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Full Length Research Paper

Serum biochemical changes accompanying prolonged administration of ethanolic extract of whole fruit of *Lagenaria breviflora* (Benth) Roberty in Wistar rats

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Toxicological evaluation of the whole fruit of *Lagenaria breviflora* was carried out using the serum biochemical changes accompanying prolonged administration of the ethanolic extract of the fruit in Wistar rats. Twenty five rats were randomly but equally divided into five groups. Rats in groups B, C, D and E were administered with ethanolic extract at 1000, 2000, 4000 and 8000 mg/kg body weight, respectively, while rats in group A received 0.9% physiological saline as the control animals. The lower doses of the extract (1000 and 2000 mg/kg body weight) lowered the serum levels of total cholesterol (TC), triglycerides (TG) and low density lipoprotein-cholesterol (LDL-C), while high-density lipoprotein-cholesterol (HDL-C) was elevated. Higher doses of 4000 and 8000 mg/kg body weight of the extract increased the serum levels of TG and LDL-C and, lowers HDL-C level in the serum. There was dose-dependent elevation of serum glucose level in rats administered with the extract. The serum value of glucose in rats administered with the extract of 8000 mg/kg body weight increased two and half-fold over the control value. The mean serum total protein value increased for all the treatment groups when compared with that of the rats in the control group. The serum creatinine (CRT) level increased dose-dependently and blood urea nitrogen (BUN) was elevated two-fold in most of the test groups. This was corroborated with histopathological findings revealing marked renal tubular degeneration. The serum level of ALP increased significantly ($P < 0.05$) in test rats administered the highest dose of the extract. It was concluded that therapeutic application of the extract of *L. breviflora* is quite safe at lower doses but it is nephrotoxic and can precipitate hyperglycaemia and dyslipidaemia at higher doses.

Key words: Toxicological evaluation, *Lagenaria breviflora*, fruit, serum biochemistry, Wistar rat.

INTRODUCTION

The fruit of *Lagenaria breviflora* Roberty is widely used in folklore medicine in West Africa as herbal remedy for the treatment of measles, digestive disorders, and as wound antiseptics (e.g. umbilical incision wound) in man, while the livestock farmers use it for treatment of Newcastle disease and coccidiosis in various animal species, especially poultry (Sonaiya, 1999). Laboratory investigations have shown evidences in support of its anti-implantation activity (Elujoba et al., 1985), miracicidal and cercaricidal activity (Ajayi et al., 2002) and antibacterial activity (Tomori et al., 2007). In addition to its medicinal application, so much have been reported on the taxonomy (Morimoto et al., 2005) and chemical constituents (Elujoba and El-Alfy, 1986; Elujoba et al., 1991; Esuoso and Bayer, 1998; Esuoso et al., 2000) of the plant, while little or no attempt has been made to evaluate the safety of medicinal application of the extract of the fruit of *L. breviflora*. Recently, there has been increased or renewed awareness in the populace of the benefit of medicinal plant in health care and more people are increasingly employing the use of these plants without any standard dose regime, which naturally is predisposed to over-dosage and its attendant toxicities.

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In the folklore medicine, herbal preparations are administered as paste, powder and concoctions made from crude extractions of medicinal plants (Tiwari and Agrawal, 2003; Ramoutsaki et al., 2002; Brusell, 2005). It is therefore logical to evaluate possible deleterious effect of *L. breviflora* in its crude state.

This study seeks to evaluate some serum biochemical changes accompanying prolonged administration of ethanolic extract fruit of *L. breviflora* with a view to having a balanced understanding of effects of *L. breviflora* on both human and animal body systems using the Wistar rat as experimental animal model.

MATERIALS AND METHODS

The plant

Fresh fruits of *L. breviflora* were obtained from Botanical Garden of University of Ibadan, Nigeria and identified at the Department of Botany and Microbiology of the University. A voucher sample of the plant was also deposited in the department.

Extraction of the fruit

Fresh fruits were washed, weighed and cut into small pieces. These pieces were collected and tied up in small portions in sieves and placed in ethanol (96%) in plastic containers. The ethanolic solution was drained regularly and replaced with fresh ethanol every 3 days. Each batch of harvested solvent were stored in plastic containers and refrigerated at 4°C.

