Global Journal of Medical research
Volume 12 Issue 1 Version 1.0 February 2012
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN : 0975-5888

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

By Ajibade V. A., Famurewa, O
The Federal Polytechnic, P.M.B 5351, Ado-Ekiti, Nigeria

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% Barium sulfate) after starvation for 6hrs . Multiple foci of tubular necrosis and haemorrhages in the kidney, marked hyperplasia of the mucosal layer of the small intestine, and a mild periportal lymphocytic cellular infiltration of the liver of the treated rabbits were observed. Plasma urea, uric acid, creatinine and blood glucose levels increased significantly ($p < 0.05$) in the treated rabbits.

Keywords : *Histopathological, Phyllanthus niruri, Saponin, Toxicological.*

GJMR-B Classification : *NLMC Code: WX 207, QV 290*

Strictly as per the compliance and regulations of:

© 2012 Ajibade V. A., Famurewa, O. This is a research/review paper, distributed under the terms of the Creative Commons Attribution-Noncommercial 3.0 Unported License http://creativecommons.org/licenses/by-nc/3.0/), permitting all non-commercial use, distribution, and reproduction inany medium, provided the original work is properly cited.

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

Ajibade V. A.[α], Famurewa, O[σ]

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% Barium sulfate) after starvation for 6hrs . Multiple foci of tubular necrosis and haemorrhages in the kidney, marked hyperplasia of the mucosal layer of the small intestine, and a mild periportal lymphocytic cellular infiltration of the liver of the treated rabbits were observed. Plasma urea, uric acid, creatinine and blood glucose levels increased significantly ($p < 0.05$) in the treated rabbits. Plasma protein, hemoglobin, red blood cell and leukocyte counts were not altered adversely. No significant changes were observed in the enzymes' activity in all the groups of rabbits tested. The extract seems to show therapeutic actions on infections caused by *E. coli* and *Salmonella typhi* without any adverse effect on the organs.

Keywords : Histopathological, Phyllanthus niruri, Saponin, Toxicological

I. Introduction

Before 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. This study is designed to determine the toxicological and histopthatological effect of crude saponin extract from *Phyllanthus niruri* on some organs excised from bacterial infected rabbits.

Author α : The Microbiology Unit, Department of Science Technology, Federal Polytechnic, P. M. B 5351, Ado-Ekiti, Ekiti-State, Nigeria. Tel: +2348035781628 E-mail : ajibvijay@yahoo.com, (corresponding author) .

Author σ : Department of Microbiology, Ekiti State University, P.M.B 5363, Ado- Ekiti, 360101, Nigeria. College of Science, Engineering and Technology, Osun- State University P.M.B4494, Oke- Baale, Oshogbo 230212, Nigeria.

© 2012 Global Journals Inc. (US)

II. Materials and Methods

a) Collection of Plant Material

Phyllanthus niruri was collected from shrubs around the Federal Polytechnic compound, Ado- Ekiti, Nigeria between the months of July and September, 2008 and identified at the Department of Plant Science, University of Ado-Ekiti, Ekiti- State, Nigeria. A voucher specimen (STD/MIC/PLT.0982) was deposited at the herbarium of the Department of Science Technology, Federal Polytechnic, and Department of Plant Science, University of Ado-Ekiti.

b) Extraction of crude saponin

The sample used for the analysis was air-dried at room temperature of $\pm 28^0$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°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 di-ethyl 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°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 ($n \geq 6$/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.

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 9th 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).

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).

f) Histopathological examination

Experimental rabbits were dissected on the 9th day after administration of saponin. The method describe by Patel *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.

g) Statistical analysis

The data were expressed as mean $\pm$ S.D., while biochemical and physiological parameters were analyzed statistically using one way ANOVA followed by Dunnet-+-test using the Statistical Package for Social Sciences for comparison with control group and saponin treated group. $P < 0.05$ was considered as significant while $P < 0.01$ and $P < 0.001$ were considered as insignificant.

III. Results and Discussion

The mean blood parameters of groups of six rabbits each treated with saponin is shown in Table 1. The table depicts the effect of saponin on blood parameters. Urea, uric acid, creatinine and blood

© 2012 Global Journals Inc. (US)

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.

