EFFECTS OF ETHANOLIC EXTRACT OF DATURA STRAMONIUM LEAVES ON THE HISTOMORPHOLOGY AND BIOCHEMICAL INDICES OF LIVER AND KIDNEY FUNCTIONS IN RATS

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Background: *Changes in histomorphology and some indices of liver and kidney functions were studied in rats administered doses of ethanolic extracts of Datura stramonium leaves.*

Methods: *Four experimental groups of rats were respectively given oral doses of 50mg/kg, 100mg/kg and 200mg/kg of the extract daily for six weeks. Rats were sacrificed at the end of the six weeks and blood samples were collected for biochemical analysis. The livers and kidneys of the rates were harvested for histological studies.*

Results: *The results showed that alanine transaminase (ALT) and bilirubin levels were significantly ($P<0.05$) higher in the groups administered 100mg/kg and 200mg/kg extracts than the control group. The extracts at similar doses also increased significantly ($p<0.05$) the serum urea and creatinine levels. Histological evaluation of the organs of localization revealed dose-dependent effects of treatment with the extract.*

Conclusion: *The study has shown that Datura stramonium leaf extracts administered with 100 200mg/kg for six weeks caused liver and kidney damages in rats.*

Keywords: *Datura stramonium, liver, kidney functions, histomorphology.*

INTRODUCTION

Datura stramonium (Jimson weed) is a common weed seen along roads, in cornfields and pastures and in waste sites. Jimson weed is native to Asia, but is also found in the tropical West Africa[1,2] and in West Indies, Canada and United States of America[3,4]. The plant grows wild in all parts of Nigeria, including the semi-arid North-eastern region. It belongs to the family Solanceae. Its generic name is Datura, and is locally called by several names: haukata yaro (hausa); Zakedi or aljan in (Kanuri), and apikan in Yoruba. Various parts of this plant have been used in folk medicine for the treatment of various ailments. These include the use of its leaves in the treatment of skin ulcer[5,6]; respiratory tract congestion[5,7], dental caries and swollen gums[8,9]. In the Northeastern States of Nigeria, a concoction of leaves and seeds of Datura is used to treat common cold, headache and asthma (Personal communication).

The leaves and seeds of Datura form major constituents of a local snuff called "matala", which is smoked to achieve total relaxation of the body (personal communication). Intentional misuse by teenagers who eat the seeds, smoke and use the leaves for tea has been reported[10]. It is predominantly ingested, smoked or absorbed topically through mucous membranes. The range of toxicity is reported to be highly variable and unpredictable[11,12].

Toxic effects of the extracts of Datura on the Central Nervous System (CNS) have been widely reported[13,14]. The atropine content in the leaves and seeds is believed to be the cause of Jimson weed poisoning[13]. When atropine (a tertiary amine) is absorbed by the central nervous system it causes inhibition of CNS receptors resulting in central anti-cholinergic syndrome of acute psychosis or delirium[1,15]. Ingestion of high doses of the seeds and leaves of this plant caused vomiting and diarrhoea in grazing animals[10,14]. Gidado *et al*[16] have reported changes in some biochemical parameters in rats fed with seeds of Datura.

The liver and kidneys are the most important organs for elimination of endogenous metabolites and foreign chemicals from the body; hence these organs face high risk of toxicity. There are, however, few studies on the effects of this often abused plant on these vital organs. This study was therefore aimed at investigating the effects of various doses of the ethanolic extract of the leaves of *Datura stramonium* on the histomorphology of the liver and kidney and some indices of liver and kidney function.

Materials and methods

Plant material

The leaves of *Datura stramonium* were collected from Forey, a suburb of Maiduguri, Borno State, Nigeria.

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Following positive identification and authentication of the plant at the Department of Biological Sciences, University of Maiduguri, Nigeria where a voucher specimen (PG 002 NS) was deposited, the leave samples were air dried.

Preparation of extracts

Air-dried leaves of the plant were reduced to coarse powder and 200g of the material was extracted by maceration in 70% ethanol for 48hr at room temperature. The extract was filtered and concentrated at 80°C using a rotary evaporator. The concentrate was then brought to complete dryness over water bath and stored in the refrigerator at 4°C. The calculated percentage yield 26.4%.

