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Full Length Research Paper

Toxicological profiles of direct administration of extract of *gossypium barbadense* (linneaus) leaves

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Abstract

This study was designed to investigate the acute and sub-chronic toxicities of direct-extract of leaves of *Gossypium barbadense* (Linn) with and without addition of lime in albino mice and wistar rats respectively, with a view to evaluating their possible toxic effects on these animals, and also its cytotoxic potential using *Allium cepa* test. Fresh leaves of *G. barbadense* were crushed and squeezed to obtain direct-extract while the limed-extract was prepared from the mixture of direct-extract and *Citrus medica* juice in ratio 3 : 1[v/v].The direct and limed-extract were then partitioned with ethyl acetate to obtained ethyl acetate fractions which was used for cytotoxicity test. The direct and limed-extracts were screened for phytochemical constitutents. Acute toxicity study was carried out by standard procedure, using mice of weight range between 19g and 25g, while in sub-chronic toxicity study using adult wistar rat of weight range 110g and 150g. The animals were treated with 250 and 500 mg/kg for sub-chronic study, every other day for a period of 30 days after which the animals were sacrificed; blood was collected and liver and kidney were excised from each animal. Haematological and biochemical parameters were analyzed in the blood while histological examination was carried out on liver and kidney using standard methods. The total protein concentration in the plasma and liver homogenates were determined using Bradford method. The plasma albumin, bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl trasferase,urea and creatinine concentration were determined using standard procedure. Cytotoxicity was carried out using *Allium cepa* with different concentrations (2.5, 5 and 10 mg/ml) of ethyl acetate fraction from direct and limed-extract, the roots of *A. cepa* were harvested and homogenized with normal saline to obtain supernatant for biochemical analyses. The results indicated that the median lethal dose (LD_{50}) was above 5000 mg/kg of body weight since no mortality was recorded in acute study. Biochemicals indices of plasma and liver homogenates, alanine aminotransferase (ALT),aspartate aminotransferase (AST) and albumin were lower in the test animals compared with the controls, in contrast, total protein concentrations in both plasma and liver homogenate were higher in test groups than in control, bilirubin concentration was also higher in all the treated animals, the difference was significant at (P <0.05) for the animals treated with direct extract. Haematological parameters maintained nearly the same level with control in all the treated groups. Additionally, the cytotoxicity test using *Allium cepa* suggested that direct-extract and ethyl acetate fractions were cytotoxic but addition of lime (*Citrus medica*) appears to have lowering effects on the cytotoxicity in the treatment. However the histological study of the liver did not reveal any damage but the kidney histology indicated partial glomerulus degeneration for animals treated with 250 mg/kg bwt in both limed and direct extracts. In conclution, this study probably showed that both direct and limed-extract of the leaves of *G. barbadense* may not have toxic effect on the animals at 250mg/kg and 500mg/kg body weight in sub-chronic toxicity. However the ethyl acetate fractions obtained from both direct and limed-extract exhibited some degree of toxic effect in cytotoxicity study using *Allium cepa,* addition of lime appear to lower this cytotoxicity. Therefore at the doses investigated in this study, the extract of *G. barbadense* may not be toxic to laboratory animals. Further investigation should be carried out using higher doses of the direct and limed-extract.

Keywords: *Gossypium barbadense,* Cytotoxicity, *Allium cepa,* Ethyl acetate

INTRODUCTION

Medicinal plants have formed the basis of health care throughout the world since the earliest days of humanity and are still widely used with considerable importance in international trade (Ebong *et al*., 2008). Also the use of

plant-derived natural compounds as part of herbal preparations as alternative sources of medicaments continues to play major roles in the general wellness of people all over the world. The African continent contains some of the richest biodiversity in the world, and abounds in plants of economic importance and plants of medicinal importance which when developed would reduce our expenditure on imported drugs to meet our health needs. Herbal-based and plant-derived products can be exploited with sustainable comparative and competitive advantage. The therapeutic action of a range of wild plants, although not scientifically proven, has been discovered by indigenous people over centuries. Developing countries are often subject to shortages of funds, medical facilities and newly developed medicine, which make them more dependent on their natural resources (Mammem and Cloete, 1996).

Plants are one of the most important sources of active substances with therapeutic potential to cure a variety of diseases in humans. The evaluation of pharmacological effects can be used as a strategy for discovering new drugs of plant origin. There is an ongoing world-wide revolution which is mainly premised on the belief that herbal remedies are safer and less damaging to the human body than synthetic drugs (Alam *et al*., 2011). According to World Health Organization about 80% of the world population relies on traditional medicine for primary health care and more than 30% of the plant species have been used medicinally. However, there is limited scientific evidence regarding the safety and efficacy to support the continued therapeutic application of these medicinal plants. Because of this renewed interest in herbal remedies and the increased use of plants extracts in food, cosmetics and pharmaceutical industries, there is a compelling need for thorough scientific safety evaluation of the medicinal plants. Laboratory animals are sensitive to toxic substances occurring in plants. Hence, the administration of the extracts in increasing amounts enables the evaluation of the acute and sub-acute toxicity limits.

