EFFECTS OF ETHANOLIC EXTRACTS OF *Daturametel* PARTS ON LIVER FUNCTION OF MALE ALBINO RATS

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Abstract: This study investigated the effects of ethanolic extracts of *Daturametel* parts on liver function of male albino rats. The plant parts are used for several reasons, including its medicinal and psychoactive activities. Thirty-five albino rats were used and distributed into 7 groups of 5 rats each. The extracts were administered to the test animals for 7 days before being sacrificed and blood collected for biochemical analysis. The serum activities of AST and ALT significantly ($p<0.05$) increased in all test groups, except (AST) in Group 4 (compared with Group 1). In Groups 3, 4, 5 and 7, ALP decreased significantly, but increased non-significantly in Groups 2 and 6 compared to the control. Total protein increased non-significantly ($p>0.05$) in Groups 2, 4, 5 and 6, but reduced non-significantly in Groups 3 and 7. Total bilirubin and indirect bilirubin increased significantly ($p<0.05$) in all groups administered the extracts. Direct bilirubin increased significantly ($p<0.05$) in Group 2 and 6, but increased non-significantly in Groups 3, 4, 5 and 7. Photomicrographs of some of the liver sections from rats administered the extracts show evidence of inflammatory cellular infiltration within the central canal and centrilobular hepatocytes, central canal rupture, degenerating hepatocytes, vacuolations, perivascular necrosis and widened sinusoids. However, portal tract and periportal hepatocytes appear fairly intact. These results show that despite the acclaimed medicinal effects of different parts of *Daturametel*, it can cause liver toxicity and may induce inflammatory cellular infiltration within some regions of the liver.

Keywords: *Daturametel*, hepatocyte, inflammation, liver, photomicrograph

Introduction

Medicinal plants are used for their high degree potential in the management and treatment of certain disease conditions. The medicinal value of plants is believed to be as a result of their bioactive phytochemical component which enables them to perform lots of physiological functions in the body of humans (Akinmoladun*et al*., 2007). Some of the chemicals include saponins, alkaloids, flavonoids, tannins, terpenoids and phenolic compounds. Although plants possess medicinal effects, there also exist some of the chemicals in the plants which may cause adverse effects to humans, especially when they are consumed in large amount; e.g. some alkaloids. In the primary health care system of some countries, they are now making use of traditional medicinal systems (Manas*et al*., 2012; Imo and Uhegbu,2015).

*Daturametel*which belong to Solanaceae family is a herbaceous and leafy plant. The common names include Devil's apple and Thorn apple. In Nigeria, it is called Miaramuo in Igbo; Apikan in Yoruba and Zakami in Hausa (Abdullahi*et al*., 2003). It is native and widely cultivated in Africa and Asia. In height, *Daturametel*can grow up to 200cm. The leaves of the plant are alternate and can be up to 21cm wide and 26cm long.*Daturametel* fruit can contain up to 300 seeds. The fruit usually split open when it is ripened to release its many seeds.

In most parts of Nigeria (especially in the northern part), *Daturametel*is sometimes cultivated, but most times seen growing as a weed in areas like dumpsites and abandoned farmlands. The leaves and seeds can be used for different purposes and in many ways because of its believed psychoactive activities (Kutama*et al*., 2010). The different parts of the plant could be abused by some youths who are more consumers or users of the plant parts and are likely to suffer the dangers of smoking parts of the plant. *Daturametel*extracts are potent poison when consumed regularly and could result to health challenges which may possibly lead to death. Nuhu (2002) reported that *D. metel*contains the tropane alkaloids and are used for some purposes such as sedative, mydriatic and anti-spasmodic agents. Some other constituents such as scopolamine, hyoscyamine and withanolides are also present in *Daturametel.* It is also believed that parts of this plant possess anaesthetic, anti-tussive, hallucinogenic, anti-asthmatic, narcotic and hypnotic effects. The leaves are often used as a local application for remedies of rheumatic swellings of the joints, eczema, allergy, scabies and glandular inflammations. It is also used externally for treating earache and usually smoked to relieve certain conditions such as spasmodic asthma. The seeds are also used externally for treating piles (Yusuf *et al*., 2009). The seeds, leaves and roots are reported to be used in conditions such as fever with catarrh, diarrhoea and skin diseases (Khaton and Shaik, 2012).