Animal treatment

Twenty-four male and female white albino rats of wistar strain, weighing between 110g and 150g obtained from the animal house of the Department of Biochemistry, University of Maiduguri were used. The animals were randomly divided into four groups of six rats each, and were fed with commercially available standard feeds (ECWA Feed, Jos) and water *ad libitum*. The rats in three groups were given 50mg/kg, 100mg/kg and 200mg/kg of the extract respectively as daily oral doses for six weeks, using feeding tube (MBI feeding tube size 8). Animals in the fourth group were given distilled water and served as control. Daily feed intake and weekly body weights of the animals were monitored in all four groups for the period of treatment after which the rats were sacrificed with overdose of chloroform anaesthetic. Blood samples were collected, allowed to clot and then centrifuged at 5000rpm in order to obtain clear sera which were used for the following biochemical assay using the calorimetric method of Reitman and Frankel[17]: serum alanine transaminase (ALT), aspartate transaminase (AST), urea, bilirubin, creatinine, protein, chloride, potassium and sodium ions. The livers and kidneys were removed for histological studies.

Biochemical analysis

Alanine transaminase and AST were assayed calorimetrically by the method of Reitman *et al*.[17] Bilirubin, urea and creatinine were assayed respectively, using the sulphanilic reaction, diacetymonoxime reaction and Jaffe reaction as described by Kaplan *et al*.[18]. Serum protein was determined by the Biuret reaction and chloride by titrimetric method as described by Harold[19]. Serum potassium and sodium were estimated by flame photometry.

Histological analysis

The liver and kidney tissues were fixed in 10% formal saline and processed by routine method for embedding in paraffin. Sections were cut at 8µm and stained with Haematoxylin and Eosin (H & E) for light microscope examination.

Statistical Analysis

All data collected were summarized as mean ± SEM and differences between groups were assessed using Student's T-test. A P-value less than or equal to 0.05 was considered statistically significant.

Results

Table 1 shows body weight gain, feed intake, liver enzymes (ALT and AST), total serum protein and bilirubin levels in rats after oral intake of ethanolic extract of *Datura stramonium* for 6 weeks. The mean gain in the body weight by the rats which received 50mg/kg of the extract was not statistically different from that of control group. However, the gain in body weight of the rats in the groups that received 100mg/kg and 200mg/kg of extract were significantly higher ($P<0.05$) than that of the control animals. ALT and bilirubin levels were significantly higher ($P<0.05$) in the groups that received (100mg/kg) and (200mg/kg) compared to control group and the 50mg/kg group. No significant effects were observed in feed intake, AST and total serum proteins in all the treated groups.

Table 1: *The effects of ethanolic extract of D. stramonium leaves on body weight, feed intake and biochemical parameters of liver function*

Parameters	Groups (n=6) A (50mg/kg)	B (100mg/kg)	C (200mg/kg)	D Control
Body wt. gain (g)	7.00±2.0[a]	23.02±1.02[b]	30.15±2.0[b]	18.55±5.2[a]
Feed intake (g/100/day)	7.35±1.55	8.0±3.11	10.00±2.10	6.50±1.05
ALT (iu/L)	63.00±2.51[a]	70.2±1.90[a]	98.11±5.40[b]	58.10±2.1[a]
AST (iu/L)	56.77±1.00	59.0±0.88	58.20±1.66	55.00±2.7
Total protein (g/L)	70.25±3.60	71.10±3.0	77.3±3.01	66.35±1.20
Total bilirubin (µmol/L)	9.05±1.50[a]	10.00±1.61[a]	16.0±2.00[b]	8.0±1.00[a]

[a,b] Values with different superscripts on the same horizontal row are significantly different ($P<0.05$), value in parentheses are daily doses of extract

Table 2 shows serum urea, creatinine, sodium, potassium, bicarbonate and chloride levels in rats treated with the extract for 6 weeks. Creatinine levels significantly ($P<0.05$) increased in rats treated with 100mg/kg and 200mg/kg body

weight of the extract. The increase in value was dose-dependent, with the rats given 200mg/kg body weight having a value of 104.21 ± 2.80 µmol/L. Serum urea level increased in the rats that were treated with 200mg/kg body weight, although the increase did not reach statistically significant level.

