Nigerian Journal of Pharmaceutical and Biomedical Research
Vol. 3, No. 2, August, 2018. All Rights Reserved. ISSN: 2579-1419

Evaluation of Haematological Parameters and Blood Glucose After a 28-day Oral Administration of Standardized Extract of *Laggera aurita* (Linn) in Rats

Julde S. Muhammad and Malami Sani*

Department of Pharmacology and Therapeutics, Bayero University, Kano, Nigeria

***Corresponding Author:** Dr. Sani Malami, Department of Pharmacology and Therapeutics, Bayero University, Kano; malamisani@gmail.com; +2348039701420

Abstract

Lagerra aurita is widely used in traditional medicine to treat various ailments in African countries like Nigeria, Senegal and Ghana. It has been reportedly used in traditional medicine for the treatment of fever, pain, epilepsy, dyspepsia and indigestion. This research is aimed at assessing safety profile of crude extract of *Laggera aurita* in rats. A total of fifty rats were employed and divided into 5 groups of 10 animals each (5 males and 5 females per group). The animals were orally administered the extract at doses of 600, 300, 150 and 75 mg/kg daily for 28 days. Food and water intake were measured daily while blood glucose level and body weight were evaluated weekly. Organ weights, biochemical and haematological indices, were assayed after 28 days. There was no significant change in food intake, water intake, glucose level, body weight and organ weight. Haematological parameters were generally not significantly ($p< 0.05$) affected by the extract, except in a few situations where a significant ($P < 0.05$) reduction in PCV of male rats at 600 mg/kg and 75 mg/kg was observed as well as a significant reduction in WBC of male rats at 300 mg/kg. Haematological parameters of the female rats did not show any significant change with the exception of eosinophils which was significantly lowered in all treatment groups. The crude extract of *Laggera aurita* is relatively safe on blood parameters.

Key words: *Laggera aurita*; Relative safety; Food intake; Haematological, Blood glucose

Introduction

Plant based traditional medicine has been used all over the world for thousands of years (Tarkang *et al*, 2012); medicinal plants and their extracts or derivatives provide limitless opportunities for discovery of new drugs (Parasuraman, 2011). However, the issue of standardization and the knowledge of extent of adverse effects associated with their use has been a major challenge (Koduru *et al.* 2006). Such plants have potentially unexpected effects and toxicity (Staines, 2011). Toxicity testing is important in evaluating the safety profile of drugs and herbs that will eventually be used by mankind for treatment of various ailments (Rajalakshmi *et al,* 2014), it is therefore important to obtain information through these studies in order to improve safety and development of new drugs (Ukwuani *et al.* 2012).

Laggera aurita is an annual plant found mostly in tropical Africa and Southeast Asia; it is widely used in African countries like Nigeria, Senegal and Ghana. It has been reportedly used in traditional medicine for the treatment of fever, pain, epilepsy, dyspepsia and indigestion (Malami *et al.* 2016). Preliminary anti tuberculosis screening suggests some activity in *aurita* species (Egharevba *et al.* 2010). Membrane stabilizing effect of the ethanol crude extract of *L. aurita* has been established (Abdullah *et al*. 2013). The anti-hyperalgesic activities of *Lagerra aurita* extract (Olurishe and Mati 2014) and its mosquito repellent properties (Sing and Mittal, 2015) have been reported; analgesic and anti inflammatory properties of the methanol extract were also reported (Shehu *et al.* 2016). Results from the anticonvulsant studies on the methanol leaf extract of *Laggera aurita* suggest its anti-epileptogenic properties (Malami *et al.* 2016). Its effect on serum biochemical parameters was shown to be relatively safe (Julde *et al.,* 2017). Also, Magaji and Malami (2018) demonstrated its antiplasmodial activity. The present study evaluates the effect of the plant extract on heamatological indices and blood glucose level in Wistar rats.

Materials and methods

Drugs and equipment

Methanol (Sigma-Aldrich), chloroform (Sigma-Aldrich), formalin (10%), distilled water, mouth cannular (for rats), syringes (1ml, 5ml and 10ml), pestle and mortar, spatula, crucible, measuring cylinders, soxhlet apparatus, oven, dessicator, electronic weight scales, Accu check glucometere and test strips,), cotton wool, blades, EDTA bottles, plastic containers.