Separation of the extract

The extract obtained was clarified by filtration through celite on water pump and was then concentrated *in vacuo* using a rotation evaporator at low temperatures. The ethanol remaining in the extract was finally removed by placing small volumes in porcelain dishes in the oven set at low temperature of 4°C. The extract came as semi-solid greenish-brown paste. A stock solution was prepared by dissolving 100 mg of the extract in 100 ml of distilled water.

Experimental animals

Fifty adult male rats were used for this study. They were housed at the Animal Housing Unit of the Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan. The rats were fed with pelletized rat ration (Guinea Feed, Nig. Ltd). Water was given *ad libitum* and the rats were allowed to stabilize for 2 weeks before commencement of the experiment. Twenty five rats were used to determine the safe dose range of the extract in an acute toxicity study which showed that doses above 8,000 mg/kg body weight of the rats caused mortality. The remaining 25 rats were randomly but equally divided into five groups.

Dosing and collection of blood samples

The rats in group A were administered with 0.9% physiological saline. While the four treatment groups B, C, D and E were administered with ethanolic extract of 1000, 2000, 4000 and 8000 mg/kg body weight of the rats, respectively. The rats were administered, orally with their various designated dosages once daily for 14 days using rat cannula. Blood was collected from each rat in fluoride oxalate bottles for glucose evaluation and in non-heparinized bottles for other serum biochemical analysis.

Serum biochemical parameters evaluated

Serum lipid profile including total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL-C) and low density lipoprotein (LDL-C) were determined according to the method of Meiathnin et al. (1978). Total protein was measured using Biuret reaction (Lanzarot et al., 2005), while glucose and albumin levels were measured by spectrophotometric estimation using the Sigma Diagnostic Kit (Sigma Diagnostics, UK). Globulin was obtained from the difference of total protein and albumin. Serum enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were determined by method of Duncan et al. (1994) using enzyme kits prepared by Randox Laboratories Ltd, UK. Serum urea and creatinine levels were determined using spectrophotometric methods described by Coles (1986). The total bilirubin and conjugated bilirubin concentrations were determined as described by Balistreri and Shaw (1987). The unconjugated bilirubin concentration was calculated as the difference between total and conjugated bilirubin.

Statistical analysis

Student t-test statistic was used to analyze the values obtained from these determinations. The differences in the values were considered significant at probability levels of $P < 0.05$ (Steel and Torrie, 1996).

Lipid profile

Total cholesterol (TC)

The mean total cholesterol values were lower in all the test groups when compared with the control group, but significant ($P < 0.05$) reductions were observed for rats in groups B (125.6 ± 7.23 mg/dl) and C (141.0 ± 8.20 mg/dl) relative to that of the rats in the control group (163.6 ± 7.58 mg/dl) (Table 1).

Triglycerides (TG)

The mean serum triglycerides values were reduced non-significantly ($P > 0.05$) in rats in groups B (62.0 ± 3.35 mg/dl) and C (69.4 ± 6.16 mg/dl), while the values were higher ($P > 0.05$) in groups D (99.5 ± 14.17 mg/dl) and E (81.6 ± 8.87 mg/dl) when compared with the values obtained for the rats in the control group (70.0 ± 5.18 mg/dl) (Table 1).

High density lipoprotein-cholesterol (HDL-C)

The values of mean serum high-density lipoproteins were observed to have be reduced in all the test groups when compared to that of the control rats (43.8 ± 5.11 mg/dl). The difference of the means is only significant ($P < 0.05$) between the control group (43.8 ± 5.11 mg/dl) and C (40.5 ± 5.04mg/dl) (Table 1).

Low density lipoprotein-cholesterol (LDL-C)

The serum level of low density lipoproteins were observed to have

Table 1. Serum lipid profile and glucose levels of rats administered with ethanolic extract of fruit of *L. breviflora*.

