

Full Length Research Paper

Evaluation of acute toxicity in mice and subchronic toxicity of hydroethanolic extract of *Parinari curatellifolia* Planch (Chrysobalanaceae) seeds in rats

Steve O. Ogbonnia^{1*}, Sunday O. Olayemi², Emmanuel N. Anyika³, Veronica N. Enwuru⁴ and Olawale O. Poluyi²

¹Department of Pharmacognosy, Faculty of Pharmacy, College of Medicine Campus, University of Lagos, Idi-Araba, Lagos, Nigeria.

²Department of Pharmacology, School of Basic Medical Sciences, College of Medicine Campus, University of Lagos, Idi-Araba, Lagos, Nigeria.

³Department of Clinical Pharmacy and Biopharmacy, Faculty of Pharmacy, College of Medicine Campus, University of Lagos, Idi-Araba, Lagos, Nigeria.

⁴Department of Pharmaceutical Technology and Pharmaceutical Microbiology, Faculty of Pharmacy, College of Medicine Campus, University of Lagos, Idi-Araba, Lagos, Nigeria.

Accepted 19 February, 2009

***Parinari curatellifolia* seeds are ethnobotanically used in the treatment of diabetes and other diseases. The seed drug preparations are administered over a long period of time in the treatment of certain disease conditions. The aim of this study was to evaluate the safety of the seed extract through acute toxicity study. Also subchronic toxicity in the animals was carried out by assessing the effects on biochemical parameters, body weight and liver, heart and renal organs following oral administration of aqueous ethanolic extract of the seeds. Toxicity of the extract was evaluated in Swiss albino mice by feeding the animals with graded doses of the extract between 1.0 to 20.0 g/kg body weight orally and observed continuously for the first 4 h and every hourly for the next 24 h, then 6 hourly for 48 h (72 h, acute toxicity). Wistar rats were also fed with different extract doses for at least 30 days and the effects on biochemical parameters evaluated (subchronic toxicity model). The median acute toxicity value (LD₅₀) of *P. curatellifolia* seeds extract was found to be 7.27 g/kg body weight. The extract reduced plasma glucose and low density lipoprotein (LDL)-cholesterol levels, but increased high density lipoprotein (HDL)-cholesterol in the treated groups compared to the control. A significant increase in the body weight was observed in all the groups treated with the extract. Aspartate aminotransferases (AST), creatinine and phosphorus levels were significantly increased only in the group treated with highest dose of the extract while significant decrease in alanine aminotransferases (ALT) level was observed in all groups. The LD₅₀ value indicated the drug to be quite safe in one dose treatment. The study also showed that the extract had good hypoglycemic effects and good reducing effects on the cardiovascular risk factors but indicated that high dose of the extract on a long term use can cause liver and kidney problems.**

Key words: *Parinari curatellifolia*, seeds, acute, toxicity, diabetes.

INTRODUCTION

Traditional use of plant drugs popularly known as herbal remedies, in the treatment of a variety of diseases, is

widely practiced in the Nigerian communities as well as in other developing countries. The exclusive use of herbal drugs for the management of certain ailments continues unabated in most developing communities due to easy access and for economic reasons. Plants, therefore, remain the main source of the active drugs from a natural

*Corresponding author. E-mail: steveogbonnia@hotmail.com.

source and are still indispensable in the traditional medicine for treating a number of diseases (Ogbonnia et al., 2008).

Herbal remedies mainly used in the traditional medicine contain active organic compounds and are employed in the treatment of diseases of diverse origins. Traditional medicines are used by about 60% of the world population both in the developing countries and developed countries where modern medicines are predominantly used (Mythilypriya et al., 2007). The practitioners most often prepare the recipes from a combination of two or more plant products, which could be used in the treatment of more than one disease condition (Pieme et al., 2006). They are administered in most disease conditions over a long period of time without a proper dosage monitoring and consideration for toxic effects that might result from such a prolonged usage. The danger associated with the potential toxicity of such therapy and other herbal therapies used over a long period of time demand that the practitioners be kept abreast of the reported incidence of renal and hepatic toxicity resulting from the ingestion of medicinal herbs (Tédong et al., 2007).

