Histopathological and Toxicological effects of crude saponin extract from Phyllanthus niruri, L (Syn. P. franternus. Webster) on Organs in animal studies

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Abstract

The histopathological view of liver, intestines and kidney of bacterial infected rabbits, fed with 100mg/ml saponin extracted from Phyllanthus niruri over a period of seven days was carried out to determine the effect of the plant extract on these organs after treatment. Saponin was administered as strawberry suspension at a dose of 10mg per day (divided into four doses) to ten rabbits, nine of which were fed with food contaminated with 0.5mL bacterial suspension obtained by McFarland standardization (10

Index terms— Histopathological, Phyllanthus niruri, Saponin, Toxicological.

1 INTRODUCTION

efore the advent of modern medicine which witnessed synthetic production of many drugs including antimicrobial agents, extract of plants were known to elicit certain reactions in human body when applied in a prescribed manner. Among such plant is Phyllanthus niruri L., (Syn. P. fraternus.Webster). It belongs to the Euphorbiaceae family and has been claimed to be an excellent remedy for jaundice and hepatitis (Qudhia and Tripathi, 2002;Tabasum et al., 2005). Based on its long documented history of uses in the Amazonian region, the plant is believed to be helpful in treating oedema, anorexia and diabetes (George and Roger, 2002;Khanna et al. , 2002.). The bark yields a bitter principle, phyllanthin, while the infusion of the root and leaves is a good tonic and diuretic when taken cold in repeated doses (Unander, 1990). Many of the active constituents found in the plant are biologically active lignands, glycosides, flavonoids, saponins, alkaloids, ellagitannins and phenylpropanoids (Dhir et al., 2002 ; Tabasum et al., 2005).), common lipids sterols and flavonoids also occur in the plant (Barros et al., 2003).

Saponins are glycosides with a distinctive foaming characteristic. They are found in various parts of the plant leaves, stems, roots, bulbs, blossom, and fruit. The name originated from soapwort plant (saponaria), the root of which was used historically as a soap. Saponins are believed to be useful in the human diet for controlling cholesterol, but some including those produced by the soapberry are poisonous if swallowed and can cause urticaria (skin rash) in many people (Otsuka, 2005). Digistalis type of saponin strengthens heart muscle contractions, causing the heart pump to work more efficiently **??**Desert, 2007). They inhibit some kind of cancer cell tumor growth in animals particularly in the lungs and blood cancers, without killing normal cells (Unander, 1990;Ray, 2007). These effects point to the potentials of saponin, including those present in the diet, as a remedy against two of the major health hazards in many countries, namely obesity and cancer (Otsuka, 2005). Saponin from P. niruri has been observed, within the range of standard antibiotics like Chloramphenicol and Gentimycin used as control;

showed high potency on E. coli and Salmonella typhi **??**Ajibade and Famurewa, 2010). Histopathological studies evaluate the conditions of organs of the body after the use of some therapeutic agents (Ambi et al., 2007). The study estimates the toxic stage and damages that could come from the use of these agents.

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II. The sample used for the analysis was air-dried at room temperature of ±28 0 C and pulverized. The saponin was extracted according to the method described by Otsuka et al. (2005). The milled plant (170g) was defatted using 700ml of Petroleum ether for 72h with the aid of Soxhlet. Seven hundred (700ml) milliliter of methanol was used to extract saponin from defatted sample and the residue was left overnight under reflux at 70 o C. It was then filtered and the filtrate evaporated to dryness. The yield was dissolved in 300ml distilled water-butanol (1:1 v/v) in a separating funnel. The set up was left for three days after which two layers were formed. The upper layer was precipitated with diethyl ether to obtain 20mg of crude saponin; this was poured into an evaporating dish and dried by evaporation for 2 weeks at room temperature. c) Experimental animals Rabbits of both sexes were maintained under standard environmental conditions at room temperature of (±28 -±1 o C) in the animal house of the Department of Science Technology, Federal Polytechnic, Ado-Ekiti. The rabbits had free access to feed and water. Prior to the experiment, rabbits were fed with standard feed for 1 week in order to adapt to the laboratory conditions. Seven days after acclimatization, the rabbits were divided into five groups of six rabbits each (n >6/group): the groups of rabbits were treated as follows: one negative control group, (water; 5ml/kg body weight); one positive control group (200mg amoxicillin; 5ml/kg body weight,) and three saponin treated groups (25-400mg saponin; 5ml/kg body weight). Prior to test, on day 1, the rabbits were fasted for 6hr, but allowed free access to water. d) Effect and toxicity of crude saponin on rabbits infected with Escherichia coli and Salmonella typhi.