Parameter	Group A Control (n = 5)	Group B 1000 mg/kg (n = 5)	Group C 2000 mg/kg (n = 5)	Group D 4000 mg/kg (n = 5)	Group E 8000 mg/kg (n = 5)
TC (mg/dl)	163.6 ± 7.58^{a}	125.6 ± 7.23^{ab}	141.0 ± 8.2^{b}	149.0 ± 10.82	144.8 ± 11.27
TG (mg/dl)	70.0 ± 5.18	62.0 ± 3.35^{a}	69.4 ± 6.16	99.5 ± 14.17^{a}	81.6 ± 8.87
HDL-C (mg/dl)	43.8 ± 5.11	49.4 ± 2.69	48.2 ± 1.53	40.5 ± 5.04	38.4 ± 2.96
LDL-C (mg/dl)	88.8 ± 4.39	79.0 ± 5.37	79.6 ± 3.84	75.5 ± 3.57	90.8 ± 6.45
Glucose (mg/dl)	92.0 ± 7.87^{abcd}	102.0 ± 14.97^{a}	104.49 ± 0.69^{b}	122.4 ± 0.26^{c}	237.23 ± 7.05^{d}

Means with the same superscripts are statistically significant at P < 0.05 on the same row.

Table 2. The mean serum protein and non-protein metabolite values of rats administered with ethanolic extract of fruit of *L. breviflora*.

Parameter	Group A Control (n = 5)	Group B 1000 mg/kg (n = 5)	Group C 2000 mg/kg (n = 5)	Group D 4000 mg/kg (n = 5)	Group E 8000 mg/kg (n = 5)
TP (g/dl)	5.06 ± 0.25^{ab}	5.68 ± 0.18^{c}	6.54 ± 0.31^{a}	5.54 ± 0.90	6.78 ± 0.26^{bc}
ALB (g/dl)	3.38 ± 0.11	3.64 ± 0.13	3.44 ± 0.14	3.42 ± 0.52	3.48 ± 0.15
Glob (g/dl)	1.68 ± 0.21^{a}	2.04 ± 0.18^{b}	3.10 ± 0.44	2.42 ± 0.50	3.30 ± 0.18^{ab}
TBil (mg/ml)	0.62 ± 8.7 x 10^{-3}	0.56 ± 8.6 x 10^{-3}	0.44 ± 6.8 X 10^{-3}	0.53 ± 6.3 x 10^{-3}	0.36 ± 8.1 x 10^{-3}
CRT (mg/dl)	0.14 ± 2.4 x 10^{-3}	0.18 ± 2.0 x 10^{-3}	0.20 ± 1.0 x 10^{-3}	0.34 ± 2.4 x 10^{-3}	0.38 ± 3.7 x 10^{-3}
BUN (mg/dl)	67.6 ± 13.04^{abcd}	115.4 ± 7.66^{aef}	145.4 ± 7.80^{be}	137.4 ± 5.12^{cf}	108.4 ± 14.06^{d}

Means with the same superscripts are statistically significant at P < 0.05 on the same row.

reduced (P > 0.05) for the treatment groups: B (79.0 ± 5.37 mg/dl), C (79.6 ± 3.84 mg/dl) and D (75.5 ± 3.57 mg/dl) when compared with that of the rats in the control group (88.6 ± 4.39 mg/dl). It was however increased (P > 0.05) for group E (90.8 ± 6.48 mg/dl) (Table 1).

Serum glucose level

Glucose levels

The mean values recorded for the glucose levels increased dose-dependently for all the treatment groups: B (102.0 ± 14.97 mg/dl), C (104.49 ± 0.69 mg/dl), D (122.4 ± 0.26mg/dl) and E (237.23 ± 7.05 mg/dl) over the values obtained for the rats in the control group (92.0 ± 7.87 mg/dl). The difference of the means is significant between the values of each treatment group and the control group (Table 1).

Plasma proteins and non-protein metabolites

Total protein (TP) levels

The mean serum total protein value increased for all the treatment groups when compared with that of the rats in the control group (5.06 ± 0.25 g/dl). The increase was significant (P < 0.05) for the rats in groups C (6.54 ± 0.31 g/dl) and E (6.78 ± 0.26 g/dl) (Table 2).

