

## Hepatic effects of aqueous extract of *Chromolaena odorata* in male Wistar albino rats.

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### Abstract

The hepatic effects of aqueous leaf extract of *Chromolaena odorata*, a plant commonly used for traditional medications, was evaluated using male Wistar albino rats. Acute and sub chronic toxicity was evaluated after 90 days of exposure. The LD<sub>50</sub> was 2154mg/kg. Serum levels of glucose, lactate dehydrogenase, aspartate aminotransferase transaminase, and total bilirubin increased significantly in the 538.5 and 1077 mg/kg dose groups. These two groups also had significantly reduced serum levels albumin and total serum protein when compared with the control group. Histopathological assessment showed degenerative changes in the liver. Aqueous leaf extract of *Chromolaena odorata* used in traditional medical practice may be safe whereas high doses may have deleterious hepatic effects

Keywords: *Chromolaena odorata*

## Introduction

There has been an upsurge in the use of medicinal plants in recent times due to their purported potencies in traditional medical practice. Although most botanical products are probably safe under normal doses, some are however known to be toxic at high doses, while others have the potential for adverse side effects [1]. *Chromolaena odorata* has been used in various parts of the world for medicinal [2] and nutritional purposes [3]. *Chromolaena odorata* is known to remediate metals [4]. These substances can therefore be readily transferred from plant through the food chain to herbivores that feed on them. Those at high risk are mainly people who rely on *Chromolaena odorata* for medications.

An in depth understanding of the toxicological profile of *Chromolaena odorata* is considered worthwhile. The present study is part of report of an in- depth risk assessment of *Chromolaena odorata* from our laboratory. This study was therefore designed to evaluate the hepatic effects of *Chromolaena odorata* in an animal model.

## Material and Methods

### Collection and of plant material

Leaves of *Chromolaena odorata* were collected between the months of June and August from the Local farms in Otolu Nnewi, Anambra State, Nigeria. Voucher specimen has been deposited at the Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, Umuahia. The botanical identification of the plant was confirmed by Prof J. C. Okafor of Tree Crops and Tropical Ecology Centre, No. 7 Dona Drive, Off Ihiala Street, Independence Layout, Enugu, Nigeria. Ground sample of about 10g was extracted with 100ml of de-ionized water by boiling. The boiled mixture was shaken vigorously for 10-15 seconds and allowed to stand for about 30 minutes and then filtered through a 150µm aperture sieve to obtain the aqueous extract. The aqueous extract was thereafter lyophilized to give a yield of 10.9%. The sample was then placed in air-tight containers and refrigerated.

Phytochemical studies: Phytochemical screening of the extract was done by the method of [5,6] for the presence of alkaloids, saponins,

glycosides, carbohydrates, flavonoids, tanins, proteins, terpenoids/steroids, resins, and oils.

### Animal husbandry

All animal experiments in this study followed the principles of laboratory Animal Care (NIH publication 1985). Male Wistar albino rats, weighing between 80-150g were obtained from University of Jos, Nigeria, Animal House and allowed to acclimatize for fourteen days at the experimental site (National Institute of Veterinary Research Vom). The animals were housed in steel cages under standard conditions of temperature, 22±3°C under light period of 12hr and 12hr darkness. Standard laboratory fresh pellets were collected weekly from Dangwon farm of National Veterinary Research Institute (NVRI), Vom, Plateau state, Nigeria, and the animals were allowed access to feed and deionized water ad-libitum before the commencement of the experiment. The LD<sub>50</sub> was determined using the method of Lorke [7].

## Sub-Chronic Toxicity Study

### Experimental design

Fifty five male albino rats weighing (80-150) g were used for the sub chronic studies. The animals were divided into four treatment groups (2, 3, 4 & 5,) and control group (1) of eleven rats- each. The animals were divided into five groups of eleven albino rats each and fed as shown below for 90 days by oral gavage

Group 1	0.5ml deionised water
Group 2	161.5 mg/kg <i>Chromolaena odorata</i>
Group 3	323mg/kg <i>Chromolaena odorata</i>
Group 4	583.5 mg/kg <i>Chromolaena odorata</i>
Group 4	583.5 mg/kg <i>Chromolaena odorata</i>
Group 5	1077 mg/kg <i>Chromolaena odorata</i>

The daily fluid and weekly feed intake of the animals were recorded. After 90 days of exposure the final body weights of the Wistar albino rats were taken, and thereafter, sacrificed under ether anaesthesia.