The aim of this present research study is to investigate the effects of ethanolic extracts of parts of *Daturametel* on liver function of male albino rats. The results of this study will aid in revealing and establishing the expected effects of parts of *Daturametel* on liver function of people who usually make use of parts of the plant.

Materials and Methods

Plant materials

The parts (leaves, fruits and seeds) of *Daturametel* were gathered at Wapan-Nghaku: popularly known as T-junction in Wukari, Nigeria. The parts of the plant were cleaned and sun-dried. Each of the dried parts was ground to powder. The powders were macerated using 70% ethanol in a clean container for 2 days and filtered. The ethanol in the filtrates was eliminated by evaporation and the concentrate reconstituted in normal saline prior to the experiment.

Experimental animals

A total of 35 healthy albino rats (males) which were eight weeks of age were used. The male albino rats were bought and kept in the Animal House section of Biochemistry Department in Federal University Wukari, Nigeria. The animals were allowed to acclimatize for a period of one week under the Institutional standard laboratory conditions peculiar

for the use of experimental animal with free access to water and feed.

Experimental design

The experimental rats were distributed into 7 different groups of 5 rats each. Group one served as normal control and received a placebo of normal saline. Group two received 300 mg/kg b.w., while group three received 600 mg/kg b.w. of the ethanolic leaf extract of *Daturametel*. Group four received 300 mg/kg b.w., while group five received 600 mg/kg b.w. of the ethanolic seed extract of*Daturametel*. Group six and group seven received 300 mg/kg b.w. and 600 mg/kg b.w. of the ethanolic fruit extract of *Daturametel*, respectively. All the test animals received the respective extracts for a period of 7 days. The administration of the different plant extracts was through oral route. Throughout the study, the albino rats were given access to water and feed *ad libitum*.

Blood collection

The albino rats were starved overnight after the administration of the plant extracts. They were anaesthetized with the use of chloroform and sacrificed. Blood from each rat was collected by means of cardiac puncture and dispensed into a clean sample tube. It was allowed to clot after standing for about fifteen minutes, thereafter spun using a centrifuge at 4000 rpm for exactly ten minutes. Pasteur pipette was used to separate the serum from the clot and the serum dispensed into sterile sample tubes for some biochemical analysis.

Biochemical and histological analysis

Serum ALT, AST, ALP, total bilirubin, direct bilirubin, indirect bilirubin and total protein levels were determined with the use of an auto-analyzer (SelectraProM). After the animal sacrifice, the liver of each animal of the seven groups were harvested and examined histologically. Photomicrographs of different sections of the liver were taken.

Statistical analysis

After the biochemical analysis, the data was analyzed statistically using ANOVA and further with Duncan Multiple Comparisons with the use of Statistical Package for Social Sciences (SPSS) version 21. The means were compared for significance at $p \leq 0.05$. Data were presented in form of mean ± SD (n=5).

Results and Discussion

The results of the study are presented below. AST and ALT increased significantly ($p<0.05$) in all the groups, except in Group 4 where AST increased non-significantly ($p>0.05$) compared with normal control. Alkaline phosphatase (ALP) decreased significantly ($p<0.05$) in Groups 3, 4, 5 and 7, but increased non-significantly in Groups 2 and 6 compared to the normal control.Total protein levels increased non-significantly ($p>0.05$) in Groups 2, 4, 5 and 6, but reduced non-significantly in Groups 3 and 7 compared with the normal control.Total bilirubin and indirect bilirubin increased significantly ($p<0.05$) in all Groups compared with the normal control. Direct bilirubin increased significantly ($p<0.05$) in Groups 2 and 6, but increased non-significantly ($p>0.05$) in Groups 3, 4, 5 and 7 compared with the normal control.

