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Toxicological Studies of Hydromethanolic Leaves Extract of *Grewia crenata*

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ABSTRACT
The acute and sub-chronic oral toxicity studies of hydro-methanolic extracts of *Grewia crenata* leaves were evaluated in rats. The extract at a single dose of 5000 mg/kg did not produce treatment related signs of toxicity or mortality in any of the animals tested during 14 days observation period. Therefore, LD_{50} of this plant was estimated to be more than 5000 mg/kg. Phytochemical screening of the leaves extract revealed the plant to contain saponins, steroids, flavonoids, anthraquinones and glycosides. In the repeated dose 28days oral toxicity study, administration of 900, 1800, 2700 and 3600 mg/kg of body weight *G. crenata* leaves extracts revealed no significantly difference ($p<0.05$) in the hematological parameter except for reduced platelet and increased differential blood count shown in some of the treated groups. Analysis of serum liver enzymes, uric acid, total protein, albumin, cholesterol, electrolytes and creatinine revealed no significant ($P<0.05$) changes in the extract treated groups compared to control group. However, significant differences ($P<0.05$) were seen in glucose, urea and bilirubin of the treated groups when compared to the control group. These results suggest that the sub-chronic administration of hydro-methanolic leaves extracts of *Grewia crenata* has no marked acute and sub-chronic toxic effect in rats.

Keywords: Toxicity, *Grewia crenata*, leaves extracts, hydromethanolic and medicinal plant.

INTRODUCTION

For centuries, medicinal plants are the basis for the treatment of various diseases. [1] Nearly 80% of people living in developing countries still depend on plant-based traditional medicine for their primary health care and almost three-fourths of the herbal drugs used worldwide are derived from medicinal plants. [2] However, the quality control of herbal medicine remains a challenge owing to the fact that there is a high variability in the active constituents involved. [3] Hence, World Health Organization (WHO) has approved fingerprint technique or standardized extract for quality assurance of herbal medicines. [4]

In recent years, phytochemicals are increasingly purported to exert potent beneficial actions to support health and may play a role in reducing synthetic drug use for the treatment of metabolic complications. To this effect, research has focused on the identification and isolation of compounds from natural products. [5] Many plants synthesize compounds that are useful for the maintenance of health in humans and animals, like aromatic substances, most of which are phenols or their oxygen-substituted derivatives such as tannins. [6] Many of these compounds extracted from natural products could be useful lead compound in the production of drugs. [7]

Herbal medicines are popular and extensively used in the developing world. In Nigeria, they offer a more wide available and affordable alternative to pharmaceutical drugs and natural food supplements. There are over a hundred chemical substances that have been derived from plants for drugs and medicines. However, many plants have also been reported to be toxic to both human and animals. [8-11] It should therefore, be emphasized that the traditional use of any plant for medicinal purposes, by no means, warrants the safety of such plant. Plants in folk medicine should therefore, be evaluated for safety or toxicity and necessary recommendations made on their use. The data of the acute and sub-chronic toxicity studies on medicinal plants or preparations derived from them should be obtained in order to increase the confidence in its safety to humans, particularly for use in the development of pharmaceuticals.

Grewia crenata Linn is a large flowering plant and is today placed by most authors in the mallow family Malvaceae. There is a dearth of information on the biological activity of

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this plant. In northwest Nigeria, *G. crenata* leaves popularly known as 'kamomowa' is used locally in the treatment of fractured bones, wound healing and inflammatory conditions. The present study was therefore carried out to evaluate the phytochemical and toxicological profile of this plant extract.

MATERIALS AND METHODS

Identification of the plants

Preliminary identification of *G. crenata* was done in the field by a botanist. The plant parts (leaves, stems-bark and roots) were collected from the wilds of various local government areas of Sokoto state, Nigeria. Herbarium specimens were prepared and photographs taken to aid in the confirmation of the identity of the plants. Voucher specimens were deposited in the Herbarium, Botany unit, Biological Science Department, Usmanu Danfodiyo University, Sokoto-Nigeria, where identity of the plants was confirmed by comparison with available voucher specimens.