Plant material and preparation of extract

The leaves of *Laggera aurita* were collected from Dajin Kudingi Zaria LGA, Kaduna state and authenticated by Mallam Namadi Sanusi of the Herbarium Unit, Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. The sample was compared with a previously deposited specimen and a voucher number (No. 2002) was collected. The leaves were washed and air dried until a constant weight was obtained. They were then crushed with the aid of a pestle and mortar to a powdered state after which 500 g of the powdered leaves was weighed and extracted with 2 Litres of methanol via soxhlet extraction. The extract was evaporated to dryness with a thermostat oven (DHG/910/1SA) at 40℃. The dried extract was weighed and stored in an air-tight container.

Animals

Wistar rats of both sexes were acquired from Animal House, Department of Pharmacology and Therapeutics, Ahmadu Bello University (ABU) Zaria. The weights of the rats were between 110-130g. The rats were kept under normal laboratory conditions with access to food and water. The animals were randomly selected, marked to allow individual identification and kept in their cages for 5 days prior to the experiment in order to allow for acclimatization to the laboratory conditions. Animals were used in compliance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (1985).

Subchronic toxicity studies

Fifty Wistar rats were divided into five groups of ten animals each (five males and five females). Group I, II, III, and IV were orally administered the freshly prepared extract daily at doses of 600, 300, 150 and 75 mg/kg respectively while group 5 which served as the control was administered distilled water. This was done continuously at the same time daily for 28 days. Food and water intake were recorded daily; weight of animals and blood glucose (with Accu check glucometere) was checked and recorded weekly. After 28 days the animals were starved overnight but were allowed access to water. The animals were then weighed and anaesthetised with the aid of chloroform after which they were sacrificed by decapitation and blood samples were collected from the jugular vein of each rat in EDTA bottles for haematological analysis.

Haematological analysis

Blood samples collected in the EDTA bottles were used for this analysis. For the haemotocrit, the blood sample was transferred into a capillary tube which was placed in the micro haematocrit centrifuge (Hawskley model no, 1657), and spun for five minutes at preset speed of 1000 rpm. The tube was placed in a micro haematocrit reader and the reading was recorded.

Haemoglobin was determined using the haemoglobin cyanide method (HCN). Whole blood was diluted to the ratio of 1 in 201 in drabkins solution. The red blood cells were haemolysed and the Haemoglobin oxidized by the ferricyanide to methaemoglobin which is then converted by the cyanide to stable haemoglobin cyanide. Absorbance of the HCN solution was read at 540 nm using a calorimeter (optima, Japan model no AC 116). The absorbance obtained was compared to that of a standard HCN solution.

For the total white blood, red blood and platelets count the blood samples were diluted with the appropriate diluting fluids; 1 in 20 for WBC, 1 in 100 for RBC and 1 in 20 for platelets. The diluted blood sample was then placed on mounted counting chamber (Hawskley model 0278) and left for a few minutes to allow the corpuscle to settle to the bottom. The WBC and platelets were counted from the large chamber while the RBC was counted from the small squares.

Differential white blood was determined by placing a drop of blood sample on one end of a glass slide; a thin blood film was made with the aid of a spreader. The blood film was then stained with Leishman stain and allowed to dry. The slide was then mounted on the microscope and examined via the oil immersion objective (x100). The WBC was counted and the number of neutrophils, basophils, lymphocytes, monocytes and eosinophils were recorded.

Statistical Analysis

Data were presented as Mean ± standard error of mean. The results were analyzed using one way ANOVA and Dunett's post hoc test with significance at $P < 0.05$.

Results

General animal behaviour and mortality

Daily administration of the methanol leaf extract of *Laggera aurita* (MLLA) for 28 days did not produce observable signs of toxicity as well as mortality. Similarly, there was no significant change in food and water consumption, as well as organ weights.

Haematological analysis

Haematological analysis and blood glucose tests carried out revealed no changes in the blood glucose level. Likewise most of the haematological parameters were not affected except in a few situations where a significant ($P < 0.05$) reduction in PCV of male rats at 600 mg/kg and 75 mg/kg was observed as well as a significant reduction in WBC of male rats at 300 mg/kg. The same can be said for the female rats as the haematological parameters did not show any significant change with the exception of esinophils which was significantly lowered in all treatment groups as shown in the table.