5 MATERIALS AND METHODS

The toxicity effect was studied using the methods of Anupama et al., (2011). Saponin was administered as strawberry suspension at a dose of 10mg per day (divided into four doses) to ten rabbits, nine of which were fed with feed contaminated with 0.5mL bacterial suspension of Salmonella typhi and Escherichia coli obtained by McFarland standardization (10% Barium sulfate) containing 10 3 (forming units/ml) cfu/ml after starvation for 6hrs .Toxicity studies were done on white blood, diff count, urine and haemoglobin analysis, blood urea nitrogen (BUN), creatinine, serum alanine transaminase (ALT) and aspartate transaminase (AST). It was performed before administration of saponin, on the third day of therapy, and at 9 th day of therapy. The levels of intact saponin were determined in specimen of urine and blood collected from the rabbits by spectrofluorometric analysis described by Schwartz et al. **??**1999).

6 e) Behavioural and toxic effects

The acute oral toxicity study was evaluated in the rabbits according to the standard methods of Litchfield and Wilcoxon (1949) described in Adesokan and Akanji (2004) and Aziza et al, (2008). Four groups of five rabbits were administered with 25, 50, 100, 200 and 400mg/kg of the saponin extract orally, while one group with the same number of rabbits served as control. The animals were observed continuously for 1hr for any gross behavioral changes, symptoms of toxicity and mortality if any, and intermittently for 6hr and 24hr after dosing with saponin extract. After 24hr, animals were sacrificed following chloroform anesthesia. Blood was collected by heart puncture. Blood samples were collected from each animal and allowed to clot for 45min at room temperature. Serum was separated by centrifugation at 600rpm for 15min and analyzed for various biochemical parameters including serum alanine transaminase (ALT) and serum aspartate transaminase (AST) (Ahmed et al., 2003).

7 f) Histopathological examination

Experimental rabbits were dissected on the 9th day after administration of saponin. The method describe by **??**atel et al. (2010) was employed for the dissecting. They were killed by chloroform anesthesia and dissected. The small and large intestine, liver and kidneys were removed separately and cut into sections, The sections were fixed directly on a slide, stained with haematoxylin and eosin, examined and photographed.

8 g) Statistical analysis

The data were expressed as mean + S.D., while biochemical and physiological parameters were analyzed statistically using one way ANOVA followed by Dunnet-+-test using the Statistical Package for Social glucose levels were significantly ($p < 0.05$) increase in group V rabbits when compared with group 1. Plasma protein, haemoglobin, red blood cell (RBC) and leukocyte counts were not significantly different in all the groups.

The percentage of saponin in urine 72hr after oral administration is shown in Table 2. The mean percentage of the saponin in the urine of the rabbits is 52.36µg/ml

The urine analysis of rabbits treated with saponin for a period of 9days is shown in Table 3. The urine area, uric acid and creatinine levels decreased significantly ($p < 0.05$) in group V animals. Urinary protein and alkaline phosphate activity were not significantly different.

The effect of saponin on the activity of serum, liver and kidney enzymes in controlled and experimental groups of rabbits is shown in Table 4 indicating the activity of marker enzymes (AST and ALT). Slight differences were observed in the activity of enzymes in all the groups of rabbits tested. The result of the histopathological studies of the liver, kidney and small intestine of treated and untreated rabbits is shown in figs 1, 2 and 3 respectively. The liver, small intestine and kidney of the untreated rabbits showed no visible lesion, but there were sectioning artifacts (figs 1(a), 2(a) and 3 (a)). In the treated liver, there was a mild periportal lymphocytic and histiocytic cellular infiltration (Fig **??**b). In the kidney, there are multiple foci of haemorrhages into the intertitium. There were few loci of tubular necrosis and presence of hyaline casts with interstitial cellular infiltration by macrophages (fig **??**b), and small intestine of the treated rabbits there were marked hyperplasias of the mucosal layer (Fig **??**b).