Extraction of plant materials

The plant materials were open air dried under the shade and chopped into smaller pieces. The dried plant materials were pulverized into moderately coarse powder. The powdered plant material (100 g) was macerated in 50% methanol in an air tight aspirator bottle for 72 hours. This was then filtered with the aid of sterile sieving cloth and evaporated using a drying cabinet at 45^{o}C. The dried extract collected was weighed, labeled and stored in an air tight bottle container.

Phytochemical screening

Chemical tests were carried out on the hydromethanolic extracts using standard procedures to identify the constituents by characteristic colour changes. [12-14] All chemicals used are of analytical grade.

Animals

Sprague - Dawley rats of both sexes (170-200g) were employed for this study. The animals were obtained from the National Veterinary Research Institute (NVRI), Vom, Nigeria. They were kept in well-ventilated environment and had free access to rodent pellets (Vital Feeds Ltd, Nigeria) and water *ad libitum*. The animals were allowed to acclimatize for 2 weeks and were fasted over night with free access to water prior to experiments.

Acute oral toxicity test (LD_{50})

After acclimatization period, the acute oral toxicity study as described by Dixon [15] was performed as per the OECD-423 guidelines (acute toxicity class method). Five (5) rats of either sex selected by random sampling technique were used for this study. The animals were fasted over night providing only water, after which the plant extract was administered orally at a dose level of 5000 mg/kg body weight to each rat at 48 hours interval respectively and subsequent observed for 14 days. The behavioral changes (abdominal constriction, hyperactivity, sedation, grooming), mortality and body weight were observed for 14 days.

Sub-chronic toxicity test

Rats were divided into five (5) groups of five (5) rats each for sub-chronic toxicity study in which daily oral administration of different concentration (based on the calculated LD_{50}) of the plant extract continues for 28 days. Group 1 received distilled water and serve as the control while Groups 2 to 5 received graded doses (900, 1800, 2700 and 3600 mg/kg body weight) of leaf extract respectively. The weights of the rats were taken weekly. The rats were fasted overnight on the 28th and on the 29th day, weights were taken and blood samples collected via cardiac puncture and centrifuged to obtain sera for further analysis.

Markers of toxicity

The following parameters were analyze from the blood samples collected at the end of the sub-chronic toxicity studies; Aspartate aminotransferase (AST) [16], Alanine aminotransferase (ALT) [16], Alkaline phosphatase (ALP) [17], Bilirubin [18], Albumin [19], Total protein [20], Urea [21], Uric acid [22], Electrolytes [23] and Creatinine. [24]

Haematological studies

Hematological ananlysis was performed using an automatic hematological analyzer (cell Dyn 3500, Abbott). The blood parameters measured were: hematocrit (PCV), hemoglobin (Hb), red blood cell (RBC), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell (WBC) lymphocytes, neutrophils, monocytes, basophils and platelets.

Statistical analyses

Statistical analysis was carried out using GraphPad Instat. Differences among the tested extract were analyzed using one-way ANOVA. Values were expressed as mean ± SEM and differences between groups were considered to be significant if $p<0.05$.

RESULTS AND DISCUSSION

Plants are recognized for their ability to produce a wealth of secondary metabolites and mankind has used many species for centuries to treat a variety of diseases. [25] Secondary metabolites are biosynthesized in plants for different purposes including growth regulation, inter and intra-specific interactions and defense against predators and infections. Many of these natural products have been shown to present interesting biological and pharmacological activities and are used as chemotherapeutic agents or serve as the starting point in the development of modern medicines. [26-27] In this study, extraction process yielded 34.6% of hydromethanolic leaves extract of *G. crenata* and the phytochemical tests revealed the presence of flavonoids, steroids, tannins, anthraquinones, cardiac glycosides and saponins mainly. The pharmacological activities of medicinal plants are usually due to their secondary metabolites. [28] There are reports on the role of flavonoids as a powerful antioxidant [29] and tannins as antinociceptive agent. [30]

In screening drugs, determination of LD_{50} is usually an initial step in the assessment and evaluation of the toxic characteristics of a substance. It is an initial assessment of toxic manifestations and is one of the initial screening experiments performed with all compounds. Data from the acute toxicity study may; (a) Serve as the basis for classification and labeling; (b) Provide initial information on the mode of toxic action of a substance; (c) Help arrive at a dose of a new compound; (d) Help in dose determination in animal studies; (e) Help determine LD_{50} values that provide many indices of potential types of drug activity. Hydromethanolic extract at a dose of 5000 mg/kg had no adverse effect on the behavioral responses of the tested rats up to 14 days of observation. There was no mortality observed at the tested dose nor affected the weight loss in the rats. The LD_{50} of this plant was therefore estimated to be more than 5000 mg/kg.