Parinari curatellifolia called "Ebere" by the Nigerian Yorubas is a valuable and cherished medicinal plant and is widely used by the traditional herbalists. The remedies may be prepared with the seeds alone or in combination with other herbs and used locally in the treatment of diabetes and other diseases. The combination therapy is more common, since most traditional medical practitioners believe that a combination of many plant products would create the desired synergy.

The preliminary phytochemical analysis of the extract indicated positive results for alkaloids, polyphenols and anthraquinone glycosides. For a plant or herbal preparation containing active organic principles to be identified for use in the traditional medicine, a systemic approach is required for the evaluation of efficacy and safety through experiment and clinical findings (Mythilypriya et al., 2007).

The aim of this study was to evaluate the phytochemical profile and safety of *P. curatellifolia* seed extract by carrying out the acute and subchronic toxicity studies in the animals. Subchronic toxicity evaluation is required to establish potential adverse effects of the valuable medicinal plant.

MATERIALS AND METHODS

Plant material

The *P. curatellifolia* seeds were bought from Mushin market in the Lagos metropolis and were authenticated at the Department of Botany and Microbiology, University of Lagos, Lagos, and voucher specimen deposited at the Department Herbarium. The seeds (with their coats removed) were dried at an ambient temperature between 35 – 45°C in an oven for four days, and powdered to coarse particles. 500 g of the powder was macerated with ethanol (80%) at room temperature for seven days with stirring at intervals.

After filtration, the solvent was removed under reduced pressure in a rotary evaporator at a temperature below 50°C and dried to a constant weight of 22.45 g (4.49 w/w% yield)

Acute toxicity study

The toxicity study was carried out using thirty-five (35) male and female Swiss albino mice weighing 20 – 25 g each. The animals were randomly distributed into one control group and six treated groups, containing five animals per group. They were maintained on animal cubes (Feeds Nigeria Ltd), provided with water *ad libitum* and were allowed to acclimatise to the laboratory conditions for seven days before the experiment. After depriving the animals food overnight, the control group received 0.3 ml of 2% Tween 80 solution orally while each treated group received orally the hydroalcoholic extract prepared by dispersing 8.0 g in 10 ml volume of 2% Tween 80 in the doses as follows: 1.0, 2.5, 5.0, 10.0, 15.0 and 20.0 g/kg. The animals were observed continuously for the first 4 h and then each hour for the next 24 h and at 6 hourly intervals for the following 48 h after administering of the extract, to observe any death or changes in general behaviour and other physiological activities (Shah et al., 1997; Bürger et al., 2005)

Subchronic test

Male and female Wistar rats weighing 160 ± 10 g were used. They were allowed to acclimatise to the laboratory conditions for seven days and were maintained on standard animal feeds and provided with water *ad libitum*. The animals were weighed and divided into five groups of five animals each. After fasting the rats overnight the control group received a dose of 0.5 ml of 2% Tween 80 solution orally once a day for 30 days. The four treated groups respectively received the following doses: 50, 100, 250 and 500 mg/kg body weight of the hydroalcoholic extract (prepared as in the acute toxicity) orally once daily for 30 days (Pieme et al., 2006; Joshi et al., 2007; Mythilypriya et al., 2007). The animals were then weighed every five days, from the start of the treatment, to note any weight variation. At the end of the treatment, they were anaesthetised with warm urethane and chloralose (25%: 1%v/v) at a dose of 5 ml/kg body weight and blood collected via cardiac puncture into two tubes: one containing EDTA for immediate analysis of haematological parameters and the other containing heparin - to separate plasma for biochemical estimations. The collected blood was centrifuged within 5 min of collection at 4000 g for 10 min to obtain plasma, which was analysed for total cholesterol, total triglyceride, and HDL-cholesterol levels by precipitation and modified enzymatic procedures from Sigma Diagnostics (Wasan et al., 2001). LDL-cholesterol levels were calculated using Friedwald equation (Crook, 2006). Plasma was analysed for alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatinine by standard enzymatic assay methods (Sushruta et al., 2006). Plasma glucose and protein contents were determined using enzymatic spectroscopic methods (Hussain and Eshrat, 2002). Haematocrit was estimated using the methods of Ekaidem et al. (2006). Haematocrit tubes were filled by capillary action to the mark with whole blood and the bottom of the tubes sealed with plasticide, and centrifuged for 4 - 5 min using haematocrit centrifuge. The percentage cell volume was read by sliding the tube along a "critocap" chart until the meniscus of the plasma intersects the 100% line. Haemoglobin contents were determined using cyanmethaemoglobin (Drabkin) method (Ekaidem et al., 2006).