Table 1: Concentrations of liver enzymes (IU) in rats administered ethanolic extracts of *Daturametel* parts

Biochemical parameters	Group 1 (Normal control)	Group 2 (300 mg/kg bw of leaf)	Group 3 (600 mg/kg bw of leaf)	Group 4 (300 mg/kg bw of seed)	Group 5 (600 mg/kg bw of seed)	Group 6 (300 mg/kg bw of fruit)	Group 7 (600 mg/kg bw of fruit)
ALT	20.75 ± 1.0^{a}	31.75 ± 2.06^{b}	28.78 ± 4.61^{b}	30.83 ± 2.16^{b}	28.55 ± 2.42^{b}	30.50 ± 0.62^{b}	29.88 ± 2.24^{b}
AST	88.05 ± 5.65^{a}	$121.10 \pm 8.67^{b,c}$	128.95 ± 7.31^{c}	95.85 ± 5.63^{a}	$120.88 \pm 5.21^{b,c}$	116.58 ± 11.52^{b}	$126.28 \pm 4.09^{b,c}$
ALP	289.60 ± 9.97^{a}	301.38 ± 12.02^{a}	208.53 ± 11.25^{b}	190.33 ± 10.39^{b}	269.85 ± 21.42^{c}	294.20 ± 8.12^{a}	204.73 ± 7.78^{b}

Results represent mean ± standard deviation (n=5).

Mean in the same row, with different letters of the alphabet as superscript are statistically significant ($p<0.05$).

ALT= Alanine aminotransaminase, AST= Aspartate aminotransaminase, ALP= Alkaline phosphatatse.

Table 2: Concentration of total proteins (g/l) in rats administered ethanolic extracts of *Daturametel* parts

Biochemical parameters	Group 1 (Normal control)	Group 2 (300 mg/kg bw of leaf)	Group 3 (600 mg/kg bw of leaf)	Group 4 (300 mg/kg bw of seed)	Group 5 (600 mg/kg bw of seed)	Group 6 (300 mg/kg bw of fruit)	Group 7 (600 mg/kg bw of fruit)
Total proteins	60.60 ± 3.80^{a}	62.00 ± 2.98^{a}	58.85 ± 2.32^{a}	62.60 ± 3.63^{a}	61.53 ± 2.30^{a}	60.75 ± 3.03^{a}	60.00 ± 4.30^{a}

Results represent mean ± standard deviation (n=5).

The entire mean is statistically non-significant ($p>0.05$) compared with normal control.

Table 3: Concentrations of bilirubin (µmol/L) in rats administered ethanolic extracts of *Daturametel* parts

	Group 1 (Normal control)	Group 2 (300 mg/kg bw of leaf)	Group 3 (600 mg/kg bw of leaf)	Group 4 (300 mg/kg bw of seed)	Group 5 (600 mg/kg bw of seed)	Group 6 (300 mg/kg bw of fruit)	Group 7 (600 mg/kg bw of fruit)
TB	5.55 ± 0.35^{a}	7.05 ± 0.19^{b}	$6.85 \pm 0.13^{b,c}$	6.60 ± 0.24^{c}	6.58 ± 0.22^{c}	7.00 ± 0.23^{b}	6.55 ± 0.29^{c}
DB	2.40 ± 0.24^{a}	3.10 ± 0.36^{c}	2.58 ± 0.10^{a}	$2.70 \pm 0.36^{a,b,c}$	$2.65 \pm 0.10^{a,b}$	$3.05 \pm 0.31^{b,c}$	2.50 ± 0.22^{a}
IB	3.15 ± 0.13^{a}	3.95 ± 0.31^{b}	4.28 ± 0.05^{c}	3.90 ± 0.22^{b}	3.93 ± 0.15^{b}	3.95 ± 0.13^{b}	$4.05 \pm 0.13^{b,c}$

Results represent mean ± standard deviation (n=5).

Mean in the same row, with different letters of the alphabet as superscript are statistically significant ($p<0.05$).

TB= Total bilirubin, DB= Direct bilirubin, IB= Indirect bilirubin.