The cotton genus (Gossypium) under study includes approximately 50 species distributed in arid to semi-arid regions of the tropic and subtropics. Included are four species that have independently been domesticated for their fiber, two each in Africa–Asia and the Americas. Gossypium species exhibit extraordinary morphological variation, ranging from herbaceous perennials to small trees with a diverse array of reproductive and vegetative characteristics. A parallel level of cytogenetic and genomic diversity has arisen during the global radiation of the genus, leading to the evolution of eight groups of diploid species. The evolutionary history of the genus included multiple episodes of trans-oceanic dispersal, invasion of new ecological niches, and a surprisingly high frequency of natural interspecific hybridization among lineages that are presently both geographically isolated and intersterile (Wendel, 2000).

Recent investigations have clarified many aspects of this history, including relationships within and among the eight genome groups, the domestication history of each of the four cultivated species, and the origin of the allopolyploid cottons. Data implicate an origin for Gossypium 5–15 million years ago and a rapid early diversification of the major genome groups. Allopolyploid cottons appear to have arisen within the last million years, as a consequence of trans-oceanic dispersal of an A-genome taxon to the New World followed by hybridization with an indigenous D-genome diploid. Subsequent to formation, allopolyploids radiated into three modern lineages, including those containing the commercially important species *G. hirsutum and G. barbadense*. Genome doubling has led to an array of molecular genetic interactions, including inter-locus concerted evolution, differential rates of genomic evolution, inter-genomic genetic transfer, and probable alterations in gene expression. The myriad underlying mechanisms are also suggested to have contributed to both ecological success and agronomic potential (Wendel, 2000).

Extracts of *Gossypium barbadense* L. has been used in the treatment of different forms of ailments such as gastric irritation, diarrhoea, dysentery, dysuria, rheumatoid arthritis and otalgia but there is no scientific information on its toxicity profile. The current study is therefore designed to investigate the acute and sub-chronic toxicities of direct-extract of leaves of *Gossypium barbadense* (Linn) with and without addition of lime as it is employed traditionally since there is limited scientific evidence regarding the safety and efficacy to support the continued therapeutic application of this plant.

MATERIALS AND METHOD

Collection and Identification of Plant

Fresh leaves of *G. barbadense* (Linneaus) were collected from Ofatedo and Ido-Osun in Egbedore Local Government Area, Osun State, Nigeria. The plant was identified and authenticated at IFE Herbarium, Department of Botany, Obafemi Awolowo University Ile-Ife, where specimen was deposited, voucher and identification specimen number 16915 was collected.

Experimental Animals

Eighteen (18) healthy matured mice with an average weight range of 19 g to 28 g selected for acute toxicity test and twenty five (25) healthy matured Wister strain rats with an average weight range of 110 g to 150 g for sub-acute toxicity were obtained from Faculty of Health Sciences, Obafemi Awolowo University Ile-Ife. The animals were acclimatized for three weeks in the Animal

House, Department of Biochemistry where they had free access to Standard pellets (Guinea Feeds, Benin City, Nigeria) and clean water was provided.

Preparation of Extracts

Fresh leaves (1.5 kg) was crushed and squeezed to obtain direct juice from the leaves. The direct juice (540 ml) was centrifuged to remove chlorophyll and then divided into two equal parts. The direct juice (270 ml) was kept as direct extract while the second portion (270 ml) was mixed with juice of *Citrus medica* in ratio 3:1 (v/v). These 2 portion were then freeze dried to obtained the direct-extract (un-limed) and limed-extract respectively.

Phytochemical Screening

Both limed and un-limed extracts of *G. barbadense* were screened for phytochemical constituents according to a procedure that was based on the methods described by Sofowora (1993), Trease and Evans (2002) and Prashant *et al*., (2011),

Determination of LD_{50} of the Extracts

Acute toxicity of *G.barbadense* was carried out according to the procedure of Lorke (1983)

Subchronic Toxicity Study

Rats (25) were divided randomly into five groups of five rats per group as follows: Group 1 served as control (received distilled water); Group 2 and 3 received 250 mg/kg bwt and 500 mg/kg bwt of limed extract respectively while Group 4 and 5 received 250 mg/kg bwt and 500 mg/kg bwt of direct *G. barbadense* extract respectivelly for 30 days in every other day.

After the treatment period, the animals were sacrificed by cervical dislocation, dissected and blood was collected into heparinized bottles, kidney and liver were excised for histological study and for other biochemical assays. Part of the blood were also collected for haematological study

Histological Investigation

The histological evaluations carried out by standard procedure, the liver and kidney tissues from control and test rats were fixed in 10% buffered formalin (pH 7.3) for 24 h, dehydrated in ascending grade of ethanol (50%, 70%, 90% then absolute, twice) for the interval of 1hr. to enable the tissue to be embedded in paraffin. Clearing in xylene preceded paraffin wax at another 1hr to remove water from tissue and the tissue was sectioned at 5 µm and stained with hematoxylin and eosin prior to examination with a zeiss EM light microscope. The histological evaluations carried out by standard procedure

Preparation of Blood Plasma

The collected blood samples were subjected centrifigation at 3000 rpm for 10 min in a Gallenkhamp junior Table Centrifuge (Denly, BS 400) at room temperature. The supernatants (plasma) were collected into sterile sample bottles, labeled and kept in deep freezer for further analyses.