In the repeated dose 28 days oral toxicity study, administration of 900, 1800, 2700 and 3600 mg/kg of body

weight *G. crenata* leaves extracts revealed a decrease in body weight of extract treated animals in week 1 and week 4 which was not significantly (P<0.05) different when compared with control (Table 1).

According to Moore and Dalley [31], an increase in organ-body weight ratio is an indication of inflammation while a reduction in the same parameter can be adduced to cellular constriction. In this study, no significant differences (P<0.05) were found in the organ weight of the extract treated groups compared to control (Table 2).

The various biochemical parameters investigated in this study are useful indices of evaluating toxicity of plant extract in animals. [32-34] Assessment of hematological parameters can be used to determine the extent of deleterious effect of extracts on the blood of an animal. It can also be used to explain blood relating functions of a plant extract or its products. [33] Such analysis is relevant to risk evaluation as changes in the haematological system have higher predictive value for human toxicity, when the data are translated from animal studies. [35] Hemogram was estimated for all the treated as well as control groups and results have shown no unusual effect on blood cell count, hemoglobin, PCV, MCV, MCH and MCHC in treated animals as compared to control group (Table 3). MCHC, MCH and MCV relates to individual red blood cells while Hb, RBC and PCV are associated with the total population of red blood cells. Therefore, the absence of significant effect of the extract on RBC, Hb, PCV, MCH, MCHC and MCV could mean that neither the incorporation of hemoglobin into red blood cells or the morphology and osmotic fragility of the red blood cells were altered. [36] However, significant differences (p>0.05) were seen in platelet, lymphocytes, monocytes and nuetrophils in the treated groups when compared with the control group.

Table 1: Body weight of rats receiving hydromethanolic leaves extract of *G. crenata* for 28days

Week	control	900mg/kg	1800mg/kg	2700mg/kg	3600mg/kg
0	222.10±16.83	219.36±11.33	195.93±5.99	220.85±22.15	194.76±12.95
1	198.68±19.29	206.74±11.08	198.46±4.53	215.82±14.24	185.84±9.23
2	210.78±15.27	186.14±7.35	189.54±4.21	192.46±13.42	196.02±7.88
3	212.58±15.03	191.28±7.72	192.64±4.23	209.54±20.46	198.30±8.38
4	198.42±13.92	179.82±4.70	179.72±3.90	185.74±15.18	178.109.14

Values are mean ± SEM (n = 5). *Significant different (P < 0.05)

Table 2: Organ weight of rats receiving hydromethanolic extract of *G. crenata* leaves

Organ	Liver	Right kidney	Left kidney
Control	6.66±0.36	0.67±0.22	0.66±0.02
900mg/kg	5.83±0.25	0.70±0.04	0.67±0.02
1800mg/kg	6.22±0.36	0.68±0.03	0.66±0.03
2700mg/kg	6.82±0.48	0.76±0.06	0.77±0.05
3600mg/kg	5.62±0.23	0.66±0.05	0.67±0.04

Values are mean ± SEM (n = 5). *Significant different (P < 0.05)

Table 3: Effect of hydromethanolic extract of *G. crenata* leaves on hematological parameters