Blood was collected for hepatic biochemical analysis, while the liver, was harvested and weighed immediately fixed in 10% buffered formal saline and processed for histopathological studies. The serum was from the blood for liver function following standard laboratory procedures.

Alkaline Phosphatase (ALP) was determined by using the modified method of Bowers and Mc Combs (1996) [8], transaminases: aspartate amino transferase (AST) and alanine amino transferase (ALT) activities (Rietman and Frankel, 1957) [9], determination of lactate dehydrogenase (LDH) using methods of Vassault, (1983) [10]. Glucose was determined using the oxidase method by Trinder (1969) [11], albumin by the method of Doumas, et al., (1971) [12]. Determination of serum total bilirubin by Jendrassik and Grof, 1938 [13] and serum cholesterol was by the colorimetric method as described by Allain et al. (1974) [14], Calcium level by Heuck and Frei (1995) [15].

The liver was harvested and fixed in 10% formalin for 24 h and dehydrated in gradual ethanol (50 - 100%) cleared in xylene and embedded in paraffin. Sections (5µm thick) were prepared and stained with H&E dye and periodic acid-Schiff (PAS) stains for photo microscopic observation.

#### Statistical Analysis:

Fluid and feed consumption, animal body and liver weights and biochemical parameters were evaluated by employing the Mann Whitney test, and groups were considered to be significantly different if  $p \leq 0.05$  using the statistical package of Social Sciences (SPSS) software version 13.0.

#### Results

There were no mortality or any behavioral changes observed up to dose level of 1600mg/kg. However the animals manifested visible toxic signs, and recorded mortalities in the dose levels of 2900 to 5000mg/kg. Behavioral patterns in the high dose groups showed functional changes, such as, gait disturbances, loss of spinal reflex breathing irregularities and passage of loose feces. Lethality was observed from week 4 to 6. These signs of toxicities were not seen in the lower dose groups and the controls in the course of the study. Table 1 shows the effect of aqueous extract of *Chromolaena odorata* on fluid and feed intake, relative liver weight and the initial/final body weights of control and treated rats. There were significant changes between the control untreated group and the 1077 mg/kg in the fluid intake  $30.16 \pm 4.39$  and  $27.55 \pm 3.43$  ml, feed intake  $21.02 \pm 1.73$  and  $18.05 \pm 1.93$ g and relative weight of liver  $3.34 \pm 0.16$  and  $4.39 \pm 0.69$  respectively.

Figure 1 shows the percentage body weight changes in wistar albino rats treated with aqueous of *Chromolaena odorata* for 90 days. There was a progressive weekly body weight increase in all the groups. However the percentage weekly bodyweight gain was least in the 1077 mg/kg *Chromolaena odorata* dose group.

Table 2 shows the serum liver enzyme markers. Serum aspartate aminotransferase activity increased significantly at 585.5mg/kg [ $372.72$  u/L ( $p < 0.05$ )] and 1077mg/kg [ $337.02$ u/L ( $p < 0.05$ )] dose groups, while increase in serum level of lactate dehydrogenase was significant in the 1077mg/kg [ $4167.82$ u/L ( $p < 0.05$ )] dose group when compared with the control group. No significant treatment-related effects were observed in alkaline phosphatase and alanine aminotransferase levels in all the treated groups when compared with the control. The AST/ALT ratio ranged from 2.25 in the control untreated group to 3.62 in the 538.5 and 1077mg/kg *Chromolaena odorata* treated groups.

The effect of the aqueous extract of *Chromolaena odorata* on some serum liver indices is shown in Table 3. Serum calcium and cholesterol in all the treated groups showed no significant ( $p < 0.05$ ) difference when compared to the control. Total bilirubin levels in the 1077mg/kg *Chromolaena odorata* [ $3.07$ µmol/L] and 538.5 mg/kg *Chromolaena odorata* [ $2.29$ µmol/L] dose groups showed a significant increase ( $p < 0.05$ ), when compared with control untreated [ $1.64$ µmol/L], while the total protein [ $74.62$ g/L] and albumin [ $33.55$ mmol/L] were significantly ( $p < 0.05$ ) decreased at 1077mg/kg *Chromolaena odorata* dose level compared with the control protein of [ $81.85$ g/L] and albumin of [ $40.66$ mmol/L].