Preparation of Liver Homogenates

The livers were washed with normal saline and subsequently blotted with tissue paper, then, one gram (1g) of each liver and kidney were homogenized separately in 10 ml 100 mM phosphate buffer, pH 7.2 and centrifuged at 6000 rpm for 30 min. The supernatants were collected into clean sterile bottles, labeled and kept in deep freezer for further analyses.

Estimation of Total Protein Concentrations (Plasma and Liver homogenates)

The total protein concentrations were determined according to the method of Bradford (1976).

Estimation of Plasma Albumin Concentration

Albumin determination was carried out the method of Grant *et al*. (1987)

Estimatiom of Total Bilirubin and Direct Bilirubin Concentration

The estimation of total bilirubin concentration was carried out as reported by Jendrassik and Grof, (1938)

Estimation of Plasma Urea Concentration

The plasma urea concentration was assayed based on (Fawcett and Scott, 1960) colorimetric method using a commercially available kit by Randox Laboratories Ltd, antrim, UK.

Estimation of Plasma Creatinine

Plasma creatinin concentration by the alkaline picrate method **of** Bartels and Bohmer, 1972

Assay of Plasma Alanine aminotransferase (ALT) Activity

The plasma and liver homogenates alanine aminotransferase activities were estimated as described by Reitman and Frankel (1957) using the Randox kit.

Assay of Plasma Aspartate aminotransferase (AST) Activity

The plasma aspartate aminotransaminase activity was estimated as described by Reitman and Frankel (1957).

Assay of Gamma Glutamyl Transferase γ-GT

This was carried out by the methos described by Szasz, 1969.

Cytotoxicity Assays (*Allium Cepa* Test)

Allium cepa test was carried out according to the method of Fiskesjo (1985) as modified by Rank and Nielsen (1994). *Allium cepa* is used to assess cytotoxic potential, environmental hazards and genotoxicity of compounds by allowing developing *Allium cepa* roots to come into contact with substances to be tested. After sufficient exposure, the roots were harvested and homogenized biochemical analysis. Toxicity is evidenced by accumulation of proline, sugar and protein.

Preparation of *A. cepa* Root homogenate

On the 5^{th} day, the roots of *Allium cepa* placed on different concentrations of both direct and limed extract, ethylacetate and aqueous fractions were homogenized with normal saline, centrifuged at 3000 rpm for 30 min. and the supernatant was removed for biochemical analyses.

Estimation of Total Soluble Sugar

The sugar content of *Allium cepa* roots grown on different concentration of each fraction of direct and limed extract was estimated using phenol/sulphuric acid reaction method of Dubois *et al.,* (1956).

Estimation of Total Protein Concentration

The protein content of each fraction was estimated according to the method of Schocterk and Pollack, 1978, using bovine serum albumin as standard.

Estimation of Proline Concentration

The accumulation of proline under various abiotic stress conditions such as heat, cold, drought, moisture and salinity in plant is considered as a tolerance mechanism. It is suggested to act as an osmolyte as well as source of nitrogen during recovery from stress Estimation of Proline Concentration by the method of Bates *et al., (*1973).

Statistical Analyses

All data obtained from various experiments were subjected to statistical analysis. The results were expressed as (X ± SEM) mean ± standard error of the mean (SEM). Statistical analysis was performed by one-way analysis of variance (ANOVA) with Turkey test to evaluate significant differences between groups. Values of P >0.05 considered significant. All statistical analyses were carried out using the Instat statistical package (Graph Pad Software).

RESULTS

Table 1 showed the results of the phytochemical constituent of both the direct and the limed extract of *G. barbadense*, Tables 2 and 3 showed the changes in plasma enzyme activities and the concentration of specific metabolite respectively following the administration of the plant extracts, Table 3 showed the result of the *allum cepa* test while Table 4 showed the results of the haematological parameters. Plate 1 and 2 showed the results of the histology of the liver and the kidney respectivelly.

From the figure below, [CV] means Central Vein of the liver of Wistar rats treated with different concentration of *G. barbadense.* Group [A] serves as control administered with distilled water, Groups [B and C] received 250 and 500 mg/kg body weight of limed juice of *G. barbadense,* while Groups [D and E] were administered with 250 and 500 mg/kg body weight of direct juice of *G. barbadense.*

From the figure below, [dG] means degenerating glomerulus and [G] means normal glomerulus in the kidney of Wistar rats treated with different concentration of *G. barbadense.* Group [A] serves as control administered with distilled water ,Groups [B and C] received 250 and 500 mg/kg body weight of limed juice of *G. barbadense,* while Groups [D and E] were

Table 1: Phytochemical Constituents of both direct and limed-extract of *G. barbadense*.