Table 1 : Blood parameters of rabbits treated with saponin at dose of 100mg/day for a period of 30 days

Parameters	Groups				
	I	II	III	IV	V
Urea(mg/dl)	16.82±2.45	17.53±3.84	16.59±3.51	18.53±2.92	22.29±2.31**
Uric acid(mg/dl)	7.53±1.67	7.42±0.85	7.22±1.56	8.76±1.56	10.42±1.90*
Creatinine(mg/dl)	1.32±0.41	1.26±0.49	1.35±0.38	1.65±0.38	2.08±0.46**
Protein (g/dl)	8.62±2.25	9.48±1.92	9.52±2.01	8.34±2.68	9.06±2.87
Blood glucose (mg/dl)	40.12±5.59	40.86±5.25	41.60±5.21	47.57±4.00**	52.50±4.25***
Hb (g/dl)	12.27±3.36	11.58±2.96	12.87±3.55	11.91±1.81	11.08±1.91
RBC X 10^6 mm^3	2.45±0.15	2.57±0.09	2.53±0.08	2.35±0.16	2.60±0.26
WBC	4410±182	4429±179	4317±191	4215±186	4388±183

Values are expressed as mean ± SD for six rabbits
Comparisons were between groups I(control) with II, III, IV and group V
*X $p < 0.05$, **XX $p < 0.01$,***XXX $p < 0.001$

Table 2 : Percentage of saponin in urine (0-72hr) after oral administration (100mg/day)

Rabbit Groups No	(% Mean (μg/ml) of the groups)
1	63.8
2	63.5
3	59.7
4	29.0
5	57.9
6	40.3
TOTAL MEAN	52.36
Standard Error (SE)	4.8

© 2012 Global Journals Inc. (US)

Table 3 : Urine analysis of rabbits treated with saponin for a period of 9 days

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

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

Table 4 : Effect of saponin on serum, liver and kidney enzymes activity in controlled and experimental groups

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±2.82***
ALT	36.25±3.5	39.41±6.04	34.16±5.62	34.16±5.62	34.94±4.94***
Liver (units/mg protein)					
AST	173.3±18.01	174.6±12.5	179.7±16.2	164.12±14.6	169.12±16.3***
ALT	37.02±5.31	37.34±5.20	39.89±3.80	39.91±6.20	41.80±5.25**
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

© 2012 Global Journals Inc. (US)

a) Results of histopathological examination

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 1b). 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 2b), and small intestine of the treated rabbits there were marked hyperplasias of the mucosal layer (Fig 3b).

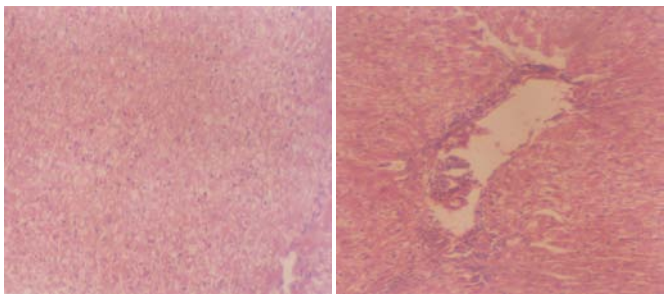
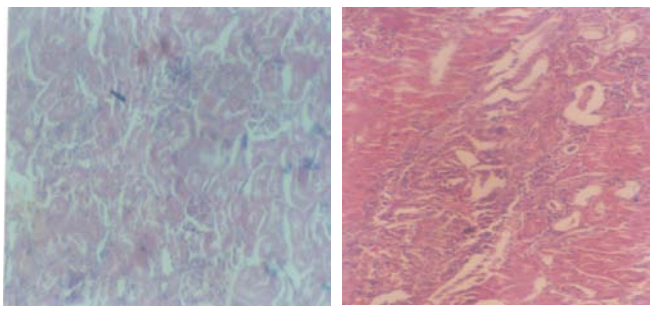


Fig. 1 : Liver of untreated (a) and saponin treated (b) rabbits.



