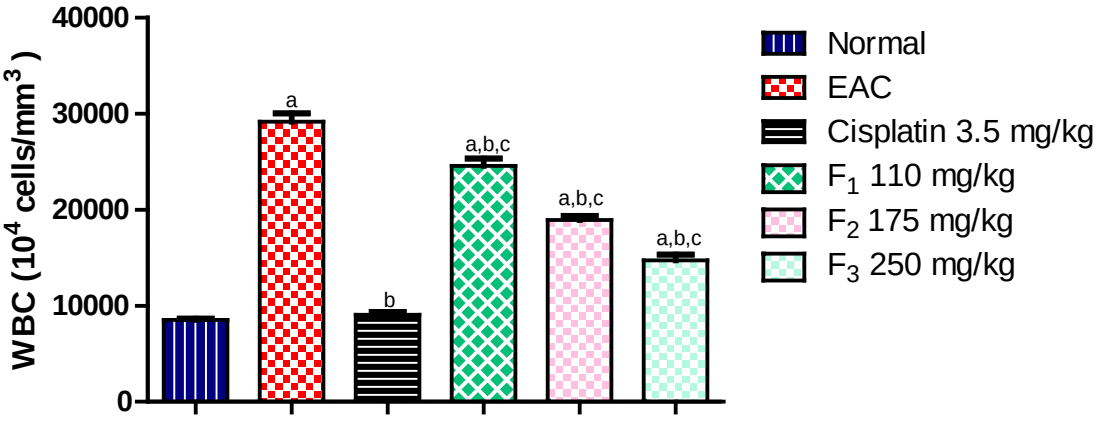

Figure 6: Effect of combination of honey bee venom and ethanol extract of *Zingiber officinale Roscoe* on WBC count in EAC tumour bearing mice

n=6, values are mean ± SEM. Data were analysed by one way ANOVA followed by post hoc test. Where P values; a< 0.05 as compared to normal, b < 0.05 as compared to EAC control and c< 0.05 as compared to cisplatin.