Phytochemicals	Direct extract	Limed extract
Saponins	+	–
Tannin	+	–
Alkaloids	–	–
Flavonoids	–	–
Terpenoids	+	+
Cardiac glycoside	+	+
Steroids	+	+
Phytosterols	+	+
Xanthoprotein	+	+
Phlobatanins	+	+
Triterpenes	+	+

Key: + = Presence and - = absence

Table 2. Changes in Plasma Enzyme after Administration of the Plant Extracts.

	GGT (U/L)	L – ALT (U/L)		L – AST (U/L)
Group	**Plasma**	**Plasma**	**Liver**	**Plasma**
1	1.54 ± 0.95[a]	71.20 ± 3.27[a]	112.35 ± 0.38[a]	217.00 ± 20.00[a]
2	0.77 ± 0.77 [a]	58.20 ± 7.33[a]	109.24 ± 0.68[a]	167.00 ± 20.82[b]
3	1.54 ± 0.95 [a]	61.20 ± 5.37[a]	108.80 ± 2.09[a]	232.00 ± 15.00[a]
4	0.77 ± 0.77 [a]	64.70 ± 8.46[a]	109 0.8 ± 0.38[a]	170.33 ± 31.80[b]
5	0.77 ± 0.77 [a]	49.20 ±3.06[a]	108.04 ± 1.14[a]	173.67 ± 38.44[b]

The table above shows Plasma gamma glutamyltranferase, alanine and aspartate amino transferase activities. Values were expressed as mean ± SEM (standard error of mean) of n = 5.
[a, b] represent different values but not statistically significant at P > 0.05
Group 1: control, was given distilled water;
Group 2; was given 250mg/ml body weight of Limed *G. barbadense* extract;
Group 3; was given 500mg/ml body weight of Limed *G barbadense* extract;
Group 4; received 250mg/ml body weight of Direct *G. barbadense* extract and
Group 5; received 500mg/ml body weight of Direct *G. barbadense* extract alone

administered with 250 and 500 mg/kg body weight of direct juice of *G. barbadense.*

DISCUSSION

This study focused on the investigation of toxicological potential of direct and limed-extract of the leaves of Gossypium barbadense. The therapeutic properties of any medicinal plant are, as a result of presence of different phytochemicals in their leaves, stem-bark, roots and fruits (Sofowora, 2006). The result of the phytochemical screening of both the direct and limed-extract of the G. barbadense revealed the presence of terpenoids, cardiac glycosides, steroids, phytosterols in both while saponins and tannins are present in the direct-extract they were found absent in the limed-extract. This probably implied that the addition of fresh lime juice may have precipitated both saponins and tannins from the limed-extract. However, alkaloids and flavonoids were absent in both direct and limed-extract as indicated in Table 1.

The toxicity studies in animals are commonly used to assess the potential health risks in humans by intrinsic adverse effects of phytochemicals in the extracts (Oyedemi et al., 2010). These adverse effects may cause significant alterations in the levels of biomolecules such as enzymes, metabolites de-rrangement and histomorphology of the organs e.t.c (Yakubu et al., 2009). Also assessment of haematological parameters could

Table 3: Effect of *G. barbadense* on Specific Plasma / Liver Metabolites

PARAMETERS	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5
Homogenate Protein (µg/ml)	181.79 ± 8.90[a]	190.86 ± 8.89[a]	169.11± 11.12[a]	203.75±14.35[a]	188.71 ± 6.85[a]
Plasma Protein (µg/ml)	109.11 ± 7.36[a]	113.28 ± 4.41[a]	109.57 ± 1.99[a]	121.57 ± 1.87[a]	123.87 ± 5.76[a]
Albumin (g/l**)**	50.90 ± 2.17[a]	48.47 ± 3.67[a]	40.50 ± 1.36[b]	38.31 ± 0.92[c]	41.09 ± 1.83[a]
Total Bilirubin (µmol/l)	11.72 ± 2.02[a]	17.81 ± 1.75[a]	15.27 ± 1.10[a]	24.05 ± 2.70[b]	26.64 ± 2.45[c]
Direct Bilirubin (µmol/l)	0.43 ± 0.06[a]	0.55 ± 0.06[a]	0.37 ± 0.07[a]	0.98 ± 0.14[b]	0.90 ± 0.16[b]
Plasma Urea (mmol/l**)**	6.17 ± 0.54[a]	7.57 ± 0.42[a]	6.55 ± 0.17[a]	7.12 ± 0.58[a]	7.25 ± 0.51[a]
Creatinine (µmol/l**)**	674.05±25.66[a]	719.68±18.20[a]	660.51±27.42[a]	693.19±26.61[a]	567.36±2.21[b]