The results obtain in this study showed an increase in the serum activities of serum aspartate aminotransaminase (AST) and alanine aminotransaminase (ALT). The elevation in the levels of liver enzymes in serum have been reported as an indication showing cellular leakage and malfunctioning of cell membrane in the liver by Moore *et al*. (1985) and Imo *et al*. (2013). The increase in AST and ALT levels in all the groups and ALP (in Groups 2 and 6: administered low doses of leaf and fruit extracts, respectively) shows the evidence of possible hepatotoxicity and the possibility of cellular leakage of these

liver enzymes into the blood which is caused by the administration of *Daturametel* leaves, seeds and fruits extracts. The liver is an organ that plays many roles in human and some other animals which includes metabolic functions. As a result, it is likely to suffer xenobiotic injury since it plays a central role in the metabolism of xenobiotic (Sturgill and Lambert, 1997). The hepatotoxicity is believed to be as a result of the toxic alkaloids present in *Daturametel* leaf, seed and fruit, since all plant parts of *Datura* has been reported to be poisonous. The poisonous effect is believed to be due to certain toxic tropane alkaloid or scopolamine, atropine and hyoscyamine present in it, which can cause neural toxicity (Ko, 1999). However, the reduction in the activity of serum ALP in the groups of rats administered the seed (300 mg/kg and 600 mg/kg), fruit (600 mg/kg) and leaf (600 mg/kg bw) extracts show that despite its hepatotoxic effects, *Daturametel* may also possess protective effects on the liver, especially on the portal tract or bile duct.

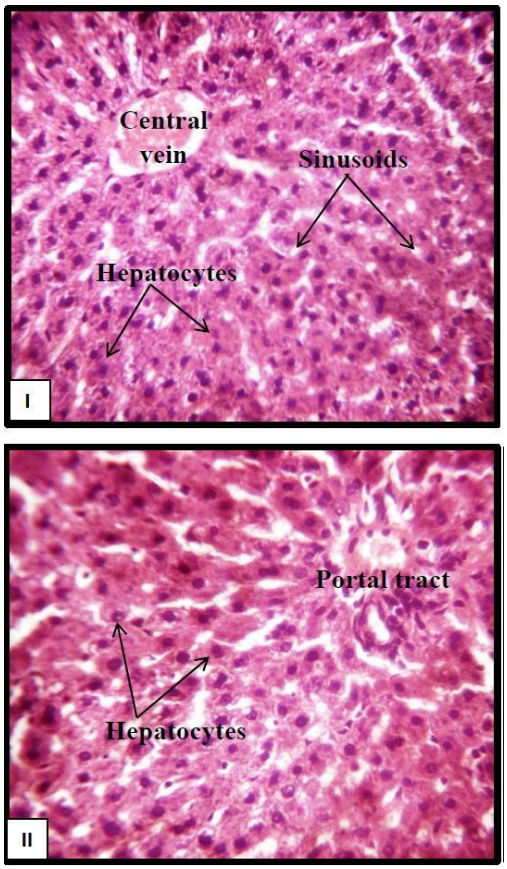

Plate 1: Photomicrographs of the liver section from normal control rat (Group 1) showing normal histoarchitecture of the hepatic tissue. Normal hepatocytes, central vein, portal tract and radially distributed sinusoids are shown (Stain: H & E; Mag: I & II-x400).

Total protein level increased non-significantly ($p>0.05$) in Groups 2, 4, 5 and 6, but reduced non-significantly in Groups 3 and 7 compared with group one (normal control). This result shows the possibility that low dose (300 mg/kg bw) of the fruit, seed and leaf extracts encouraged protein synthesis, with evidence of an increased protein concentrations when compared with the normal control. The effect of the different extracts of *Daturametel* on protein level was dose-dependent (the higher the concentration of the extracts, the lower the protein level of the animals). Higher dose (600 mg/kg bw) of the fruit and leaf extracts caused reductions in the serum protein levels, showing there is an interference in the processes of protein synthesis.