Phytochemical evaluation of the crude extracts

Phytochemical screening of the extract for the presence of secon-

Table 1. The acute toxicity of the aqueous ethanol extract of *P. curatellifolia* in mice.

Group	No of mice	Doses of extract (g/kg)	Number of dead mice	%Cumulative of dead mice
1	5	control	0	0.0
2	5	1.0	0	0.0
3	5	2.50	0	0.0
4	5	5.0	1	9.10
5	5	10.0	2	27.30
6	5	15.0	3	54.50
7	5	20.0	5	100.00

Control group each animal received a dose of 0.3 ml 2% Tween 80 solution orally.

dary metabolites was performed using the following reagents and chemicals: alkaloids with Mayer reagent and Dragendorff's reagent (Farnsworth, 1966; Harborne, 1998), flavonoids with the use of Mg and HCl (Silva et al., 1993; Houghton and Raman, 1998); tannins with 1% gelatin and 10% NaCl solutions and saponins with ability to produce suds (Houghton and Raman, 1998).

Statistical analysis

Differences in total blood glucose, total plasma cholesterol and triglyceride, LDL-cholesterol, HDL-cholesterol concentrations, AST, ALT and creatinine levels and body weight for all treated and control rats were determined using the analysis of variance (ANOVA). Significant differences were determined using the student's t-test where differences were considered significant ($p < 0.05$). All data are expressed as mean \pm standard error of the mean.

RESULTS

In the acute toxicity study (Table 1) 100% death was recorded for all the animals that received 20.0 g/kg b.w.t of the extract while 54.5, 27.3 and 9.1% death respectively for animals that received 15.0, 10.0 and 5.0 g/kg b.w.t of the extract. There was no death in the animals that received 2.5.0 g/kg b.w.t. The median acute toxicity (LD_{50}) of the aqueous ethanolic extract of the seeds was determined to be 7.27 g/kg b.w.t.

The effect of the extract on the body weights of the control and treated animals is shown in Table 2. Generally, there was significant increase ($p < 0.05$) in the body weights of all the treated animals compared with the control. A drop in the weight of the control was observed in day 10 and a dramatic increase was witnessed there after till the end of the experiment. The reason for this anomaly can not be easily deduced. The mean percentage increase in the weights of all the treated animals compared to the control was very significant and is shown in Figure 1.

The effects of the extract on the organs were presented in Table 3. Significant changes ($p < 0.01$) in the weights of various organs were observed only in the animals treated with the highest dose of the extract (500 mg/kg bwt), but macroscopic examinations did not show any changes in

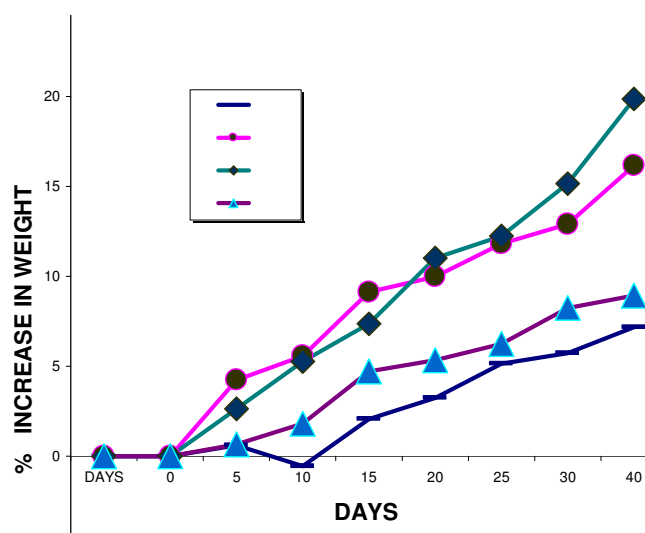


Figure 1. Percentage mean body weight increase of the control and the treated rats for 40 days in subchronic toxicity study. — Control, • 100 mg/kg body weight, ■ 250 mg/kg body weight and ▲ 500 mg/kg body weight.

colour of the organs of the treated animals compared with the control.