Albumin (Alb) and globulin (Glob) levels

The serum level of albumin fractions increased non-significantly (P > 0.05) for group B (3.64 ± 0.13 g/dl), C (3.44 ± 0.14 g/dl) and E (3.48 ± 0.15 g\dl), while that of group D was reduced (P > 0.05) relative to value obtained for the control group (3.38 ± 0.11 g/dl). The mean value of the globulin fraction increased in rats in all the treatment groups and the difference of the means were significant (P < 0.05) between the control and group D (Table 2).

Total bilirubin

The serum total bilirubin values decreased non-significantly (P > 0.05) for rats in all the treatment groups: B (0.56 ± 8.6 x 10^{-3} mg/dl), C (0.44 ± 6.8 x 10^{-3} mg/dl), D (0.53 ± 6.3 x 10^{-3} mg/dl) and E (0.36 ± x 10^{-3} mg/dl) when compared with those of the rats in the control group (0.62 ± 8.7 x 10^{-3} mg/dl) (Table 2).

Creatinine (CRT) level

The serum creatinine levels of the rats increased non-significantly and dose-dependently in all the treatment groups: B (0.18 ± 2.0 x 10^{-3} mg/dl), C (0.20 ± 1.0 x 10^{-3} mg/dl), D (0.34 ± 2.4 x 10^{-3} mg/dl) and E (0.38 ± 3.7 x 10^{-3} mg/dl) when compared with the values obtained for the rats in the control group (0.14 ± 2.4 x 10^{-3} mg/dl) (Table 2).

Blood urea nitrogen (BUN) level

The mean blood urea nitrogen (BUN) values significantly (P < 0.05) increased in rats from group B (115.4 ± 7.66 mg/dl), C (145.4 ± 7.08 mg/dl), D (137.4 ± 5.2 mg/dl) and E (108.4 ± 14.06 mg/dl) when compared with that of rats in control group (67.6 ± 13.04 mg/dl).

Serum enzymes

Aspartate aminotransferase (AST) level

There was no significant change in serum AST levels in the rats in

Table 3. Serum enzyme levels of rats administered with ethanolic extract of fruit of *L. breviflora*.

Parameter	Group A Control (n = 5)	Group B 1000 mg/kg (n = 5)	Group C 2000 mg/kg (n = 5)	Group D 4000 mg/kg (n = 5)	Group E 8000 mg/kg (n = 5)
AST (U/L)	30.8 ± 1.88	30.8 ± 3.17	27.6 ± 1.57[a]	32.0 ± 1.68a	31.2 ± 2.92
ALT (U/L)	26.6 ± 1.50	26.4 ± 2.58	22.2 ± 1.46	24.3 ± 1.93	26.0 ± 3.27
ALP(U/L)	90.6 ± 3.31	74.2 ± 6.48[a]	82.0 ± 2.63[b]	101.3 ± 5.81	108.2 ± 6.18[ab]

Means with the same superscripts are statistically significant at $p<0.05$ on the same row.

all treatment groups when compared to that of the control rats (Table 3).

Alanine aminotransferase (ALT) level

There was a marginal difference between the mean serum levels of ALT of the rats in each of the treatment groups: B (26.4 ± 2.58 U/L), C (22.2 ± 1.46 U/L), D (24.3 ± 1.93 U/L) and E (26.0 ± 3.27 U/L), and when compared to that of the control group (26.6 ± 1.50 U/L) (Table 3).

Alkaline phosphatase (ALP) level

The mean value of serum level of ALP obtained for rats in the control group (90.6 ± 3.31 mg/dl) was non-significantly higher than those of group B (74.2 ± 6.48 U/L) and C (82.0 ± 2.63 U/L) but lower than that of group D (101.3 ± 5.81 U/L) and E (108.2 ± 6.18 U/L) (Table 3).

Histopathology

No visible lesion was observed in the liver, kidney, heart and intestine of rats in group A. There was marked epithelial necrosis with goblet cell hyperplasia in the intestines of rats in groups B, C, D and E. Marked renal tubular degeneration and necrosis were observed in the rats in groups C and E with no visible lesions in other organ samples.