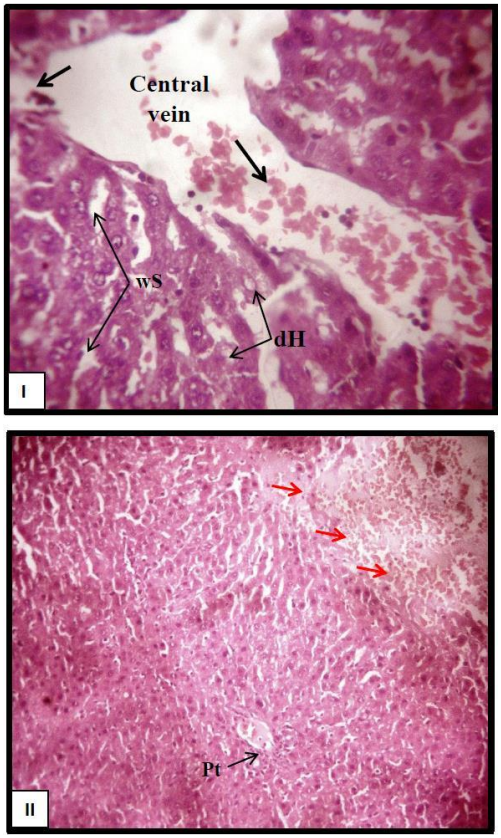

Plate 2: Photomicrographs of the liver section from rat administered 300 mg/kg bodyweight of *Daturametel* leaf extract (Group 2) showing evidence of central canal rupture (black thick arrows), degenerating hepatocytes (dH), perivascular necrosis (red arrows) and widened sinusoids (wS). However, the portal tract (Pt) and periportal hepatocytes appear fairly intact (Stain: H & E; Mag: I–x400; II-x200).

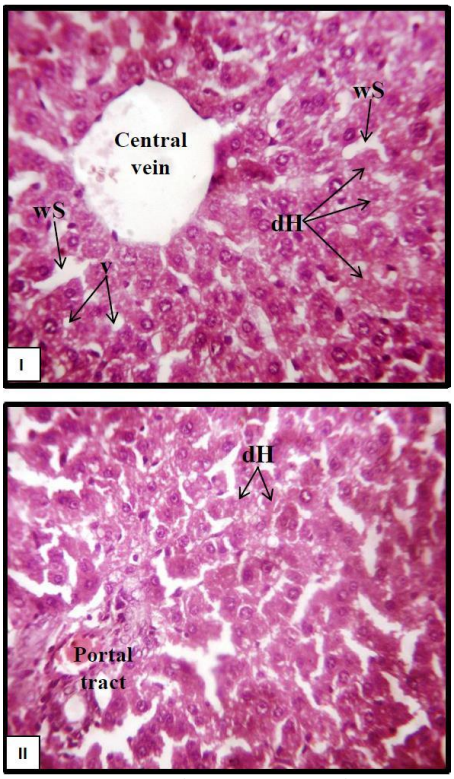

Plate 3: Photomicrographs of the liver section from rat administered 600 mg/kg bodyweight of *Daturametel* leaf extract (Group 3) showing dilated central vein, degenerating hepatocytes (dH), vacuolations (v), widened sinusoidal spaces (wS), but fairly intact portal tract (Pt) (Stain: H & E; Mag: I & II-x400)

It is possible that the reductions in protein levels in Groups 3 and 7 may be due to the destruction of more haemoglobin (as a result of the high dosage and toxic phytochemicals present in *Daturametel*) and degenerations of some hepatocytes, among other causes, since most proteins found in the plasma are mostly synthesized by the hepatocytes and thereafter secreted into circulation. Nair(2006) has reported that serum protein level is a marker of the synthetic function of the liver and is used as a very helpful guide in assessing the severity of the liver damage. It therefore means that low concentrations of the fruit, seed and leaf extracts of *Daturametel* may improve the synthetic function of the liver.