Values were expressed as mean ± SEM of n = 5.
Values with different superscript are statistically different at P<0.05

Table 4: Effect of *G. barbadense* on Biochemical Parameters of *Allium cepa* Root

Fractions	Percentage inhibition	Glucose(mg/ml)	Protein(µg/ml) 10^{-4}	Proline(mg/ml)
Crude Juice	95.97% ± 0.60	64.50 ± 12.50	1.25± 0.73	3.93± 0.02
Crude Juice + lime	92.94% ± 0.36	60.75 ± 3.75	0.66± 1.26	5.07 ± 0.14
Control		55.67 ± 13.09	0.35 ± 3.32	2.36 ± 0.01

Values were expressed as mean ± SEM of n = 3.

also be used to evaluate the deleterious effects of foreign compounds including plant extracts on the blood constituents of an animal. In this study, the result of acute toxicity recorded no death when the extract was administered up to 5,000 mg/kg for mice. This implied that the oral LD_{50} of leaf extract of *G. barbadense*is greater than 5000 mg/kg body weight for mice. Similarly, in the sub-chronictoxicity test, the direct and limed-extract of the leaves of G .barbadense at doses of 250 mg/kg and 500 mg/kg body weight, administered every other day for a period of 30 days did not result in death of the animals and there were no observable signs of toxicity during the experimental period. Also from this study, the rats in different groups, treated with different doses of direct and limed-extract of the leaves of G barbadense was observed to have progressive increase body weight. The increase in final body weight when compared to initial body weight was found to be significant ($P < 0.05$), even in the control animal (Table 2). The progressive increase in body weight at doses of 250 mg/kg and 500 mg/kg of rats during 30 days oral administration of the extract probably reflect the nutritional status of the animals, which probably means that the extract did not affect the nutritional state of the treated animals. Additionally, the relative liver-body weight of the rats did not show any significant difference at ($P >0.05$) between the control and the treated groups, the little increase in the liver-body weight was proportional to an increase in body weight in both direct and limed-extract administered at different doses.

Interestingly too, the results of biochemical analyses of both direct and limed-extract of the leaves of G. barbadense as shown in different tables for the plasma and 10% liver homogenate after a period of 30 days of oral administration of the direct and limed-extract. From Table 4, there were no significant changes in the activities of gamma glutamylaminotransferase (GGT) and liver homogenate alanine aminotransferase (ALT) in the animals administered with both direct and limed-extract. In plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST), a non-significant difference ($P > 0.05$) were observed in plasma alanine aminotransferase (ALT) activities in all the groups when compared with the control group. The decrease in the activity was more pronounced in group 5 (49.20 ± 3.06) which was administered with 500 mg/kg body weight of direct extract of the leaves of G.barbadense, whereas control group was (71.20 ± 3.27). Since activity of the enzymes was found to be decreased instead of being increased, it cannot be concluded that the plant extract was toxic to the liver. The same decrease in activity was also observed in plasma aspartate aminotransferase this was also not statistically significant ($P > 0.05$). ALT and AST are two important liver marker enzymes that are associated to the hepatocellular damage, with ALT being more specific. AST and ALT areof higher concentrations in the hepatocytes, however only ALT is remarkably specific for liver function since AST is also present in the myocardium, skeletal muscle, brain and kidneys (Witthawasku et al., 2003). A mild elevation of AST level