DISCUSSION

In this study, there was a biphasic response by the serum lipid profile to the administration of ethanolic extract of whole fruit of *L. breviflora*. It was discovered that the lower doses of the extract (1000 and 2000 mg/kg body weight) lowered the serum levels of TC, TG and LDL-C, while HDL-C was elevated. Higher doses of 4000 and 8000 mg/kg body weight of the extract increased the serum levels of TG and LDL-C, and lowers HDL-C level in the serum. The serum values of TC for doses of 4000 and 8000 mg/kg body weight of extract though lower than that of the control, were nevertheless found to be comparatively higher than the serum level of TC recorded for the lower doses of the extract.

It is inferable from the foregoing that the extract of the fruit of *L. breviflora* exhibits lipolytic effect at lower doses or put in another way; it does not have dyslipidaemic potential at lower doses, which is actually a desirable quality for a plant like *L. breviflora* that is extensively used as antiviral and antibacterial remedies in both man and animal (Burkill, 1985; Tomori et al., 2007). With this finding, the plant therefore compares favourably with many other medicinal plants like extracts of *Calotropis procera* (Akinloye et al., 2001), *Allium cepa* Linn (Kumari and Augusti, 2007), *Indigofera tinctoria* (Puri et al., 2007) and Gui Qui mixture (Zhang et al., 2006) that have been reported to exhibit some degree of lipolytic effect. This lipolytic effect of the whole fruit of *L. breviflora* would have added to many other existing claims of therapeutic effects of *L. breviflora* except for the fact that the lipid lowering effect was reversed at higher doses of the extract. At higher doses, the extract exhibited a high lipogenic effect which is believed to have arisen from the high triacylglycerol and fatty acid constituents of the fruit (Oshodi, 1996; Esuoso and Bayer, 1998). It should therefore be mentioned at this point, that administration of the extract of the plant as natural remedy should not be done wantonly because of its tendency to precipitate hyperlipidaemia and increase atherogenic indices characterized by elevation of serum TG and LDL-C, glucose and lowered HDL-C levels.

Dose-dependent hyperglycaemic effect was observed in rats administered with the extract. Hyperglycaemia has been consistently associated with subsequent development of dyslipidaemia (Parikh et al., 2001; Emral et al., 2005). There is need to find out how extract of *L. breviflora* elevates serum glucose level. Is the hyperglycaemia related to the effect of the extract on insulin, glucocorticoids and other physiological processes or hormones associated with control of blood glucose level or was it for the fact that the extract is a rich source of metabolic sugar? One established fact is that the fruit has been reported to have a high amino acid content (Esuoso and Bayer, 1998) which may lead to increase gluconeogenesis through the intermediate metabolic pathways (Lehninger et al., 1993). The excess glucose formed can be converted to lipids additionally, thus leading to eventual rise in lipid profile as clearly observed in this study.

Administration of extract of *L. breviflora* was also accompanied with elevated serum total protein level and the increase was observed for both the ALB and GLB fractions. Plasma proteins are predominantly synthesized by hepatocytes (Liang and Grieninger, 1981; Jefferson et al., 1983), which is the basis of the assumption that the increased level of serum proteins in this study is a direct effect of *L. breviflora* on hepatic functions, especially on

protein synthesis. Of more significance is the globulin fraction which strongly infers that the extract is also immuno-stimulant.

It was observed that the serum total bilirubin was actually lowered in the test rats which are an indication that the extract did not exhibit hepatotoxicity. This was corroborated by histopathology report of no lesion or degeneration of hepatic tissue in this study. However, the serum CRT level increased dose-dependently and BUN was elevated two-fold in most of the test groups. These are strong indices of renal injury (Mazze et al., 2000; Waikar and Bonventre, 2006), which was confirmed by histopathological findings. Saba et al (2009) had earlier hinted on the nephrotoxic tendency of whole fruit of *Lagenaria breviflora.* The serum enzymes did not show any remarkable changes except for the serum ALP which was significantly increased in test rats admini-stered with the highest dose (8,000 mg/kg body weight) of the extract. The elevated serum ALP level may be an indication of hepatic injury (Jansen and Muller, 1999; Moseley, 1999) by the extract at the highest dose administered but this is not supported by the histopatho-logical findings; however, injury of the gastrointestinal tract such as necrosis of the intestinal epithelium with goblet cell hyperplasia observed in this study is also known to be accompanied by elevated serum ALP (Wiwanitkit, 2001).