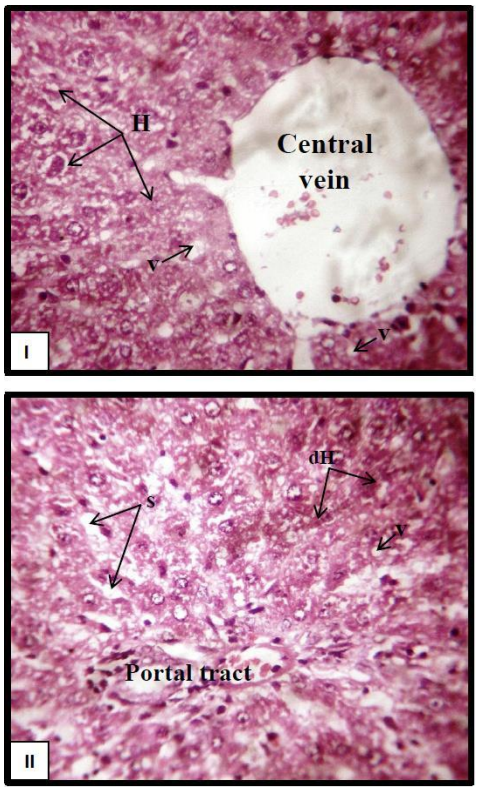

Plate 4: Photomicrographs of the liver section from rat administered 300 mg/kg bodyweight of *Daturametel* seed extract (Group 4). The portal tract and sinusoids (s) shown are fairly intact, whereas the central vein appears markedly dilated. Most of the hepatocytes are degenerating (dH) and some appear vacuolated (v) (Stain: H & E; Mag: I & II-x400).

The concentrations of total bilirubin and indirect bilirubin increased significantly ($p<0.05$) in all groups administered the different parts of *Daturametel* extracts when compared with Group 1 (the normal control). Direct bilirubin increased significantly ($p<0.05$) in Groups 2 and 6, but increased non-significantly ($p>0.05$) in Groups 3, 4, 5 and 7 when compared with Group 1. The elevation of bilirubin concentration above normal level in the serum or tissues is known as jaundice. Jaundice can exist due to toxic or infectious diseases of the liver. The disease conditions may include obstruction of the flow of bile from bile duct and hepatitis B. Bilirubin is produced in the liver as an intermediate product of haemoglobin breakdown (Reham*et al*., 2009). The elevated levels of total, indirect and direct bilirubin in all groups administered the ethanolic fruit, seed and leaf extracts of *Daturametel* is believed to be caused by toxic effect of the phytochemicals present in the plant parts, thereby resulting to destruction of some haemoglobin and possibly damaging some red blood cells. If the hepatocytes are destroyed, the liver may therefore not have the adequate ability to handle bilirubin properly.

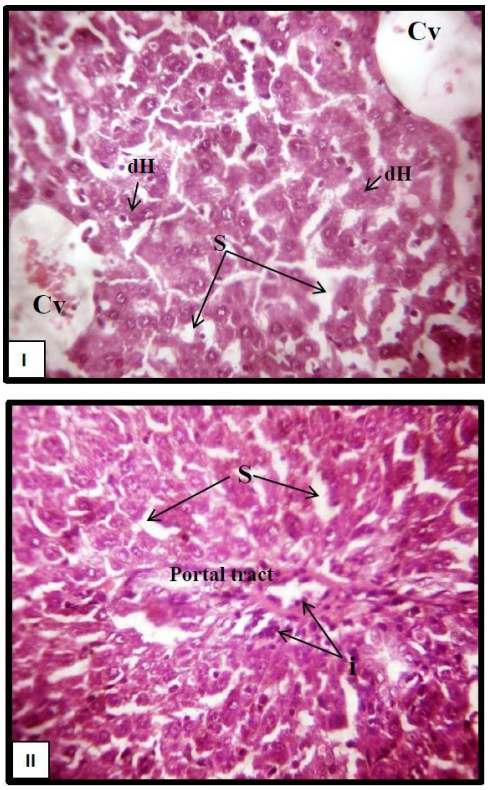

Plate 5: Photomicrographs of the liver section from rat administered 600 mg/kg bodyweight of *Daturametel* seed extract (Group 5). Dilated central veins (Cv), degenerating hepatocytes (dH) and mildly increased sinusoids (s) are observed**.** The portal tract appears fairly distorted and mild inflammatory cellular infiltration (i) is seen (Stain: H & E; Mag: I & II- x400).