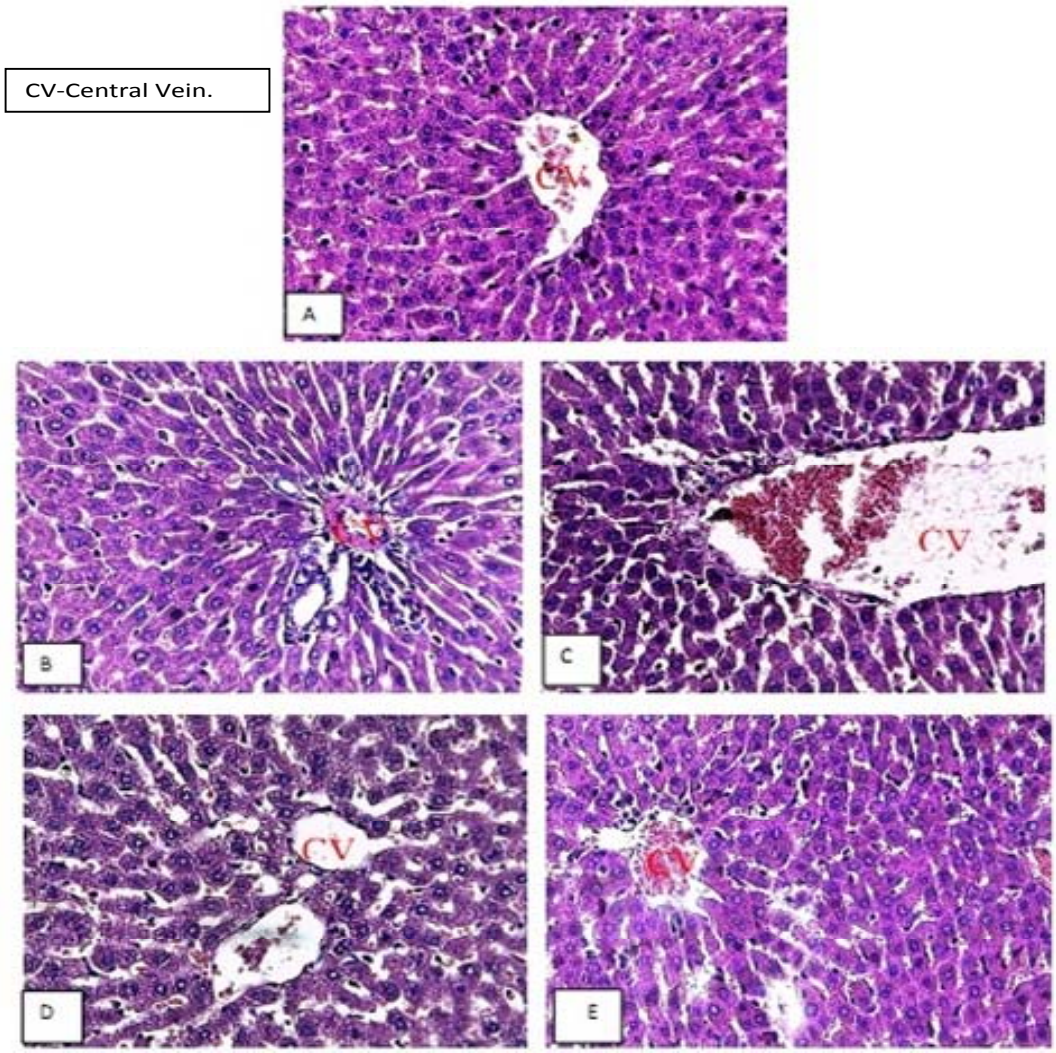

Plate 1: Microscopic view of the Liver of Control and Treated groups

has been shown to be associated with liver injury or myocardial infarctions. In this study, the administration of the extract did not lead to elevation of these enzymes activity. This suggests that both direct and limed-extract of the leaves of G. barbadense may not be toxic at the doses considered in this study.

Bilirubin is formed by the breakdown of haemoglobin in the spleen, liver and bone marrow. In the liver, bilirubin is conjugated with glucuronic acid to form a soluble compound, conjugated bilirubin passes down the bile duct and are excreted into the gastrointestinal tract. An unconjugated, albumin bound form is also present in the circulation. It is insoluble and does not normally pass through the kidneys into the urine. An increase in bilirubin concentration in the serum or tissues is called jaundice. High level of conjugated bilirubin indicates that bile is not being properly excreted therefore an obstruction may be present in the bile duct or gall bladder **(**Jendrassik and Grof, 1938**).**In this study, plasma bilirubin concentration, Table 4 was greater in animals treated with direct-extract than limed-extract of G. barbadense, the difference is statistically significant at ($P < 0.05$)

Albumin is the most abundant serum protein representing 55 – 65 % of the total protein. It is synthesized in the liver and has half-life of 2 to 3 weeks. The main biological functions of albumin are to maintain the water balance in serum and plasma and to transport and store a wide variety of ligands like fatty acid, calcium, bilirubin and hormones such as thyroxine. It also provides an endogenous source of amino acids. The study revealed low level of plasma albumin concentration than the control; the difference is not statistically significant at ($P < 0.05$)

The protein concentration in liver homogenate and plasma (Table 3) showed an increased level, which indicated that the plant extract was not toxic to liver, by