Figures 2A to 2D show the dose dependent lesions characterized by circulatory disturbances, degenerative, necrotic and inflammatory changes, produced in the liver by the administration of the aqueous extract of *Chromolaena odorata* for 90 days. Sections from untreated (control) rats, show classical liver lobules, with histomorphological normal hepatocytes radiating from the central vein to the portal areas (Figure 2A). Similarly, a dose -dependent depletion of glycogen was also observed (Figure 3A to 3C).

## Discussion

The phytochemical screening of the aqueous extract revealed the presence of several active ingredients identified in the plant similar to what was earlier isolated by [16]. This may account for the pharmacological and nutritional properties associated with the plant leaves. The LD<sub>50</sub> value of 2154mg/Kg suggest that aqueous extract of *Chromolaena odorata* may not possess significant acute toxic effects. Van Leeuwen and Vermie (2007) [17], suggested that chemical substances with LD<sub>50</sub>>500mg/Kg but <5000mg/Kg are at worst slightly toxic or not significantly toxic. Effect of the administration of *Chromolaena odorata* extract on fluid and feed in this study showed treatment related effects and tends to agree with Orisakwe et al. (2004) [18], who found a statistically significant reduction in daily fluid and feed intake that was dose dependent after administration of *Hibiscus sabdariffa* aqueous extract to male rats for 90days. The decreased rate of fluid and feed consumption intake would probably have contributed to the functional changes within the first 3weeks of the study, and consequently high rate of mortality recorded in the 1077mg/kg dose group from the 4th up to the 6<sup>th</sup> week of the exposure.

The relative nontoxic nature of the leave was buttressed by the fact that adverse toxic effects were observed only in the very high dose groups. Some of the adverse toxic effects observed were: breathing irregularities, piloerection, respiratory depression, gait disturbances, and loss of spinal and pain responses. Body weight is an important factor that is used to monitor the health of the animals [19]. Decrease in body weight is frequently the first indicator of the onset of adverse effect. A dose, that causes 10% or more reduction in body weight, is considered to be a toxic dose. It is considered to be a dose, which produces minimum toxic effect, irrespective of whether or not it is accompanied by any other changes [19]. The progressive increase in body weight as shown by all the animals in the very low (161.5mg/kg) dose group which differed from the control group throughout the experimental period, may be indicative of a growth inducing effect of *Chromolaena odorata* leave at very low dose. This observation tends to agree with the findings of [20], who investigated the nutritional potential of *Chromolaena odorata* leaf on laying

hens and found that up to 5% dietary intake had no serious health implications, but was rather beneficial. The high dose group exhibited toxic weight reduction of more than 10%.

Reduced growth rate and mortality observed in the highest dose group were not totally unexpected as death of livestock that consumed *Chromolaena odorata* leaf has previously been reported [21]. Nwokolo, (1987)[22] investigated the digestibility of minerals and proteins in the leaf meals of Siam weed and reported that the average to low availability of the leaf meal was due to anti-nutritional factors like saponin content in the leaves. The presence of saponins has been associated with poor growth rate and depressed nutrient utilization in monogastric animals, as observed in this study [23].

Liver is the major site of xenobiotic metabolism in mammals. Several hepatotoxicants have been established to stimulate liver cell division and tissue repair, since tissue repair is a simultaneous biological response that accompanies injury [24]. All the major functions of the liver can be detrimentally altered by liver injury resulting from acute or chronic exposure to toxicants. Biochemical assays provide a relatively simple method for screening populations for potential liver necrosis or damage caused by occupational or environmental toxins [25]. Principal target organ of toxicity in this study is the liver, probably because it is the first organ to encounter nutrients, phytotoxins.

Administration of the extract was associated with dose related changes in the liver: dose related hepatomegaly, significantly ( $p<0.05$ ) elevated serum liver marker enzymes (LDH and ALT) and bilirubin, with a significant ( $p<0.05$ ) reduction in the serum total protein and albumin fraction. Yakubu et al. (2005)[26] made similar observations concluding that this was an indication of hepatotoxicity. These effects were confirmed by the dose dependent histological findings of inflammatory, multi focal centrilobar degenerative changes seen in the high dose groups. But there were some minimal histological changes at these dose groups. However toxicity was more evident at the 538.5 and 1077mg/kg dose groups. Decrease in protein level may be a reflection of the catabolic potentials of *Chromolaena odorata* at very high doses, and may also account for the decrease in the animal total body weight at this dose level.