PARAMETERS	CONTROL	Hydromethanolic extract of *G. crenata* leaves(mg/kg)			
		900	1800	2700	3600
Hematocrit(%)	51.15 ± 1.66	54.03 ± 2.40	55.78 ± 2.30	53.10 ± 3.03	50.03 ± 2.41
RBC(x 10^6cells/mm^3)	7.82 ± 0.47	8.42 ± 0.32	8.55 ± 0.46	7.93 ± 0.63	8.10 ± 0.38
WBC(x 10^6cells/mm^3)	16.85 ± 4.08	9.30 ± 1.66	12.03 ± 1.01	13.83 ± 2.27	20.73 ± 2.89
Hemoglobin(g/dL)	13.45 ± 0.52	14.13 ± 0.33	14.83 ± 0.49	14.60 ± 0.66	13.75 ± 0.51
MCV(µm^3/redcell)	65.88 ± 2.74	62.73 ± 1.91	65.35 ± 0.93	67.33 ± 3.27	61.78 ± 0.57
MCH(pg/redcell)	17.33 ± 0.70	16.83 ± 0.39	17.40 ± 0.42	18.50 ± 0.79	17.02 ± 0.44
MCHC(g/dL RBC)	26.28 ± 0.28	26.83 ± 0.17	26.60 ± 0.29	27.53 ± 0.47	27.50 ± 0.51
Platelet(x10^3cells/mm^3)	749.00 ± 25.62	421.00 ± 49.49**	481.50 ± 28.70**	565.00 ± 59.03*	472.75 ± 42.31**
Lymphocytes (%)	83.08 ± 2.18	81.0 ± 6.36	75.73 ± 1.92	64.67 ± 3.04*	79.68 ± 2.74
Monocytes(%)	4.45 ± 0.50	5.07 ± 1.05	6.28 ± 0.80	9.00 ± 1.41*	6.15 ± 1.74
Nuetrophils(%)	12.48 ±1.72	13.93 ± 5.42	18.00 ± 1.41	26.33 ± 3.13*	14.18 ± 1.88

Values are mean ± SEM (n = 5). *Significant different (P < 0.05) and **Significant different (P < 0.01) compared with control

Table 4: Effect of hydromethanolic extract of *G. crenata* leaves on biochemical parameters

PARAMETERS	CONTROL	Hydromethanolic extract of *Grewia crenata* leaves (mg/kg)			
		900	1800	2700	3600
Total bilirubin (µmol/l)	1.16 ± 0.10	1.17 ± 0.33	0.35 ± 0.03*	0.35 ± 0.02*	0.92 ± 0.25
Aspartate Aminotransferase (U/L)	108.75 ± 3.95	114.67 ± 8.19	106.33±3.84	123.00 ± 8.15	94.00 ± 2.89
Alanine Aminotransferases (U/l)	46.50 ± 2.18	47.00 ± 4.36	50.00 ± 7.51	68.50 ±2.53	51.25 ± 16.47
Alkaline phosphatase(U/l)	32.95 ± 8.25	35.35 ± 3.90	40.00 ± 2.48	43.50 ± 2.40	42.50 ± 1.56
Total protein (g/dl)	9.49 ± 0.32	8.92 ± 0.48	9.42 ± 0.48	9.99 ± 0.36	9.14 ± 0.57
Serum albumin (g/dl)	4.02 ± 0.20	3.73 ± 0.13	3.75 ± 0.24	3.82 ± 0.22	3.61 ± 0.08
Cholesterol (mg/dl)	82.50 ± 4.79	82.50 ± 7.50	75.00±15.00	85.00 ± 2.89	77.50 ± 7.50
Glucose (mg/dl)	63.06 ± 2.57	63.89 ± 4.24	81.25 ± 2.09	98.15±2.45**	96.11±8.93**
Creatinine (mg/dl)	1.24 ± 0.14	0.80 ± 0.30	1.10 ± 0.03	0.98 ± 0.14	1.12 ± 0.00
Urea (mg/dl)	54.10 ± 1.38	44.95 ± 0.92*	47.23 ± 0.36	54.98 ± 1.42	45.48 ± 0.76*
Uric acid (mg/dl)	3.98 ± 0.38	4.05 ± 0.51	4.14 ± 0.55	4.89 ± 0.32	3.63 ± 0.15
Sodium (mEql/l)	144.75 ± 2.81	146.25 ± 2.59	142.25±1.67	140.25 ± 3.88	145.75 ± 3.71
Potassium (mEq/l)	6.00 ± 0.65	6.65 ± 0.42	8.60 ± 1.58	8.45 ± 0.51	7.68 ± 0.57
HCO_3^-	22.75 ± 0.48	23.00 ± 1.35	23.00 ± 0.82	22.25 ± 0.63	23.50 ± 0.96