9 DISCUSSION

Tolerance and toxicity studies of the treated rabbits included analyzing levels of crude saponin in blood between 1 and 24hr, investigating blood parameters e.g., urea, uric acid, creatinine, protein, glucose, white blood differential, haemoglobin, urinalysis, alanine transaminase (ALT), aspartate transaminase (AST) in serum, liver and kidney before and after administration of saponin. None of the experimental rabbits exhibited microbiologically active or chemically detectable saponin in the serum or urine before therapy. The mean level of crude saponin in the blood after administration to the rabbits reduced saponin in urine at 72h after administration. The percentage of saponin reduced significantly. The analysis of the blood parameters of the experimental rabbits treated with saponin showed that there was a significant increase in the values of urea, uric acid, creatinine, plasma protein and blood glucose with significant decrease in urea, uric acid and creatinine levels in urine. This may explain the use of P. niruri saponin to remove uric acid from urine (Nishiura et al., 2005). Plasma protein, hemoglobin, red blood cell and leukocyte counts were not significantly different. This findings correlates with that of Lee et al. (2000) and **??**oshikawa et al.(2001) where it was reported that the use of P. niruri do not affect the blood cells adversely.. The saponin was also found to significantly and dosedependently inhibit gastric emptying. This observation was earlier reported by **??**da et al. (2000), Shim et al. (2000), and Zhongguo et al. **??**2005) who opined that the inhibitory activity of saponin on gastric emptying was dependent on the level of serum glucose and mediated at least in part by the capsaicin-sensitive sensory nerves and the central nervous system.

It has been reported that renal dysfunction may be the cause of raised plasma, urea, uric acid and creatinine level accompanied by lowered urine urea, uric acid and creatinine level in high dose of drug treated rabbits (Adesokan and Akanji, 2004). Raised urea and non-protein nitrogen level in blood have been observed with impaired renal function or in acute renal failure **??**Adebayo et al., 2003). In the present study, the observed differences in the urinary contents are not significant. This difference may be due to the concentration of saponin used in the treatment. Zhongguo et al. (2005) found that concentrationdependent response was noticed when Quallaja saponin was used to treat E. coli K-12-infected wistar rats and that saponin from various sources differ in their biological activity. The initial increase observed in the blood glucose level was suspected to be due to the high percentage of sugar moiety that makes the chemical structure of saponins (Francis et al., 2002).

There was however a gradual reduction of the blood glucose to an insignificant level ($p < 0.001$) after 9 days. This could be due to constancy of distribution, metabolism and excretion of the saponin (Zhongguo et al., 2005). The presences of transaminase (AST and ALT) are good indices of liver and kidney damage (Nishiura et al., 2005). In this study, saponin did not induce any damage to any of the organs which could be inferred from the normal values of these enzymes. Reduction in the level of AST and ALT is an indication of the stabilization of plasma membranes as well as repair of hepatic tissue damage. This in effect conforms with the commonly accepted view reported earlier by Francis et al. (2002) that serum levels of transaminase return to normal with healing of hepatic parenchyma and the regeneration of hepatocytes. It thus, means that in this study, the saponin therapy did not lead to liver inflammation and/or kidney dysfunction.

The appearance of mild periportal lymphocytic and histiocytic cellular infiltration in the liver of the saponin-treated rabbits is an indication of a cellular immunological response brought about by infiltration of polymorphornuclear leucocytes to the site of infection induced by Salmonella typhi (Pooneh et al., 2010).

The presence of hyaline cast in the kidney is normal and has been ascribed to the use of medicines (Medline Plus Medical Encyclopaedia). The appearance of a few loci tubular necrosis in the kidney has been observed to be a reflection of the initial pathogenesis of the infection; indicating damage to the renal tubular epithelial cells. This condition is normal and not caused by the saponin therapy but a clinical manifestation of the disease. The description of renal tubular necrosis as one of the pathogenesis of clinical manifestation of typhoid fever has been made (Nishiura et al., 2005) and this substantiates the observations made in this study. The binding of saponins to bile acids in the intestine could reduce the availability of bile acids to the microbial population, thus reducing the formation of carcinogenic substances in the colon (Nishiura et al., 2005) that may lead to necrosis.

V.

10 CONCLUSION

With the information available and the observation recorded in this study, the extract seems not to show any adverse effect on the organs despite its positive therapeutic actions on infections caused by E. coli and Salmonella typhi.