Elevation of serum levels of ALT is usually more than that of AST in extra hepatic obstruction, acute hepatitis, and toxic hepatitis, while the reverse is case in cirrhosis of liver and alcoholic liver disease, intrahepatic neoplasm, and hemolytic jaundice [27]. In this study the observed greater increase in serum AST is suggestive of cirrhotic process going on in the liver, and the histological findings tend to confirm this finding. Decrease in the serum level of total protein and albumin might be attributable to derangement in the normal metabolic and synthetic pathways of proteins in the liver. Consequently, the raised total bilirubin is also suggestive of a toxic insult of the plant extract on the secretory ability of the liver and normal functioning of the organ.

Histological findings from this study also showed lesions in the liver presenting as severe inflammatory multi-focal centrilobar degenerative changes probably indicative of a toxic effect, resulting from the administration of the aqueous extract by a mechanism that is not very clear. Fatty change in the liver is usually associated with enlargement of the organ: hepatomegaly [28]. Several toxins and drugs have been found to induce fatty liver through different mechanisms [29]. Sheah et al. 1997 [30] postulated that increased synthesis of fatty acids in the liver, increased delivery of free fatty acids to the liver and decreased  $\beta$ -oxidation of free fatty acids may be responsible for the accumulation of fat in the liver. Fat in hepatocytes causes cellular dysfunction may render the liver more vulnerable to any factor that leads to inflammation [31]. This may well explain the dose dependent hepatomegaly at 538.5 and 1077mg/kg dose groups in this study. The histological observation of depletion of glycogen storage that was dose dependent may be an indication of hepatotoxicity in the 538.5 and 1077mg/kg dose groups which could be associated with the high concentrations of terpenoids found in the phytochemical studies of the aqueous extract of *Chromolaena odorata*. The ability of triptolide, a diterpenetriepoxide that was isolated from a Chinese medicinal plant to induce hepatotoxicity has also been reported [32].

The findings showed a non-significant decrease in serum cholesterol level which may be an indication of decreased synthetic activity of the

liver, though on the surface this may seem protective. Phenols and tannins constituents of the leaf extract have been reported to possess antilipidemic activities [33], and may therefore account for this particular observation. In conclusion therefore, although the present study reveals that *Chromolaena odorata* harvest from Nnewi, Nigeria, has environmental, nutritional and medicinal values; its ability to produce toxicity of the liver at high dose levels in Wistar albino rats suggest that human exposure for a long time needs to be closely monitored.

## Reference

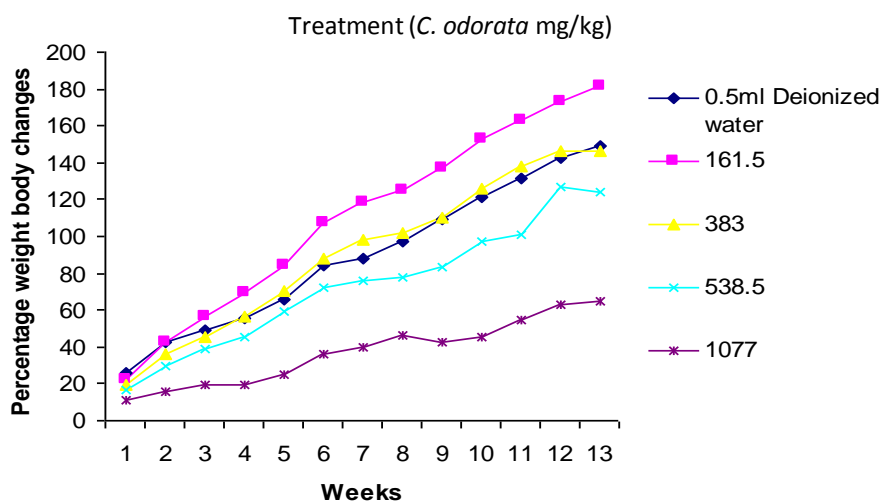
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**Table 1:** Effect of aqueous extract of *Chromolaena odorata* on fluid /feed intake, relative weight and the initial/final body weights of control and treated rats