Table 1: Effects of Sub-chronic Oral Administration of Methanol Leaf Extract of *Laggerra aurita* on Weekly Food Consumption (g/week) of Wistar Rats

Treatment (mg/kg)	Food (g/week) W1	W2	W3	W4
D/water	222.95±4.26	244.66±5.58	173.15±6.63	218.47±3.31
75	208.18±8.63	248.53±3.83	177.97±3.32	217.83±3.31
150	220.55±7.11	251.44±6.00	178.94±6.85	213.93±5.37
300	217.88±11.09	267.59±8.94	210.35±4.15	206.12±3.07
600	214.56±12.01	255.02±7.33	215.89±6.17	206.22±3.72

Data was analyzed using one way ANOVA and Dunnett's post hoc test, n = 10
Dunnett t-tests treat one group as a control, and compare all other groups against it.

Table 2: Effects of Subchronic Oral Administration of Methanol Leaf Extract of *Laggera aurita* on Weekly Water (ml/week) Consumption of Wistar Rats

Treatment (mg/kg)	Water (ml/week)			
	W1	W2	W3	W4
D/water	144.60±2.46	153.8±1.92	166.8±5.33	143.2±4.12
75	144.60±3.37	142.40±2.81	138.8±1.74	144.70±2.02
150	142.00±3.32	142.20±4.03	144.6±1.36	145.95±2.37
300	149.00±4.37	135.70±4.30	148.40±2.65	138.83±3.45
600	146.50±3.56	136.80±3.33	148.40±3.74	137.73±2.63

Data was analyzed using one way ANOVA and Dunnett's post hoc test n=10
Dunnett t-tests treat one group as a control, and compare all other groups against it.

Table 3: Effects of Subchronic Oral Administration of Methanol Leaf Extract of *Laggera aurita* on Body Weight of Wistar Rats

Treatment (mg/kg)	Weight (g)				
	W0	W1	W2	W3	W4
D/water	110.20±6.96	119.40±6.41	122.80±7.44	133.20±8.85	148.50±6.30
75	119.20±4.06	128.20±3.08	133.40±3.15	135.90±3.32	142.10±4.16
150	117.20±4.89	129.90±5.41	137.50±4.79	143.00±5.19	150.80±5.52
300	119.10±3.43	130.20±3.48	133.40±3.83	138.90±4.08	141.80±4.55
600	118.60±2.35	125.70±1.98	130.90±2.33	136.90±2.85	139.90±2.73

Data was analyzed using one way ANOVA and Dunnett's post hoc test n = 10
Dunnett t-tests treat one group as a control, and compare all other groups against it.

Table 4: Effects of Subchronic Oral administration of Methanol Leaf Extract of *Laggera aurita* on Organ Weight of Wistar Rats

Treatment	Organ			
mg/kg	Kidney	Liver	Heart	Brain
Male n= 5				
D/water	0.99±0.11	4.95±0.09	0.83±0.09	1.73±0.01
75	1.08±0.10	4.95±0.09	0.88±0.08	1.73±0.01
150	1.05±0.09	5.04±0.09	0.83±0.09	1.76±0.02
300	1.11±0.11	5.04±0.09	0.93±0.03	1.76±0.04
600	1.08±0.10	5.13±0.00	0.88±0.08	1.77±0.04
Female n= 5				
D/water	1.01±0.01	4.10±0.06	0.89±0.02	0.99±0.01
75	1.01±0.01	4.13±0.07	0.91±0.01	1.01±0.01
150	1.01±0.01	4.13±0.03	0.88±0.02	0.99±0.01
300	1.00±0.00	4.03±0.03	0.89±0.02	1.02±0.01
600	1.01±0.01	4.10±0.06	0.89±0.02	1.00±0.02

Data was analyzed using one way ANOVA and Dunnett's post hoc test n = 5
Dunnett t-tests treat one group as a control, and compare all other groups against it.