Values are mean ± SEM (n = 5). Means with the same superscript across the row for each parameter are not significantly different (P > 0.05)

Platelets have a key role in maintaining vascular integrity. They aggregates at and adhere to exposed collagen to form a physical barrier at the site of vessel injury, accelerate the activation of coagulation proteins and release stored granules that promote vasoconstriction and wound healing. Certain drugs have been associated with antiplatelet activity. Aspirin for instance, acetylates and thus inactivate cyclo-oxygenase (COX), the enzyme responsible for the first step in the formation of prostaglandins from arachidonic acid. It also prevents both the formation of thromboxane A_2 (TXA) and protacyclin (PGE_2). Our previous studies on this plant revealed significant antinociceptive activity. [37] Therefore,

the low platelet recorded in this study could be as a result of its effect on COX activity which is also a platelet activating factor (PAF). [38] Reduction in platelet number has also been associated with infections, deficiency of folate or vitaminB_{12}, drugs and some herbal remedy. [39] A differential WBC provides information on the different white cells present in the circulating blood. Significant (P >0.05) abnormality seen particularly in group 4 of this study, could be as a result of localized acute bacterial infection.

Liver is considered the key organ in the metabolism, detoxification, and secretory functions in the body, and its disorders are numerous with no effective remedies. The biochemical indices monitored in the serum such as the electrolytes and other secretory substances of the liver and kidney can be used as 'markers' for assessing the functional capacities of the organs. [32] These parameters of organ function if altered will impair the normal functioning of the organs. Renal function indices are usually required to assess the normal functioning of different parts of the nephrons. [40] Similarly, the serum concentrations of electrolytes, uric acid and creatinine could give an insight into the effect of plant extract on the tubular and or glomerular part of the kidney. Therefore, the non effect of *G. crenata* leaves extract at all the doses investigated on the renal function indices may suggest that the normal functioning of the nephrons at the tubular and glomerular levels were not affected when compared with the control (Table 4). However, a significant decrease in urea (group 2 and 5) could be as a result of water overload. [39]

The expression of toxicity of xenobiotics is usually determined biochemically by monitoring of some plasma enzymes and lipids. A rise in AST, ALT, ALP and cholesterol are commonly measured as indices of the damage of the liver cells. [41] These enzymes are mainly localized in the cytoplasm and any damage in hepatic cells may result in alteration in the serum level. Thus, the changes in activity and concentration of tumour marker enzymes like AST, ALT and ALP in tissue such as liver could reflect the state of hepatotoxicity. [42] Our results revealed no significant changes (P<0.05) in the liver enzymes of the treated groups when compared with the control. This suggests that sub-chronic administration of *G. crenata* leaves extract has no hepatotoxic effects in rats. Also significant dose dependent increase (P<0.05) in blood glucose have been recorded in this study. However, these changes were still within the normal range. [43]

Alterations in the concentration of major lipids can give useful information on lipid metabolism and predisposition of the heart to atherosclerosis and its associated coronary heart diseases. [34, 44] The non effect of the extract on the serum cholesterol concentration may be explained by non-impairment in the biosynthesis of cholesterol. Bilirubin is an endogenous anion derived from hemoglobin degradation from the RBC. Bilirubin, a metabolic breakdown product of heme derived from senescent red blood cells, is also one of the most commonly used liver function tests. [45] The reduction (P<0.05) in the total bilirubin (groups 3 and 4) could be adduced to impairment in the secretory function of these proteins. It may also adversely affect the functional activity of the liver. The liver is the major source of most the serum proteins. [46] The parenchymal cells are responsible for synthesis of albumin, fibrinogen and other coagulation factors and most of a and b globulins. [47]

Albumin is quantitatively the most important protein in plasma synthesized by the liver and is a useful indicator of hepatic function. Albumin synthesis is affected not only in liver disease but also by nutritional status, hormonal balance and osmotic pressure. The result of this study have revealed no significant (P<0.05) difference in the serum albumin and total protein of the extract treated groups as compared to the control.

It can be concluded from findings of present study that leaf extract of *Grewia crenata* is safe even at high oral acute dose level and well tolerated for the 28-day study period. Thus have potential for safe use in oral formulations. Further studies are needed to isolate and characterize the active component present in this plant.

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