<i>C.odorata</i> mg/kg	Fluid ml/animal/day	Feed g/animal/day	relative weight	Initial body weight(g)	Final body weight(g)
0.5ml deionised water	30.16±4.39	21.02±1.73	3.34±0.16	117.00±31.00	314.80±32.20
161.5	28.42±3.08*	20.00±1.84*	3.46±0.40	102.70±17.41	286.96±22.30
323	28.22±2.96*	19.07±1.24*	3.76±0.66	110.17±13.31	261.33±18.81
583.5	27.47±4.16*	18.35±1.45*	3.96±0.05*	106.09±21.88	230.43±85.10
1077	27.55±3.43*	18.05±1.93*	4.39±0.69*	142.00±1.73	248.43±23.79

Values are expressed as mean ± SD for n=11 \*significantly different from control (p< 0.05).

**Fig. 1:** Effect of aqueous extract of *Chromolaena odorata* on weekly body weights of Wistar Rats

Marker Enzymes	Control (0.5ml) deionised water	161.5 mg/kg	323 mg/kg	538.5 mg/kg	1077mg/kg
Alanine aminotransimase U/L	104.23 ± 39.57	81.63 ± 22.97	82.00 ± 21.89	102.89 ± 35.71	93.16 ± 22.55
Aspartate aminotransaminase U/L	234.96 ± 71.72	281.71 ± 58.42	287.01 ± 54.00	372.72 ± 21.91*	337.02 ± 51.82*
AST/ALT ratio	2.25	3.45	3.50	3.62	3.62
Alkaline Phosphatase U/L	178.00 ± 58.21	192.45 ± 68.21	169.18 ± 83.74	181.64 ± 69.91	151.09 ± 28.26
Lactate Dehydrogenase U/L	2373 ± 1147.10	2682.09±855.33	2826.45±652.42	2783.18±4986.48	4167.82±1046.38

Data expressed as mean ± SD  
\*Significantly different from control (n = 11)

**Table 2:** Marker Enzymes in control and treated rats of sub-chronic toxicity study

Marker Enzymes	<i>Chromolaena odorata</i> mg/kg/enzyme activity				
	Control (0.5ml) deionised water	161.5 mg/kg	323 mg/kg	538.5 mg/kg	1077mg/kg
Alanine aminotransaminase U/L	104.23 ± 39.57	81.63 ± 22.97	82.00 ± 21.89	102.89 ± 35.71	93.16 ± 22.55
Aspartate aminotransaminase U/L	234.96 ± 71.72	281.71 ± 58.42	287.01 ± 54.00	372.72 ± 21.91*	337.02 ± 51.82*
AST/ALT ratio	2.25	3.45	3.50	3.62	3.62
Alkaline Phosphatase U/L	178.00 ± 58.21	192.45 ± 68.21	169.18 ± 83.74	181.64 ± 69.91	151.09 ± 28.26
Lactate Dehydrogenase U/L	2373 ± 1147.10	2682.09 ± 855.3	2826.45 ± 652.4	2783.18 ± 4986.	4167.82 ± 1046 .38

Data expressed as mean ± SD

\*Significantly different from control (n = 11)