Table 5: Effects of Sub-chronic Oral Administration of Methanol Leaf Extract of *Laggera aurita* on Blood Glucose of Wistar Rats

Treatment (mg/kg)	Blood glucose (mg/dl)				
	W0	W1	W2	W3	W4
D/water	117.80±7.94	106.74±4.58	111.40±2.18	115.70±5.48	107.10±5.00
75	107.00±3.56	104.20±1.90	113.90±1.57	108.30±2.28	111.60±2.37
150	107.90±2.39	102.10±3.14	110.20±2.32	107.20±1.55	109.90±1.52
300	107.60±2.60	117.70±5.18	108.80±2.50	103.70±2.39	107.50±1.80
600	109.70±4.22	106.80±2.93	114.50±1.59	104.00±1.13	114.70±1.81

Data was analysed using one way ANOVA and Dunnett's post hoc test n=10
Dunnett t-tests treat one group as a control, and compare all other groups against it.

Table 6: Effects of Sub-chronic Oral Administration of Methanol Leaf Extract of *Laggera aurita* on Haematological Parameters of Male Wistar Rats

Parameter	Group I 600 mg/kg	Group II 300 mg/kg	Group III 150 mg/kg	Group IV 75 mg/kg	Group V D/water
PCV (%)	33.60±2.18*	34.60±2.25	37.00±1.30	33.20±1.39*	40.80±1.24
HB (g/dl)	11.34±0.60	11.58±0.84	12.42±0.52	11.04±0.50	13.26±0.46
WBC (x10'9)	4.36±0.22	2.66±0.22*	4.06±0.23	3.98±0.24	4.12±0.52
RBC (x10'6)	5.78±0.34	5.78±0.07	6.20±0.14	5.94±0.08	6.04±0.08
PTC (x10'5)	7.06±0.14	7.28±0.15	7.14±0.05	7.10±0.08	7.04±0.10
Neutrophils	15.40±1.44	16.80±1.77	16.80±1.07	16.80±1.43	15.80±1.66
Lymphocytes	80.00±1.52	80.40±1.94	79.40±1.08	80.80±1.39	79.80±2.24
Mesophils	2.40±0.75	1.60±0.40	2.00±0.32	1.20±0.20	2.40±0.24
Esinophils	2.00±0.32	1.20±0.58	1.40±0.24	1.00±0.32	1.80±0.20
Basophils	0.20±0.20	0.00±0.00	0.40±0.24	0.20±0.20	0.40±0.09

Data was analysed using one way ANOVA and Dunnett's post hoc test
* The mean difference is significant at $P < 0.05$ level. n=5
Dunnett t-tests treat one group as a control, and compare all other groups against it.

Table 7
Effects of Subchronic Oral Administration of Methanol Leaf Extract of *Laggera aurita* on Haematological Parameters of Female Wistar Rats

Parameter	Group I 600mg/kg	Group II 300mg/kg	Group III 150mg/kg	Group IV 75mg/kg	Group V D/water
PCV (%)	32.80±1.88	29.00±3.38	35.00±1.52	36.00±4.51	39.00±0.71
HB (g/dl)	10.46±0.67	9.78±1.27	11.50±0.50	11.90±1.38	13.07±0.19
WBC x10'9	4.08±0.38	2.84±0.29	3.26±0.17	3.12±0.19	3.66±0.16
RBC (x10'6)	6.08±0.13	5.62±0.22	6.04±0.05	6.02±0.08	5.96±0.02
PTC (x10'5)	7.18±0.09	7.32±0.13	6.90±0.03	7.04±0.05	7.04±0.05
Neutrophils	14.00±0.45	16.00±1.41	13.60±0.51	14.40±0.75	13.00±0.71
Lymphocytes	82.40±1.03	80.20±1.28	83.00±0.63	82.40±1.12	83.00±1.00
Mesophils	1.80±0.80	2.20±0.37	2.00±0.32	2.00±0.45	2.20±0.37
Esinophils	1.60±0.24*	1.40±0.24*	1.60±0.24*	1.20±0.20*	2.40±0.24
Basophils	0.00±0.00	0.20±0.20	0.00±0.00	0.00±0.00	0.40±0.24

Data was analysed using one way ANOVA and Dunnett's post hoc test
*. The mean difference is significant at $P < 0.05$ level. n=5
Dunnett t-tests treat one group as a control, and compare all other groups against it.

Discussion

Methanol leaf extract of *Laggera aurita* did not produce mortality throughout the 28 day period of the study and no abnormal behaviour and physical changes observed. The extract did not produce significant change in the food and water consumption in treatment groups compared to the control, no significant changes were observed in the weights of the animals as well as organ weight; this indicates that the extract had no toxic effect on these indices. Blood glucose level monitored weekly was neither elevated nor reduced to a significant level.