a b

Fig. 2 : Kidney of untreated (a) and saponin treated (b) rabbits

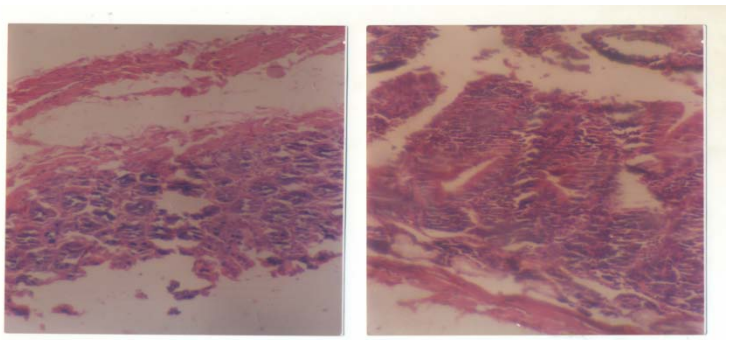


Fig. 3 : Small intestine of untreated (a) and saponin treated (b) rabbit

IV. 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 between 1h and 24h. This observation substantiates the constancy of absorption, distribution, metabolism and excretion of the saponin. Ingested saponins are exposed to many potential lignands in the intestine such as bile salts, dietary cholesterol and membrane sterols of the mucosal cells, and nutrients or antinutrients in body of ruminants (Flaoyen *et al.* 2001; Meagher *et al.*, 2001) and human subjects (Lee *et al.,* 2000) has however been demonstrated. Absorption was further

© 2012 Global Journals Inc. (US)

substantiated in the mean percentage of detectable 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 Yoshikawa *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 dose-dependently inhibit gastric emptying. This observation was earlier reported by Oda *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 concentration-dependent 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. 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*.

References Références Referencias

1. Adebayo, J. O., Yakubu, M. T., Egwin, E. C., Owoyele, B. V and Enaibe, B. U (2003). The effect of extract of Khaya senegalensis on some biochemical parameters of rat kidney J. Ethnopharmacol 88:69-72.
2. Adesokan, A.A and Akanji, M.A. (2004). Responses of selected rat kidney enzyme activities to administration of aqueous extract of Enantia chloranta. Nig. J. Pure and Appl. Sci 19:1592-1596.
3. Ahmed, B., Al-Hoiriny, T.A., and Siddigui, A.B. (2003). Antihepatotoxic activity of seeds of Chichorium intybus Journal of Ethnopharmacology.87:237-240
4. Ajibade, V. A and Famurewa, O (2011). Antibacterial activity of saponin and alkaloid extraction from whole plant of Phyllanthus niruri L. (Syn. P. franternus Webster) Pak. J. Sci Ind. Res. 54 (1): 47-52.
5. Ajibade, V.A., Adanlawo, I.G and Komolafe, O.D. (2006).Pharmacokinetic studies of saponin extracted from Phyllanthus niruri in rabbits with

© 2012 Global Journals Inc. (US)