Table 4 summarized the results of the effects of the extract on the biochemical parameters. There was significant decrease ($p < 0.05$) in the plasma glucose level especially at the highest dose treated rats compared with control. A significant decrease in the plasma protein levels and also a significant increase ($p < 0.05$) in the plasma creatinine and AST levels were observed only in the animals treated with the highest extract dose (500 mg/kg b.w.t.). A significant decrease in ALT was observed in all treated animals. Also significant decreases ($p < 0.05$) in the plasma total cholesterol (TC), triglyceride (TG) and LDL-cholesterol levels and significant increase ($p < 0.05$) in HDL-cholesterol levels were observed in all the treated animals compared with the control.

The result of the extract effects on the blood components and the electrolytes was presented in Table 5.

Table 2. The effects of the extract on weight changes in the control and treated rats in the subchronic toxicity study.

Dose	Day 1	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30	Day 40
Control	170.01±2.7	171.03±2.18*	169.06±1.80	173.51±1.10	175.60±2.5	178.75±2.7	179.75±2.2	182.2±1.5
100 mg/kg	165.01±2.1	172.20±0.4*	174.30±1.2*	180.06±1.82*	181.51±0.20*	184.50±0.2*	186.30±0.3*	191.7±3.3
250 mg/kg	152.01±0.30	156.20±1.50*	160.02±7.5**	163.20±6.32	168.75±4.97*	170.61±1.2*	175.04±1.5*	182.21±3.5
500 mg/kg	160.20±6.12	161.25±6.92**	163.10±4.8	167.75±4.97	168.75±4.97	170.05±2.25*	173.5±1.2*	174.52±2.5

Mean values of 5 animals ± sem, *p<0.05; ** p<0.01 vs. control group. Control group received 0.5 ml 2% Tween 80 solution.

Table 3. The effects of the extract on kidney, heart, liver and brain in the control and the treated rats in the subchronic toxicity study.

Organ	Control	100 mg/kg	250 mg/kg	500 mg/kg
Heart (g/100 g)	0.31 ± 0.02	0.33 ± 0.05	0.34 ± 0.01	0.36 ± 0.01*
Kidney (g/100 g)	0.65 ± 0.04	0.79 ± 0.03	0.69 ± 0.05	0.82 ± 0.04*
Liver (g/100 g)	3.22 ± 0.02	3.38 ± 0.41	3.35 ± 0.17	3.71 ± 0.21*
Brain (g/100 g)	0.64 ± 0.07	0.64 ± 0.10	0.75 ± 0.08	0.74 ± 0.03*

Mean ± SEM, (n = 5), *p<0.05; ** p<0.01 vs. control group. Control group received 0.5 ml 2% Tween 80 solution.

Table 4. Effect of daily administration of the extract for 40 days on biochemical profiles of control and treated rats in the subchronic toxicity study.

Parameter	Control	100 mg/kg	250 mg/kg	500 mg/kg
Glucose (mg/dl)	90.60 ± 0.12	87.62 ± 0.62	85.20 ± 0.1**	81.06 ± 0.32*
Cholesterol (mg/dl)	37.62 ± 0.60	23.82 ± 0.03*	30.58 ± 0.42	26.04 ± 1.4*
Triglyceride (mg/dl)	24.60± 0.45	12.69 ± 1.50*	18.29 ± 0.02*	24.37± 0.02
HDL (mg/dl)	140.31± 0.002	178.5 ± 0.007*	223.67 ± 0.07*	155.39 ± 0.49*
LDL (mg/dl)	81.44 ± 2.50	30.75 ± 2.55	very low	very low
Protein (g/dl)	3.56 ± 0.32	3.63 ± 0.25	3.26 ± 0.60	1.50 ± 0.20*
Creatinine (mg/dl)	2.51 ± 0.003	2.17 ± 0.001	1.29 ± 0.05**	4.70 ± 0.01*
AST (IU/L)	8.18 ± 1.20	7.69 ± 2.50**	7.14 ± 0.02	76.50 ± 2.30
ALT (IU/L)	52.54 ± 1.80	10.60 ± 2.52**	36.20 ± 2.6	12.00 ± 3.6

Mean ± SEM (n = 5), *p<0.05; ** p<0.01 vs. control group. Control group received 0.5 ml 2% Tween 80 solution.