Generally, the haematological indices evaluated were not affected by the extract except for male PCV (packed cell volume), male WBC (white blood cells) and female Eosinophils. Haematological parameters are those factors in the blood whose levels are usually determined in order to assess the degree of well being of an animal. (Ajayi and Raji, 2012). Thus, haematological parameters are good indicators of the physiological status of animals (Khan and Zafar, 2005). PCV which is also known as haematocrit (Ht or Hct) or erythrocyte volume fraction (EVF), is the percentage (%) of red blood cells in blood (Purves *et al.*, 2003). It measures the percentage volume of red blood cells in the blood; anaemic condition is associated with low production of red blood cells (Guenter and Lawrence, 2005). Also, Packed Cell Volume is involved in the transport of oxygen and absorbed nutrients. Increased Packed Cell Volume shows a better transportation and thus results in an increased primary and secondary polycythemia (Isaac *et al.*, 2013).

The major functions of the white blood cell and its differentials are to fight infections, defend the body by phagocytocis against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune response. Thus, animals with low white blood cells are exposed to high risk of disease infection, while those with high counts are capable of generating antibodies in the process of phagocytocis and have high degree of resistance to diseases (Soetan et al., 2013) and enhance adaptability to local environmental and disease prevalent conditions (NseAbasi et al., 2014). Eosinophils are white blood cells that are located in the cells with relatively low numbers in circulation (Navabi and Upton, 2016). Although low level of eosinophils is not associated with significant toxic effect but a study suggested that low levels of eosinophils and lymphocytes were seen among patients with heart failure, thus could probably be associated with onset of cardiovascular diseases (Shah *et al.* 2016).

Blood glucose determination is a basic tool for diagnosing and monitoring diabetes mellitus (LeRoith and Smith 2005); its changes in the blood are dependent on the glucose consumption in the peripheral tissues (Togashi *et al.,* 2016). It is widely recognized that higher peak postprandial glucose levels are associated with type 2 diabetes, obesity, cardiovascular diseases, liver steatosis, and cancer (Marques et al., 2016). The extract did not significantly change the weekly blood glucose levels, thus, devoid of potential risk to produce these untoward effects.

Subchronic toxicity studies on methanol leaf extract of *Laggera aurita* suggested that it is relatively safe.

Acknowledgement: The authors appreciate the contribution of Department of Pharmacology and Therapeutics, Bayero University, Kano, for providing some reagents and equipment used in this study.

References

Abdullah MA, Lutfi MP, Baket AO and Mohamed AH (2013). Evaluation of Anti-Inflammatory Effects of *Blumea aurita*. *Global Journal of Medical Research*. 13(4), 23-29.

Ajayi AF and Raji, Y. (2012). Haematological and serum biochemical indices of prepubertal male rabbits fed with graded level of bloodwild sunflower forage meal mixture. *African Journal of Biotechnology*, 11(35): 8730-8734.

Egharevba HO, Peters O, Okhale ES, Ibrahim I, Kunle OF, Okwute KS, and Okogun JI (2010). Preliminary Anti-tuberculosis Screening of Two Nigerian Species (*Laggera petrodonta* and *Laggera aurita*). Journal of Medicinal Research. 4(12), 1235-1237.

Guenter W, and Lawrence TG (2005). Anaemia of Chronic Disease, the New England Journal Of Medicine. 352, 1011-1023.

Isaac LJ, Abah G, Akpan B, and Ekaette IU (2013). Haematological properties of different breeds and sexes of rabbits (p.24-27). Proceedings of the 18th Annual Conference of Animal Science Association of Nigeria.

Julde S, Kyari H and Malami S (2017). Assessment of Serum Parameters After28 day Oral Daily Administration of Methanol Leaf Extract of Laggera aurita (Linn) in Wistar Rats. *Tropical Journal of Natural Product Research*, 1(6): 251-254.

Khan TA and Zafar F (2005). Haematological Study in Response to Varying Doses of Estrogen in Broiler Chicken. International Journal of Poultry Science, 4(10), 748-751.

Koduru S, Grierson DS and Afolayan AJ (2006). Antimicrobial Activity of *Solanium aculaeastrum*. Pharmaceutical Biology. 44(4), 283-286.