gastroenteritis. Biomedical Research 17(1): 1725-1730.
6. Anupama T, Mani C and Rajest S (2011) A 28-days Sub-Acute Toxicity Study in some Albino mice to evaluate toxicity profile of Neurotol plus (Mannitol and Glycerol combination) International Journal of Biomedical Science 5(4):428-433.
7. Ambi A .A, Abdurrahmal E.M, Sule M.I, Patch U.U, Abdurrahman Y.R and Ibrahim N.D.G (2007) Phytochemical Screening and histopathological Studies on the seed of Colocynthis citrullus in Albino rats Nigeria Journal of Pharmaceutical Sciences 6 (2): 7-13
8. Aziza M.H., Wagdy K.B>K and Kawkab A.A (2008) Genetic and histopathology studies on mice; effect of Fenugreek oil on the efficiency of Ovarian and liver tissues African journal of Biotechnology 6(6) 477-483.
9. Barros, M. E., Schor, N and Boin, M. A. (2003) The effect of an aqueous extract from Phyllanthus niruri on calcium oxalate crystallization in vitro. Urol. Res. 30(6):367-373.
10. Dersert, K. C. (2007. Triterpenoid saponins from Acacia victoriae (Bentham) induce apoptosis by mitochondrial perturbation. Proceedings of the Nat Acad of Sci 98:103-107
11. Flaoven, A., Wilkins, A.L., Deng, D. and Brekket, T. (2001) Ovine metabolisms of saponin: evaluation of a method for estimating the ovine uptake of steroridal saponins from Narthecium ossifragnum. Veterinary Research Communications 25: L 225-238.
12. Francis, G.,Kerem, Z., Makkar, H.P.S and Becker, K. (2002). The biological action of saponins in animal system: a review. Br. J. of Nutri 88 (6): 587-605.
13. George, D. P and Roger, M. D .(2002). Encyclopaedia of Medicinal Plant 5th print of the original edition Spanish1.
14. Khanna, A.W., Rizvi, F and Chander, R. (2002). Lipid lowering activity of Phyllanthus niruri in hyperlipemic rats J. Ethnopharmacol, 82 (1): 19-22.
15. Lee, K.T., Sohn I.C., Kim, D.H., Choi, J.W and Kwon, S.H. (2000) Hypoglycaemic and hypolipidemic effects of tectorigenin and kaika-saponin III in the streptozotocin-induced diabetic rat and their antitoxidant activity in vitro. Arch Pharmacol. Res. 23:461-466.
16. Meagher, L.P., Smith, B.L and Wilkins, A.L. (2001). Metabolism of diosgenin-derived saponins: implications for hepatogenous photosensitization diseases in ruminants. Animal Feed Science and Technology 91:157-170.
17. Nishiura, J; Campus, A; Boim, M and Schor, N .(2005). Effect of Phyllanthus niruri on urinary calcium levels in calcium stone forming patients Journal of Clinical and Laboratory Investigation of Urolothies and Related Areas 32 (15):362-366.
18. Oda, K; Matsuda, H; Murakami, T; Katayama, S; Ohgifani, T. and Yoshikawa, M. (2000) Adjuvant and haemolytic activities of 47 saponins derived from medicinal and food plants. Biological Chemistry 381:67-74.
19. Otsuka, E. A. (2005). Analysis and isolation of saponin from plant materials. Saponin in food, feedstuffs and medicinal plants. Annual proceeding of the phytochemical society pg 1-12 (W. Oleszed and A. Mars ton, edition Oxford and London, Clarendon process.
20. Patel, P; Sweiss, K; Shatavi, S; Peace, D; Clark, N; Rondelli, D (2010). The impart of novel influenza A (H1N1) after hematopoietic SCT. Bone Marrow Transplant, J. Exp. Med. 45: 181-187.
21. Pooneh, R; Amir, S; Javad, A; Rosita, E; Mehran, A; Mohammadhossein, M; Saied, M; Kayhan, A (2010) Emergence of African swine fever virus, Northwestern Iran. 16(12): 1946-1949.
22. 22. Qudhia, P and Tripathi, R. S (2002) Prospect of cultivation of medicinal plant in Chattisgarh, Indian. P211-236. in: recent progress in medicinal plants. Vol 5, Crop Improvement, Production Technology, Trade and commerce. Sci Tech Public, U.S.A
23. Ray, S. M. D .(2007). The sapogenin of the seed of flour lupin species. Journal of Plant Food 3: 181-186.
24. Shim, I; Javaid, J.I and Kim, S.E. (2000). Effect of ginseng total saponin on extracellular dopamine released elicited by local infusion of nicotine into the striatum of freely moving rats. Planta medica 66:705-708.
25. Tabasum, N., Chatterrredi, S.,Agrawal, S. S and Ahmed, N. (2005). Hepatoprotective studies of Phyllanthus niruri on Paracetamol- induced liver cell damage in albino mice. J. Exp. Med. 12 (14): 211-213.
26. Unander, D. W., VenKateswaran, P. S., Mill man, I., Bryan, H. H and Blumberg, B. S. (1990). Phyllanthus species: Sources of new antiviral compounds. Advances in new crops. Timber Press, Portland, p518-521.
27. Yoshikawa, M; Murakami, T; Kishi, A; Kageura, T and Matsuda, H. (2001). Medicinal flowers 111, marigold (1): hypoglycaemic gastric emptying inhibitory, and gastroprotective principles and new oleanane-type triterpene oligoglycosides, calendasaponins A,B,C and D, from Egyptian Calendala officinalis, Chemical and Pharmacecitical Bullentin 49:863-870.
28. Zhong Yao Za Zhi. (2005). Antispasmodic saponins from bulbs of red onion Allium cepa (L.var.Tropea) J Agric Food Chem 53 (4): 935-940

© 2012 Global Journals Inc. (US)

This page is intentionally left blank

© 2012 Global Journals Inc. (US)