Table 5. Effect of the extract on haematological parameters of the control and treated rats in the subchronic toxicity study.

Parameter	Control	100 mg/kg	250 mg/kg	500 mg/kg
Haemoglobin (mg/dl)	12.20 ± 0.3	13.60 ± 0.40*	11.80 ± 0.40*	10.79 ± 0.40*
RBC(10 ⁶ /mm ³)	9.07 ± 0.04	9.67 ± 0.06	8.06 ± 0.05	7.93 ± 0.06
WBC (10 ³ /mm ³)	12.30 ± 0.03	5.60 ± 1.65	10.40 ± 0.02	11.8 ± 0.02
PCV %	39.0 ± 2.20	41.05 ± 1.52	36.85 ± 2.50	33.55 ± 0.50**
Calcium (mg/dl)	9.05 ± 0.04	9.05 ± 0.05	9.88 ± 0.04	9.37 ± 0.03
Phosphorus (mg/dl)	7.41 ± 0.03	7.41 ± 0.95	7.94 ± 0.001	8.47 ± 0.04

Mean ± SEM (n = 5), *p<0.05; ** p<0.01 vs. control group. Control group received 0.5 ml 2% Tween 80 solution.

Significant changes ($p<0.01$) were observed in the haemoglobin contents, in the packed cell volume (PCV), red blood cells (RBC), and white blood cells (WBC) contents were observed in the group treated with 100 mg/kg b.w.t and a significant decrease of the parameters occurred in the group treated with 500 mg/kg b.w.t compared with the control. There was no significant change in the calcium level in all the treated animals compared to the control while a significant increase ($p<0.01$) in the level of phosphorus was observed only in the animals treated with the highest dose of the extract.

The results of the phytochemical screening of the plant extract not tabulated revealed the presence of alkaloids as the major active secondary metabolites, while free and bound anthraquinones and polyphenols other than flavonoids were the minor constituents. Saponins were conspicuously absent.

DISCUSSION

Herbal medicines have received a great attention as alternatives to synthetic pharmaceutical products in recent times, leading to the increase in their demand (Mythilypriya et al., 2007). The exclusive use of herbal drugs, prepared and dispensed by unscientifically trained herbalists, for treatment of diseases is still very common in rural Nigerian communities. Experimental screening method is, therefore, important to ascertain the safety and efficacy of herbal products as well as to establish the active components of these herbal remedies (Ogbonnia et al., 2008).

The median acute toxicity value (LD_{50}) of the extract was determined to be 7.27 g/kg body weight. According to Ghosh (1984) and Klassen et al. (1995) the extract can be classified as being slightly toxic, since the LD_{50} was found to be between 5 - 15.0 g/kg. The gram equivalence of the LD_{50} in an average adult man (65 kg) would translate to 472.55 g dose of the extract, which makes it relatively safe for use. The viscera of the dead animals did not show any macroscopic changes compared with the control for possible clues to the cause of death. However, since the animals did not convulse before

dying, it could be postulated that mortality did not result from some action of the extract on the nervous system (Ogwal- Okeng et al., 2003)

The increase in weight was remarkable in the treated animals that received the extract dose of 250 mg/kg b.w.t. This was clearly shown by the mean percentage increase in the weights of the treated animals compared to the control. The observed increase in the weights might be attributed to the appetite stimulation effect of the extract on the animals which was at peak at the dose of 250 mg/kg body weight and declined there after with the increase in dose. Various changes in the organs weights occurred only in the animals that received the highest dose of the extract

The extract had a remarkably decreasing effect on the plasma glucose level, especially at the highest dose in the treated rats indicating the presence of hypoglycemic components in the extract. This observation gives credence to the use of *P. curatellifolia* herbal preparation as a hypoglycemic agent. The decrease in the plasma protein level observed in very high doses could be a sign of impaired renal function (Kachmar and Grant, 1982). Also the elevation in the plasma creatinine concentration indirectly suggests kidney damage, specifically by renal filtration mechanism (Wasan et al., 2001). The decrease in protein level and increase in creatinine level occurred only in the animals that received the highest dose of the extract (500 mg/kg body weight). This is an indication that the extract at this dose and beyond may cause kidney damage.