In conclusion, it appears that therapeutic application of the extract of the whole fruit of *L. breviflora* is quite safe at doses below 2,000 mg/kg body weight in rats. There is a need to ascertain if the extract is potent at these doses for its ethno-medicinal applications. It is not advisable to take the extract at higher doses for long period because of its hyperglycaemic and dyslipidaemic effects which can easily predispose to diabetes mellitus, atherosclerosis and cardiopathy among other sequela. It is recommend-ed that further studies be carried out to firmly establish metabolic basis of the hyperglycaemic and dyslipidaemic effects of the extract of whole fruit of *L. breviflora*.

REFERENCES

Ajayi GO, Awujo NC, Abulu LE (2002). The miracicidal and cercaricidal activity of the methanolic extracts of *Lagenaria breviflora* Robert Family *Cucurbitaceae* fruit on *Schistoma mansoni*. Nigerian Q. J. Hosp. Med. 12: 57-59.

Akinloye AK, Abatan MO, Onwuka SK, Alaka OO, Oke BO (2001). Lipolytic effect of *Calotropis procera* on the skin of Wistar rats. Afr. J. Biomed. Res. 4: 139-140.

Balistreri WR, Shaw LM (1987). Liver function, in: Fundamental of Clinical Chemistry, Tietz NW (Ed). 3rd ed, W.B. Saunders Company, Philadelphia. pp. 729-761.

Brusell DE (2005). A medicinal plant collection from Montserrat, West Indices. Econ. Bot. 58: 203-220.

Burkill HW (1985). The useful plants of West Tropical African. University Press, Virginia, USA, pp. 594-601.

Coles EH (1986). Veterinary Clinical Pathology 4th ed. Saunders Company Philadelphia. pp. 126-127.

Duncan JR, Prasse KW, Mahaffey EA (1994). Veterinary Laboratory Medicine (Clinical Pathology) 3rd ed. Iowa State University Press, Ames, pp. 94-98.

Elujoba AA, El-Alfy TS (1986). Providing some pharmacopoieal standards for *L. breviflora* fruit. Acta Hortic. 188: 247-252.

Elujoba AA, Olagbende SO, Adesina SK (1985). Anti-implantation activity of the fruit of *Lagenaria breviflora* Robert. J. Ethnopharmacol. 13: 281-288.

Elujoba AA, Fell AF, Linley PA (1991). Chromatographic and spectroscopic analysis of bound and unbound phenolic acids in *L. breviflora* fruit. J. Pharm. Biomed. Anal. 9: 711-715.

Esuoso KO, Bayer E (1998). Chemical composition and potentials of some tropical underutilized biomass. Note II *Andenopus breviflorus* and *Curcumeropsis edulis*. Rivista-Italiana. Sostanze. Grasse, 95: 191-196.

Esuoso KO, Lutz H, Bayer E, Kutubuddin M (2000). Unsaponifiable lipid constituents of some underutilized tropical seed oils. J. Agric. Food Chem. 48: 231-234.

Emral R, Koseogulari O, Tonyukuk V, Uysal AR, Kamel N, Corapcioglu D (2005). The effect of short-term glycemic regulations with gliclazide and metformin on postprandial lipemia. Exp. Clin. Endoc. Diabetes, 113: 80-84.

Jansen PL, Muller M (1999). Early events in sepsis-associated cholestasis. Gastroenterology, 116: 486-488.

Jefferson LS, Liao WS, Peavy DE, Miller TB, Appel MC, Taylor JM (1983). Diabetes-induced alterations in liver protein synthesis. Changes in relative abundances of mRNAs for albumin and other plasma proteins. J. Biol. Chem. 258: 1369-1375.

Kumari K, Augusti KT (2007). Lipid lowering effect of S-methyl cysteine sulfoxide from *Allium cepa* Linn in high cholesterol diet fed rats. J. Ethnopharmacol. 109: 367-371.