Histological analysis

Figures 1 and 4 are light micrographs of liver and kidney sections from the control animals. Examination of the liver and kidneys sections revealed a dose-dependent effect of treatment with the extract. There was a mild but generalized hepatocytic necrosis, which was more pronounced at the periphery and regions of portal triad in the liver of 50mg/kg extract-treated rats. The necrotic changes were marked at the dose level of 100mg/kg with cytoplasmic disintegration and eccentric nuclei (Fig 2). Many large and oval Kupffer cells were seen in the sinusoids at extract dose of 200mg/kg, in addition to

Table 2: *Effects of ethanolic extracts of D. stramonium leaves on some biochemical parameters of kidney function*

Parameters	Groups (n=6) A (50mg/kg)	B (100mg/kg)	C (200mg/kg)	D Control
Na^+ (mmol/L)	143.00±3.00	143.05±2.31	143.00±3.22	143.50±2.0
K^+ (mmol/L)	6.00±2.00	5.50±1.00	8.55±3.00	5.82±0.94
HCO^-_3(mool/L)	59.25±3.00	58.01±2.00	60.00±2.50	61.00±3.45
Cl^-	142.05±1.56	146.00±1.85	148.01±2.11	142.00±2.00
Urea (mmol/L)	7.0±2.00	7.51±1.78	8.00±1.22	7.6±1.20
Creatinine (µmol/L)	62.00±1.00[a]	89.66±1.57[b]	104.21±2.80[b]	56.0±2.18[a]

[a,b] Values with different superscripts on the same horizontal row are significantly different (P<0.05), value in parentheses are daily doses of extract.

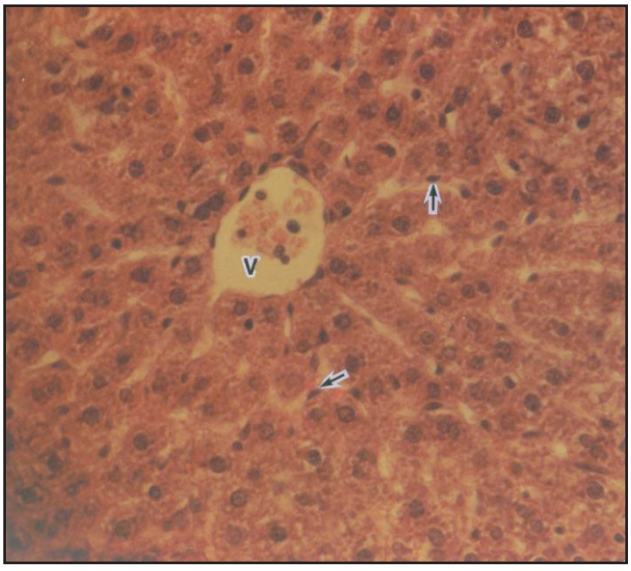

Fig: 1: *Light micrograph of liver tssue from a control rat, showing normal histologic structure, with a large central vein (V) from which radiates several sinusoids, containing elliptical Kupffer cells (arrows) H & E stain x 400*