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Figure 1: Fig. 2 :Fig. 3 :

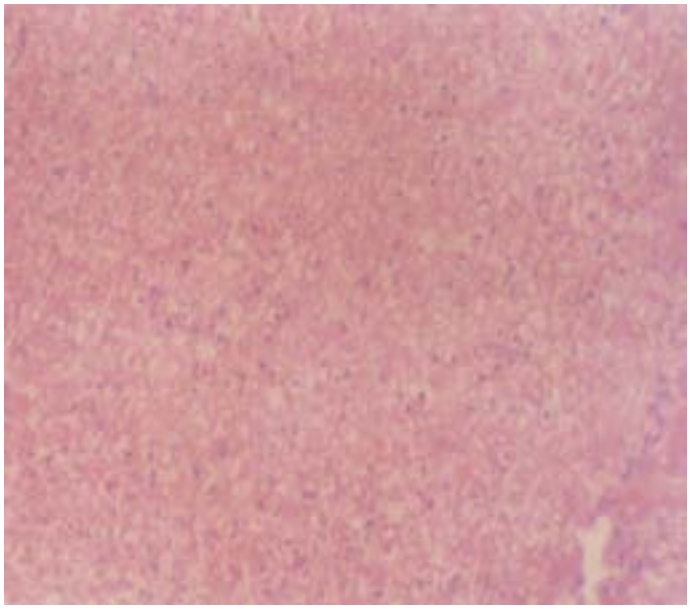

Figure 2:

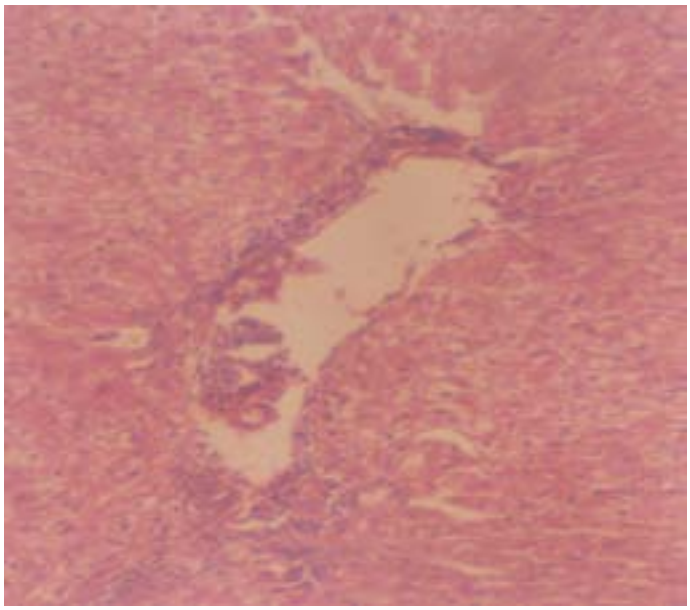

Figure 3:

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Figure 4: Table 1 :

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Figure 5: Table 2 :

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Values are expressed as mean + SD for 6 rabbits
Comparisons were made between groups I (control) with II, III, IV and group V
p< 0.05, ** p< 0.01 ,*** p< 0.001

Figure 6: Table 3 :

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Parameters	Groups				
	I	II	III	IV	V
Urea	3.18+0.43	3.06+0.51	3.51+0.45	2.99+0.31	2.56+0.47
Uric acid	0.73+0.12	0.78+0.24	0.69+0.17	0.61+0.13	0.46+0.04*
Creatinine	0.81+0.27	0.87+0.35	0.83+0.31	0.62+0.21	0.52+0.19**
Protein	5.02+1.85	5.56+1.4	6.06+1.22	6.18+1.55	5.47+0.97
Alkaline phosphate	0.45+0.08	0.43+0.07	0.45+0.07	0.52+0.06	0.54+0.12

Parameters Groups					
	I	II	III	IV	V
Serum (units/ml)					
AST	25.62+5.2	27.14+5.5	26.60+4.98	22.6+4.81	24.95+2.**
ALT	36.25+3.5	39.41+6.04	34.16+5.62	34.16+5.62	34.94+**
Liver (units/mg protein)					
AST	173.3+18.01	174.6+12.5	179.7+16.2	164.12+14.6	169*
ALT	37.02+5.31	37.34+5.20	39.89+3.80	39.91+6.20	41.80
Kidney (units/mg protein)					
AST	29.10+5.10	26.60+3.51	33.25+5.05	26.43+5.08	24.6+4.3***
ALT	26.82+3.27	25.32+2.61	29.44+3.50	24.71+2.96	24.33+4.6**

Values are expressed as mean + SD for six rats Comparisons were between groups I with II, III, IV and group V
*p<0.05, **p<0.01, ***p<0.001

Figure 7: Table 4 :

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