**Table 3:** Biochemical markers in control and treated rats of sub-chronic toxicity study

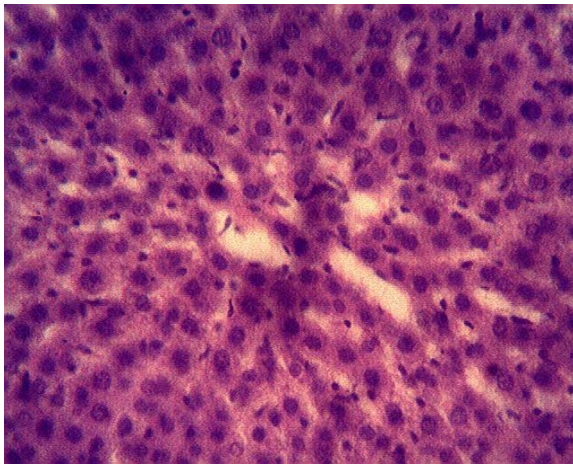
Parameter	<i>Chromolaena odorata</i> mg/kg/Enzyme Activity				
	Control (0.5ml) deionised water	161.5 mg/kg	323 mg/kg	538.5 mg/kg	1077mg/kg
Calcium mmol/L	2.35 ± 0.21	2.21 ± 0.25	2.36 ± 0.17	2.36 ± 0.14	2.32 ± 0.12
Total bilirubin umol/L	1.64 ± 0.39	2.00 ± 0.95	2.06 ± 0.69	2.29 ± 0.49	3.07 ± 0.96*
Total protein g/L	81.85 ± 5.78	81.89 ± 4.35	82.82 ± 5.47	79.16 ± 8.65	74.62 ± 1.84*
albumin mmol/L	40.66 ± 3.47	42.32 ± 3.13	42.46 ± 3.29	40.67 ± 4.52	33.55 ± 5.13*
cholesterol mg/dl	78.82 ± 11.17	69.91 ± 8.31	72.55 ± 14.97	69.09 ± 9.21	83.18 ± 14.37
glucose mmol/L	4.96 ± 0.74	5.96 ± 2.66*	5.16 ± 0.94*	7.09 ± 2.00*	6.83 ± 0.40*

Data expressed as mean ± SD

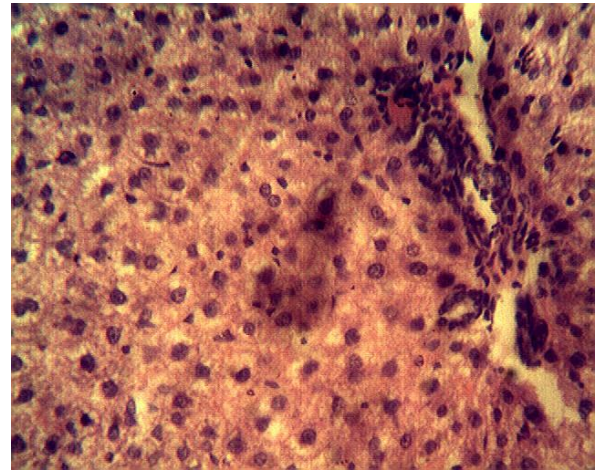
\*Significantly different from control (n = 11)



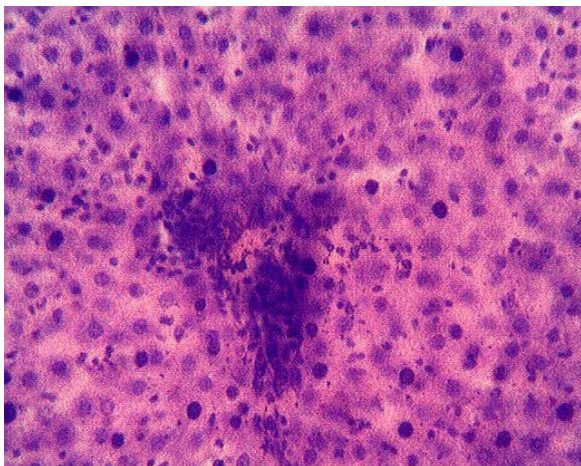
## Histopathology



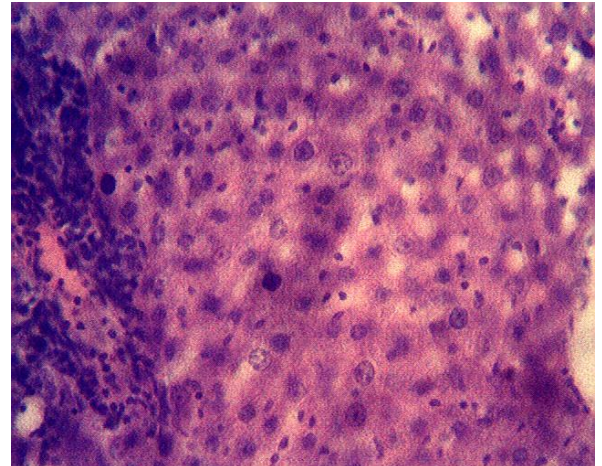
**Fig 2:[A]** H & E X 400 Section of liver from control showing classical normal liver lobules, with histomorphological normal hepatocytes radiating from the central vein (cv) to the portal areas.