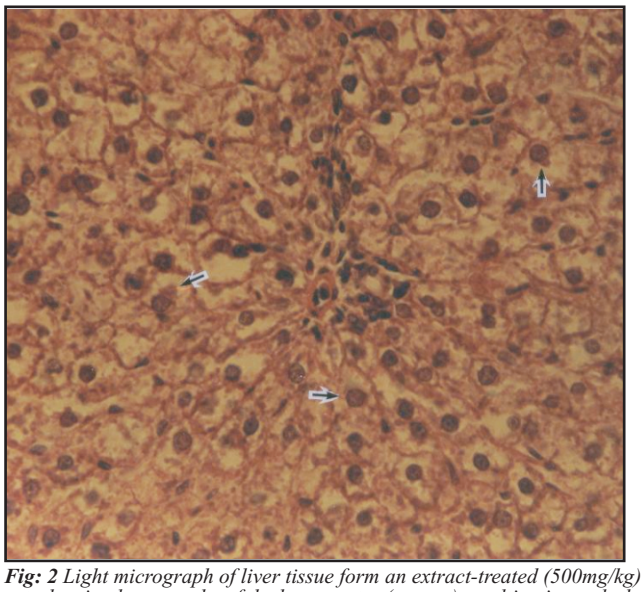

Fig: 2 *Light micrograph of liver tissue form an extract-treated (500mg/kg) rat, showing hypertrophy of the hepatocytes (arrows) resulting in marked congestion of the sinusoids. Note the portal triad in the centre of the micrograph and the vacuolations and disintegration of the cytoplasm (arrow) H & E stain, x 400.*

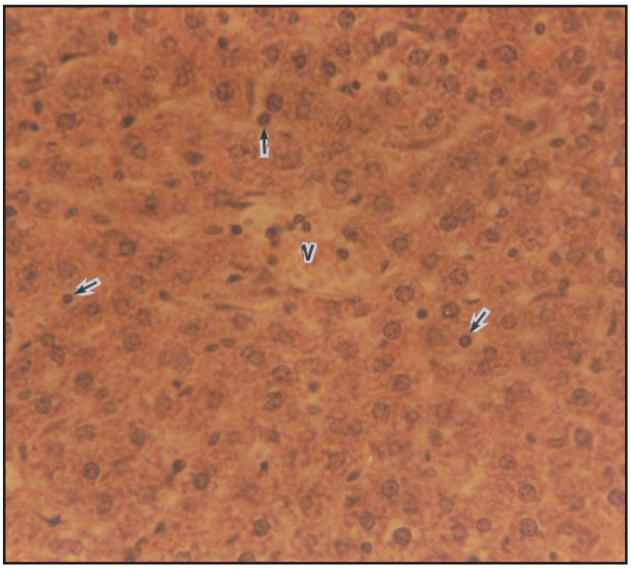

Fig: 3: *Light micrograph of liver tissue from extract-treated (200mg/kg) rat, showing many large and oval Kupffer cells (arrows) in sinusoids. Cytoplasmic vacuolations seen in Figure 2 are still present. V = central vein with leucocytes. H & E stain. x 400.*

leucocytic infiltration of the central veins (Fig. 3).

The kidney tissues of extract-treated (50mg/kg-200mg/kg) rats were characterized by generalized haemorrhage, tubular constriction and leucocytic infiltration (Fig. 5-7). The effect was much marked at the 200mg/kg dose levels with necrotic damages affecting the renal corpuscles in

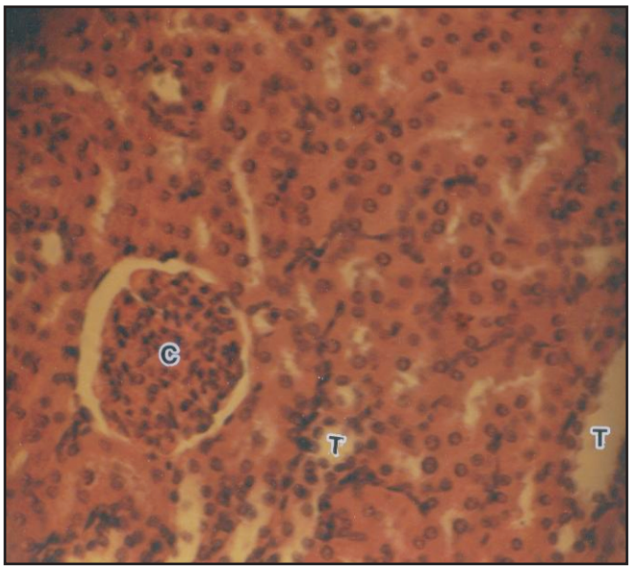

Fig: 4: *Light micrograph of the renal cortex from the kidney of a control rat, showing a renal corpuscle (C) and renal tubules (T) cut in different planes. H & E stain, x 400*