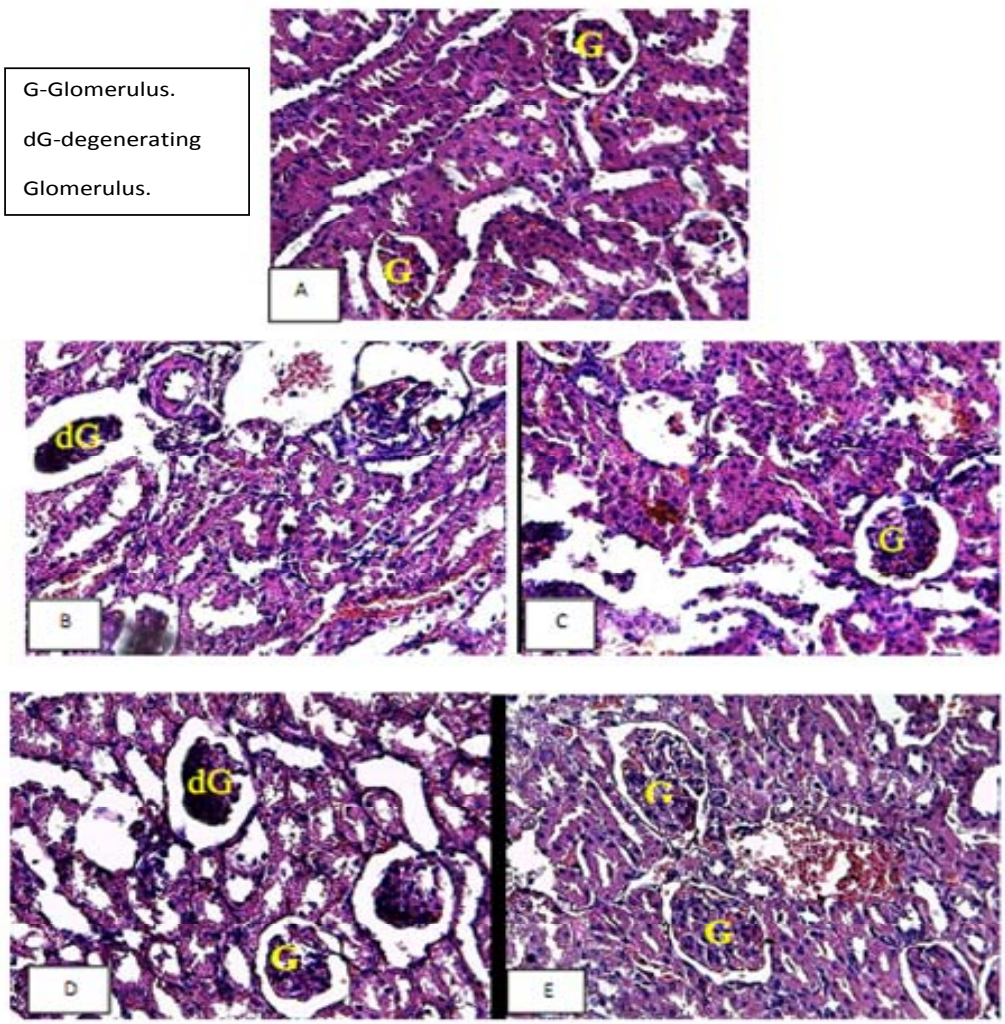

Plate 2: Microscopic view of the Kidney of Control and Treated groups

not affecting protein synthesis in the liver. If the extract was toxic to animals, it would have caused damage to liver, which might have affected the hepatocytes and lead to significant reduction in protein synthesis, because hepatocytes synthesize proteins for the whole organism, the majority of the circulating proteins are synthesized by hepatocytes. Therefore an increase in protein concentration at both liver homogenate and plasma was probably an indication that both limed and direct-extract of G. barbadense, at the doses investigated in this study (250 and 500mg/kg) may not have toxic effect.

The Kidneys are highly susceptible to toxicants because, a high volume of blood flows through it and its ability to filter large amounts of toxins which can concentrate in the kidney tubules. It can result in systemic toxicity causing: decreased ability to excrete body wastes, inability to maintain body fluid and electrolyte balance and decreased synthesis of essential hormones. Blood urea nitrogen is derived in the liver protein / amino acid from diet or tissue sources and is normally excreted in the urine. In renal disease, the serum urea accumulates because the rate of serum urea production exceeds the rate of clearance (Mayne and Mayne, 1994). Creatinine is derived from creatinine and creatine phosphate in muscle tissues and may be defined as a nitrogenous waste product. Creatinine is not reutilized but is excreted from the body in the urine via the kidney. It is produced and excreted at a constant rate which is proportional to the body muscle mass. Creatinine is measured primarily to assess kidney function and has certain advantages over the measurement of urea. The plasma level of creatinine is independent of protein ingestion, water intake, rate of urine production and exercise. Since its rate of production is constant, elevation of plasma creatinine is an indicative of under-excretion, suggesting kidney impairment. In this study, there were no significant changes in the level of creatinine between control and treated groups which

Table 5: Effect of Administration of Limed and Direct Extract of *G. barbadense* on Some Haematological Parameters of Wistar rats

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
White blood cells (10^3/L)	7.72 ± 1.88	7.93 ± 0.87	8.40 ± 0.60	7.20 ± 0.80	8.06 ± 1.63
Red blood cells (x10^6/L)	4.53 ± 0.05	6.09 ±0.19	5.99 ± 0.29	6.96 ± 0.78	5.63 ± 1.05
Packed cell volume	51.33 ± 0.33	58.20 ± 1.89	55.40 ± 2.25	51.00 ± 1.58	52.00 ± 2.35
Haemoglobin (g/dL)	14.46 ± 0.66	16.75 ± 0.25	15.17 ± 0.93	14.53 ± 0.52	13.53 ± 2.05
MCHC (g/dL)	29.14 ± 1.03	27.35 ± 0.57	28.09 ± 1.70	28.36 ± 0.84	25.42 ± 5.26

Group 1: control, was given distilled water;
Group 2; was given 250mg/ml body weight of Limed *G. barbadense* extract;
Group 3; was given 500mg/ml body weight of Limed *G barbadense* extract;
Group 4; received 250mg/ml body weight of Direct *G. barbadense* extract and;
Group 5; received 500mg/ml body weight of Direct *G. barbadense* extract.

means that the extract is safe at doses investigated. Also in the case of plasma urea, there was no significant difference P > 0.05 between the values of control group and treated groups, which implies that there was no elevation of plasma urea due to administration of the plant extract. Despite the fact that creatinine is more reliable than urea to assess kidney function, both creatinine and urea indicated that the plant extract was not nephrotoxic.