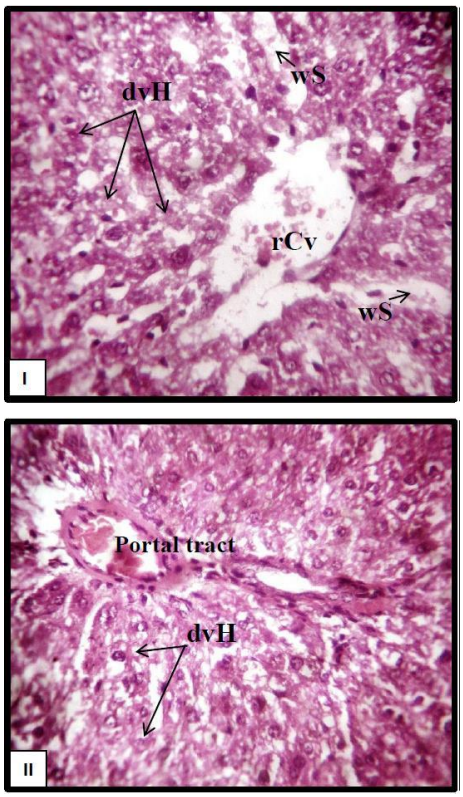

Plate 6: Photomicrographs of the liver section from rat administered 300 mg/kg bodyweight of *Daturametel* fruit extract (Group 6) showing central canal rupture (rCv), degenerating and vacuolated hepatocytes (dvH) and widened sinusoids (wS). However, intact portal tract is observed (Stain: H & E; Mag: I & II- x400).

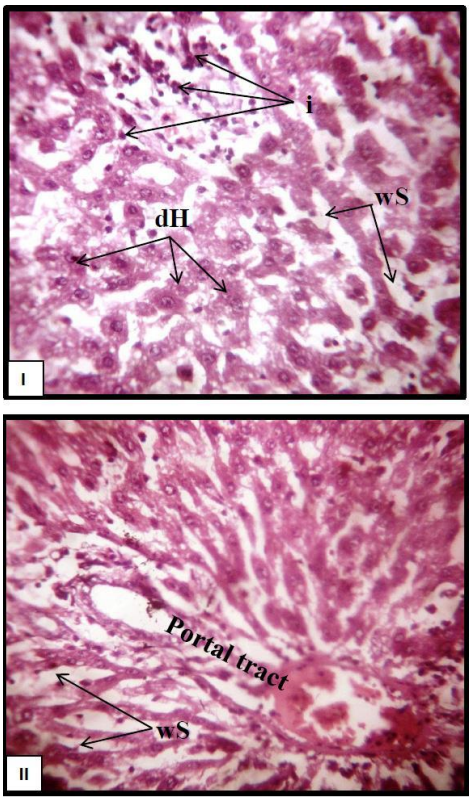

Plate 7: Photomicrographs of the liver section from rat administered 600 mg/kg bodyweight of *Daturametel* fruit extract (Group 7) showing inflammatory cellular infiltration (i) within the central canal and centrilobular hepatocytes. Degeneration (dH) and shrinking of most hepatocytes are observed and resultant marked sinusoidal widening (wS) is noted (Stain: H & E; Mag: I & II- x400).

Conclusion

The results of this study show that administration of the fruits, seeds and leaves extracts of *Daturametel* caused liver toxicity, alteration of some of the liver functions and improved protein synthesis at low dosage. This shows that despite the acclaimed medicinal effects of parts of *Daturametel*, it can induce liver toxicity and may cause inflammatory cellular infiltration within some regions of the liver, thereby causing deleterious effect on the liver and altering its functions. However, low doses of the extracts encouraged protein synthesis, showing the possibility that it may improve liver function. It is possible that the individual phytochemicals present in the crude plant parts extracts used in this study possesses different effects and different mechanisms of actions. Therefore, the use of *Daturametel* extracts should be specifically based on medical or pharmacological needs since it possesses both good and deleterious effects. Further research is therefore necessary on the use of parts of *Daturametel* to actually isolate and experiment the different phytochemical components and to ascertain the chemical(s) responsible for its positive effects and the mechanism of action.

Conflict of Interest

All contributing authors declare no conflict of interest.

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