LeRoith D and Smith DO (2005). Monitoring glycemic control: The cornerstone of diabetes care. *Clinical Therapeutics*, 27:1489–1499.

Magaji SY and Malami S (2018). Antiplasmodial Properties of Methanol Leaf Extract of Laggera aurita Linn in Experimental Animals. *Nigerian Journal of Pharmaceutical and Applied Science Research*, 7(2): 46-53.

Malami S, Kyari H, Danjuma NM, Ya'u J and Hussaini IM (2016). Anticonvulsant Properties of Methanol Leaf Extract of *Laggera aurita Linn*. F (Astraceae). Journal of Ethnopharmacology. 191, 301-306.

Marques ACR, Schiavon FPM, Travassos PB, Eik VF, Godoy G, Schamber CR and Bazotte RB (2016). Evaluation of the impact of orally administered carbohydrates on postprandial blood glucose levels in different pre-clinical models. *Brazilian Journal of Pharmaceutical Sciences*, 52 (4): 761-769.

Navabi B and Upton JE (2016). Primary Immunodeficiency Associated with Esinophilia. Allergy, Asthma and Clinical Immunology, 12:27.

NseAbasi NE, Mary EW, Uduak A and Edem EAO (2014). Haematological Parameters and Factors Affecting Their Values. *Agricultural Science*, 2(1): 37-47.

Olurishe TO and Mati FG (2014). Anti Hyperalgesic Potentials of *Laggera aurita* in Swiss Albino Mice. Pakistan Journal of Pharmaceutical Sciences. 2(1), 169-172.

Parasuraman S (2011). Toxicological Screening. Journal of Pharmacology and Pharmacotherapeutics. 2(2),74-79.

Purves, W. K., Sadava, D., Orians, G. H., & Heller, H. C. (2003). Life: The science of Biology (7th ed., p.954). Sinauer Associates and W. H. Freeman.

Rajalakshmi A, Jayachitra A, Gopal P and Krithiga N (2014). Toxicity Analysis Of Different Medicinal Plant Extracts In Swiss Albino Mice. Pharmacology and Toxicology Research. 1(2): 1-6.

Shah AD, Denaxas S, Ncholas O, Hingorani AD, and Hemingway H (2016). Low Esinophil and Low Lymphocyte Counts and the Incidence of 12 Cardiovascular Disease: A Caliber Cohort Study, Open Heart 3(2), 477.

Shehu A, Temidayo O, Zezi AU and Ahmad A (2016). Acute Toxicological, Analgesic and Anti inflammatory Effects of Methanol Extract of *Laggera aurita Linn F* (compositae) in Mice. African Journal of Pharmacology and Therapeutics. 5(2), 65-73.

Singh SP and Mittal PK (2015). Mosquito Repellent and Oviposition Deterrent Activities of *Laggera aurita* Plant Extract against Malaria Vector *Anopheles stephensi*. Journal of Enthomology and Applied Science. 1, 18-22.

Soetan KO, Akinrinde AS and Ajibade TO (2013). Preliminary studies on the haematological parameters of cockerels fed raw and processed guinea corn (Sorghum bicolor) (p. 49-52). Proceedings of 38th Annual Conference of Nigerian Society for Animal Production.

Staines SS (2011). Herbal Medicines: Adverse Effect of Drug - Herb Interaction. Journal of Malta College of Pharmacy. 17, 38-42.

Tarkang PA, Agbor GA, Armelle TD, Yamthe TLR, David K and Mengue-Ngadena Y (2012). Acute And Subchronic Toxicity Studies Of The Aqueous And Ethanol Leaf Extract Of *Citrus Sinensis* (Linnaeus) Osbeck (Prosp.) in Wistar Rats. Der Pharmacia Lettre. 4(5): 1619-1629.

Togashi Y, Shirakawa J, Okuyama T, Yamazaki S, Kyohara M, Miyazawa A, Suzuki T, Hamada M and Terauchi Y (2016). Evaluation of the appropriateness of using glucometers for measuring the blood glucose levels in mice. *Scientific Reports*, 6: 1-9.

Ukwuani AN, Abubakar MG, Hassan SW and Agaie BM (2012). Toxicological Studies of Hydromethalonic Leaves Extract of *Grewia crenata*. International Journal of Pharmaceutical Sciences and Drug Research. 4, 245-2012.