The haematological parameters in (Table 5) after 30 days of oral administration of both direct and limed-extract of the leaves of *G.barbadense*, the doses investigated in this study did not significantly (P> 0.05)alter the red blood cells (RBC),white blood cells, packed cell volume (PCV) and haemoglobin (Hb). However there was a slight decrease in mean corpuscular haemoglobin concentration (MCHC) at group 5 (500 mg/kg body weight of direct-extract of *G. barbadense*) (25.42 ± 5.26) when compared to control (Group 1) (29.14 ± 1.03). The little changes in values observed in haematology study did not show any dose responsiveness. Thus direct extract of *G. barbadense* has a slight effect on the haematological parameters.

In cytotoxicity test, proline accumulates in many plant species in response to environmental stress. Proline is a proteinogenic amino acid with an exceptional conformational rigidity, and is essential for primary metabolism; also it acts as an osmolyte as well as a source of nitrogen during recovery from stress. Proline accumulation has been reported during conditions of drought, high salinity, high light and UV irradiation, heavy metals, oxidative stress and in response to biotic stresses, (Fabro, 2004). From the estimation of total soluble sugar concentration, total soluble protein and proline concentration using *Allium cepa* (Table 5), proline concentration was high in all the fractions/samples (ethylacetate fraction [EAF] of direct-extract, ethylacetate fraction [EAF] of limed-extract, direct-extract, limed-extract, aqueous of direct-extract and aqueous of limed-extract – 5.83 ± 0.58, 4.00 ± 0.25, 3.93 ± 0.02, 5.07 ± 0.14, 4.50 ± 0.41 and 3.83 ± 0.11) when compared with control (2.36 ± 0.01), although this concentration of proline was low in limed EAF extract and limed aqueous extract than the direct-extract but still higher than the control while limed extract has high proline concentration than its direct extract as shown in (Table 4). This implies that addition of lime (*Citrus medica*) to direct extract of *G. barbadense* may have lowering effect on its cytotoxicity.

Concentrations of glucose and protein were high in all the fractions/samples when compared with control except direct EAF extract (30.75 ± 3.75) and limed EAF extract (0.08± 63s) which have low concentrations of glucose and protein respectively when compared with their control (55.67 ± 13.09) for glucose and (0.35 ± 3.32) for protein.Gross examination of internal organs like liver and kidney were normal except (Group B and D) for kidney which received 250 mg/kg body weight of limed and direct extract of *G. barbadense* in which degenerating glomerulus was detected. This degeneration of glomerulus may not be as a result of extract administered, because the level of degeneration would have been increased at 500mg/kg body weight if this were to be caused by the extract.

The fact that the extract of *Gossypium barbadense* is toxic in *Allium cepa* test is an indication that it may have therapeutic property in cancer therapy. The cytotoxic compound, ‘’gossypol’’ present in the cotton plant is more predominant in the cotton seed than any other cotton tissues, this is evident in the statement of Kandylis *et al*., 1998 which says ‘cotton (*G. hirsutum* and *G. barbadense*) tissue, particularly the seeds, can be toxic if ingested in excessive quantities because of the present of anti-nutritional and toxic factors including gossypol and cyclopropenoid fatty acids (including dihydrosterculic, sterculic and malvalic acids). The presence of gossypol and cyclopropeoid fatty acids in the cotton seed limits its use as protein supplement in animal feed’.

From this study, there was little difference between direct and limed-extract of *G. barbadense,* differences were detected in the phytochemicals, in which tannin and

saponin were absent in limed but present in direct-extract of the plant. These phytochemicals (tannin and saponin) are respectively responsible for astringency and bitter-tasting in plant extracts, this might be one of the reasons people squeeze *G. barbadense* with lime locally. More so, bilirubin concentration was high in direct-extract when compared with limed and control groups. Additionally, the proline content was low in ethylacetate fraction of limed-extract (EAF_L) and aqueous of limed-extract (AF_L) than the direct-extract fractions, since accumulation of proline is a sign of stress in cytotoxicity test, addition of lime tends to reduce cytotoxic effect of *G. barbadense* extract on meristematic cells.

In this study both limed and direct extract of the leaves extract of *G. barbadense* may not have toxic effect on the animals treated with 250 mg/kg and 500 mg/kg body weight. Therefore at the doses that were investigated in this study, the extract of *G. barbadense* may be safe for laboratory animal, although further toxicological investigation may still be carried out perhabs at higher doses.

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