The liver releases alanine aminotransferase (ALT), with an elevation in the plasma concentration indicating liver damage. The liver and heart release AST and ALT, with elevation in plasma concentration as indicators of liver and heart damage (Wasan et al., 2001; Crook, 2006). There was a significant increase in AST level only in the animals treated with the highest dose (500 mg/kg bwt), while a significant decrease in ALT was observed in all treated groups. It implies that the extract might not have caused any toxic effect on the liver and heart tissues at low and moderate doses but could have some deleterious effects on the heart tissue in high doses (Crook, 2006). The decrease in the plasma total cholesterol (TC)

and triglyceride (TG) levels could be attributed to the presence of hypolipidemic agents in the extract.

The increase in HDL-cholesterol levels and a reduction in LDL-cholesterol levels observed in all the treated animals are indicators that the extract can reduce the cardiovascular risk factors, which contribute to the death of diabetic subjects (Barnett and O'Gara, 2003). The reduction of the cardiovascular risk factors gave further support to the traditional use of the herbal formulation of *P. curatellifolia* as a hypoglycaemic agent.

The observed increase in the haemoglobin levels could be due to the increased absorption of iron. Also the increase in the haemoglobin level coupled with the increase in WBC count emphasized the beneficial effect of the extract to the general well being of the animals. The maximum benefits were derived at 100 mg/kg bwt dose and declined at the highest dose. The calcium levels were not affected in all the treated animals while a significant increase in the level of phosphorus ($p < 0.01$) was observed only in the animals treated with the highest dose of the extract. The increase in the level of phosphorus may be associated with renal failure (Tietz, 1982). Since significant increase in creatinine and phosphorus levels and a decrease in the protein level were observed in the animals that received the highest dose of the extract (500 mg/kg b.w.t.), it implies that the extract at this dose could cause kidney damage and subsequent renal failure (Wasan et al., 2001; Crook, 2006).

Phytochemical screening helps to reveal the chemical nature of the constituents of the plant extract and the one that predominates over others. It is also be used to search for bioactive lead agents that would be used in the partial synthesis of some useful drugs (Yakubu et al., 2005). The presence of alkaloids as the major secondary metabolites might have contributed immensely to the bioactivity of the extract. Also present were polyphenols and anthraquinone glycosides. Polyphenols have been associated with antioxidant activities which might be contributing to the ethnobotanical use of the seeds as an antidiabetic drug.

Conclusion

The high LD₅₀ value (7.27 g/kg) obtained was a clear indication that *P. curatellifolia* herbal preparations could be safe for use. The study showed that the extract had some hypoglycemic activity and high capacity to reduce cardiovascular risk factors. The study also revealed that the extract at low and moderate doses did not provoke toxic effects in the animals' tissues. But higher doses could cause kidney damage, leading to renal failure and toxic effects in the heart during long term treatment. Heart and kidney functions should therefore be monitored on the long term treatment of diseases with high doses of *P. curatellifolia* herbal preparations.