Lanzarot PM, Barahona VM, Manuel IS, Manuel F, Cassidy R (2005). Haematologic, protein electrophoresis, biochemistry and cholineste-rase values of free-living black stork nestlings (*Ciconia nigra*) J. Wildlife Dis. 41(2): 379-386.

Lehninger AL, Nelson DL, Cox MM (1993). The citric acid cycle. Principles of Biochemistry. Worth Publishers: New York, USA, pp. 598-611.

Liang TJ, Grieninger G (1981). Direct effect of insulin on the synthesis of specific plasma proteins: biphasic response of hepatocytes cultured in serum- and hormone-free medium. Proc. Natl. Acad. Sci. USA, 78: 6972-6976.

Mazze RI, Callan CM, Galvez ST, Leticia DRN, Mayer DB (2000). The effects of sevoflurane on serum creatinine and blood urea nitrogen concentrations: retrospective, twenty-two-centre, comparative evalua-tion of renal function in adult surgical patients. Anesth. Anal. 90: 683-688.

Meiathnin F, Bardelli F, Giamini G, Tarli P (1978). The 4-Hydroxybenzoate-4-aminophenazone chromogenic system used in the enzymic determination of serum cholesterol. Clin. Chem. 24: 2161-2165.

Morimoto Y, Maundu P, Fujimaki H, Morishima H (2005). Diversity of landraces of the white-flowered gourd (*Lagenaria siceraria*) and its wild relatives in Kenya: Fruit and seed morphology. Genet. Res. Crop Evol. 52: 737-747.

Moseley RH (1999). Sepsis and cholestasis. Clin. Liver Dis. 3: 465-475.

Oshodi AA (1996). Amino acid and fatty acid composition of *Adenopus breviflorus* Benth seed. Int. J. Food Sci. Nutr. 47: 295-298.

Parikh P, Man U, Iyer U (2001). Role of spirulina in the control of glycemia and lipidemia in type 2 diabetes mellitus. J. Med. Food 4: 193-199.

Puri A, Khaliq T, Rajendran SM, Bhatia G, Chandra R, Narender T (2007). Antidyslipidemic activity of *Indigofera tinctoria*. J. Herbal Pharmacother. 7: 59-64.

Ramoutsaki IA, Dimitriou H, Kalmanti M (2002). Management of childhood diseases during Byzantine period: II-Exanthematic disease. Paediatric Int. 44: 338-340.

Saba AB, Oridupa OA, Ofuegbe FO (2009). Evaluation of haematological and serum electrolyte changes in Wistar rats administered with ethanolic extract of whole fruit of *Lagenaria breviflora*. J. Med. Plants Res. 3(10): 758-762.

Sonaiya EB (1999). Family Poultry and Food Security. Research requirement in Science, Technology and Socio-economics. http:///www.fao.org/WAICENT/fao/INFO/AGRICULT/AGAInfo/subjects/e n/infpd/documents/papers/2000/4SONAIYA.DOC.

Steel RGD, Torrie JII (1996). Principles and procedure of Statistics: A biometric approach. McGraw Hill: New York, USA, pp. 6-15.
Tiwari L, Agrawal DP (2003). All purpose turmeric: Uses, chemistry, IPR. Essay in History of Indian Science and Technology. Indian J. Trad. Knowledge, 1: 17-21.
Tomori OA, Saba AB, Dada-Adegbola HO (2007). Antibacterial activity of ethanolic extract of whole fruit of *Lagenaria breviflora* Roberts. J. Anim. Vet. Adv. 6: 752-757.
Waikar SS, Bonventre JV (2006). Can we rely on Blood urea nitrogen as biomarker to determine when to initiate dialysis? Clin. J. Am. Soc. Nephrol. 1: 903-904.
Wiwanitkit V (2001). High serum alkaline phosphatase levels; a study in 181 Thai adult hospitalized patients. Biol. Med. Central. Fam. Pract. 2: 1-2.
Zhang YW, Xie D, Chen YX, Zhang HY, Xia Zx (2006). Protective effect of Gui Qi mixture on the progression of diabetic nephropathy in rats. Exp. Clin Endocrinol. Diabetes, 114: 563-568.