**Fig. [2B]**- X 400. Liver section of rat treated with low dose (323mg/kg) of the extract, showing mild hepatocyte necrosis (arrows) and mild leucocyte aggregation (ml) around the portal vein (pv).

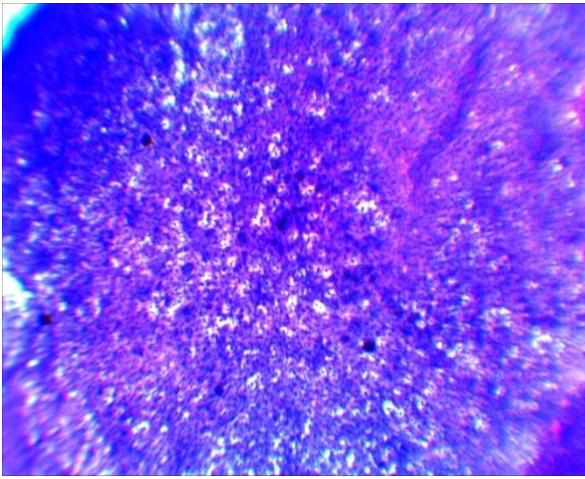


**Fig 2[C]**- X 400. Liver section of rat treated with moderate dose (538.5mg/kg) of extract. The lesions in this section are similar to those of the high dose but milder showing isolated hepatocytes necrosis (arrows) and leucocytic infiltration restricted to the periportal areas.

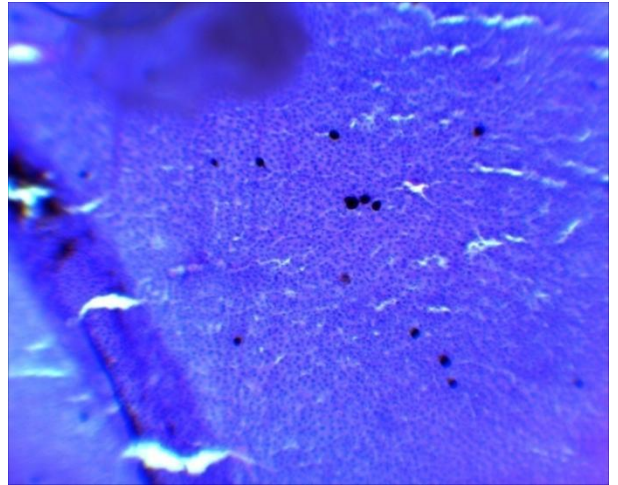


**Fig 2[D]** H & E X 400. Liver section of rats treated with high dose (1077mg/kg), showing the central vein (cv), isolated hepatocyte necrosis, (arrows) and moderate mononuclear leucocytes infiltration of the portal area(ml). The lobules are generally mildly to moderately hyperemic, especially in the centrilobular area, with hypertrophy of the Kupffer cells.

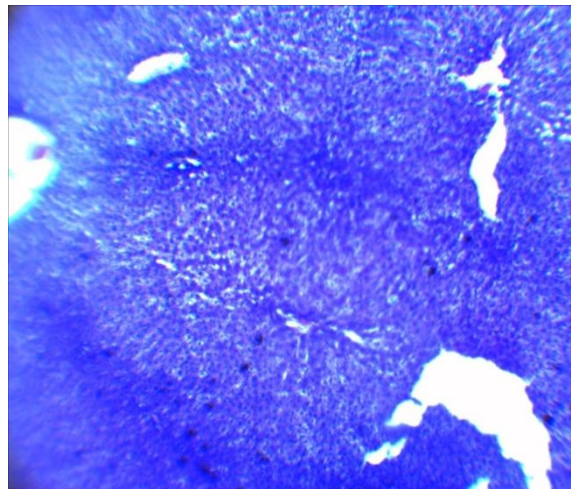
### Histopathology



**Fig 3[A]:** Liver section of untreated rats showing normal liver glycogen storage



**Fig 3[B]:** Liver section of rat treated with low dose 323mg/kg of extract showing slight glycogen depletion.



**Fig 3[C]:** Liver section of rat treated with high dose 1077mg/kg of extract showing moderate depletion of glycogen.