REFERENCES

- Bürger C, Fischer DR, Cordenunzi DA, Batschauer de Borba AP, Filho VC, Soaresdos Santos AR (2005). Acute and subacute toxicity of the hydroalcoholic extract from *Wedelia paludosa* (Acmela brasiliensis) (Asteraceae) in mice. *J. Pharm. Sci.* (www.cspscanada.org) 8(2): 370-373.
- Barnett HA, O'Gara G (2003). *Diabetes and the Heart. Clinical Practice Series.* Churchill Livingstone .Edinburgh UK., pp. 7-30.
- Crook MA (2006). *Clinical Chemistry and Metabolic Medicine.* 7th edn. Hodder Arnold, London, p.426.
- Ekaidem IS, Akpanabiatu MI, Uboh FE, Eka OU (2006). Vitamin B₁₂ supplementation: effects on some biochemical and haematological indices of rats on phenytoin administration. *Biochem.* 18(1): 31- 37.
- Farnsworth RN (1966). Review on Biological and Phytochemical screening of plants. *J. Pharm. Sci.* 55: 225-276.
- Ghosh MN (1984). *Fundamentals of experimental pharmacology.* 2nd edn. Scientific Book Agency, Calcutta, p. 154-157.
- Harborne JB (1998). *Phytochemical Methods: A guide to modern techniques of plant analysis.* 3rd edn. Chapman and Hall, London, p. 235.
- Houghton PJ, Raman A (1998). *Laboratory handbook for fractionation of natural extracts.* Chapman and Hall, London, p. 199.
- Hussain A, Eshrat HM (2002). Hypoglycemic, hypolipidemic and antioxidant properties of combination of Curcumin from *Curcuma longa*, Linn and partially purified product from *Abronia augusta*, Linn. in streptozotocin induced diabetes. *Indian J. Clin. Biochem.* 17(2): 33-43.
- Kachmar JF, Grant GH (1982). *Proteins and Amino Acids.* In: Tietz NW, (Ed.) *Fundamentals of Clinical Chemistry.* 2nd ed, WB. Saunders Company, Philadelphia, USA. pp. 849-944.
- Klassen CD, Amdur MO, Doull J (1995). *Casarett and Doull's Toxicology: The basic science of poison.* 8th edn. Mc Graw Hill, USA, pp. 13-33.
- Joshi CS, Priya ES, Venkataraman S (2007). Acute and subacute studies on the polyherbal antidiabetic formulation Diakyur in experimental animal model. *J. Health Sci.* 53(2): 245-249.
- Mythilypriya R, Shanthi P, Sachdanandam P (2007). Oral acute and subacute toxicity studies with Kalpaamruthaa, a modified indigenous preparation on rats. *J. Health Sci.* 53(4): 351-358
- Ogbonnia S, Adekunle AA, Bosa MK, Enwuru VN (2008). Evaluation of acute and subacute toxicity of *Alstonia congensis* Engler (Apocynaceae) bark and *Xylopiya aethiopica* (Dunal) A. Rich (Annonaceae) fruits mixtures used in the treatment of diabetes. *Afr. J. Biotechnol.* 7(6): 701-705.
- Ogwal-Okeng WJ, Obua C, Anokbonggo WW (2003). Acute toxicity effects of the methanolic extract of *Fagara zanthoxyloides* (Lam.) root-bark. *Afr. Health Sci.* 3(3): 124-126.
- Pieme CA, Penlap VN, Nkegoum B, Taziebou CL, Tekwu EM, Etoa FX, Ngongang J (2006). Evaluation of acute and subacute toxicities of aqueous ethanolic extract of leaves of (*L*) *Roxb(Ceasalpinaceae)*. *Afr. J. Biotechnol.* 5(3): 283- 289.
- Silva LG, Lee IS, Kinghorn DA (1993). Special problems with the extraction of plants. In: *Methods in Biotechnology Natural product isolation.* Cannell JPR (ed) Humana, Press Inc., Totowa, New Jersey, USA., 4: 329-363.
- Shah Ayub MA, Garg SK, Garg KM (1997). Subacute toxicity studies on Pendimethalin in rats. *Indian J. Pharm.* 29: 322-324.
- Sushruta K, Satyanarayana S, Srinivas N, Sekhar RJ (2006). Evaluation of the blood-glucose reducing effects of aqueous extracts of the selected Umbellifereous fruits used in culinary practice. *Trop. J. Pharm. Res.* 5(2): 613- 617.
- Tédong L, Dzeufiet PDD, Dimo T, Asongalem EA, Sokeng SN, Flejou JF, Callard P, Kamtchouing P (2007). Acute and Subchronic toxicity of *Anacardium occidentale* Linn (Anacardiaceae) leaves hexane extract in mice. *Afr. J. Tradit. Altern. Med.* 4(2): 140-147.
- Tietz NW (1982). *Blood Gases and Electrolytes.* In: Tietz NW (Ed.) *Fundamental of Clinical Chemistry,* 2nd edn, W.B Saunders Company ,Philadelphia, USA, pp. 849-944.
- Wasan KM, Najafi S, Wong J, Kwong M (2001). Assessing plasma lipid levels, body weight, and hepatic and renal toxicity following chronic oral administration of a water soluble phytostanol compound FM-

- VP4, to gerbils. J. Pharm. Sci. (www.ualberta.ca/~csps) 4(3): 228-234.
- Yakubu MT, Akanji MA, Oladiji AT (2005). Aphrodisiac potentials of the aqueous extract of *Fadogia agrestis* (Schweinf. Ex Hiern) stem in male albino rats. Asian J. Androl. 7(4): 399-404.