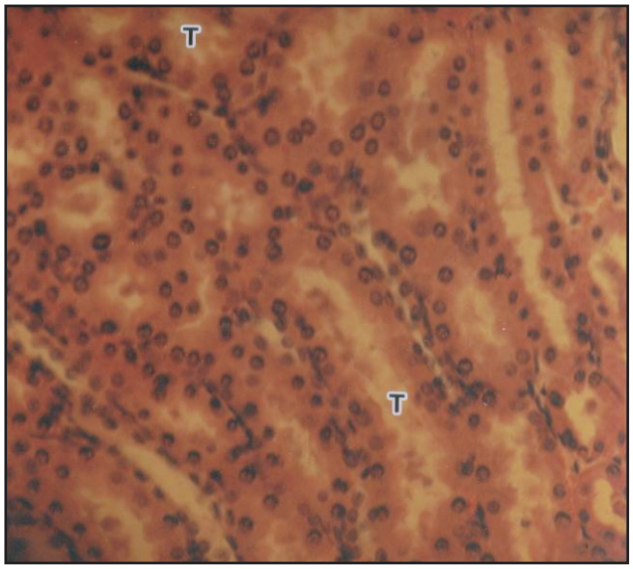

Fig: 5*:Light micrograph of corticomedullary region form the kidney of an extract-treated (100mg/kg) rat, showing massive haemorrhage in the renal tubules (arrows) H & E stain, x 400*

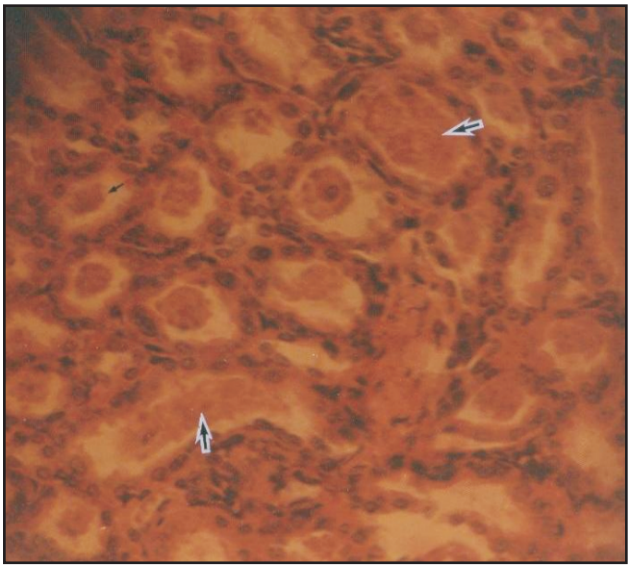

Fig: 6: *Light micrograph of the renal medulla from the kidney of a control rat, showing collecting ducts with normal cuboidal cells, resting on basement membrane, and tubules (T). H & E stain, x 400*

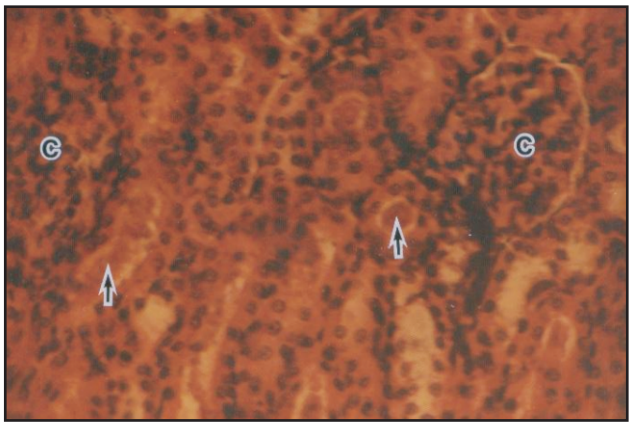

Fig: 7: *Light micrograph of the renal cortex form the kidney of an extract treated (200mg/kg) rat, showing tubular congestion and haemorrhage (arrows) and degenerating renal corpuscles and tubules (T) H & E stain, x 400*

Discussion

Oral LD_{50} of the ethanolic extract of *D. stramonium* leaves was previously determined to be 525mg/kg in rats[11]. This showed that the extract is moderately toxic, since substances whose oral LD_{50} values between 50mg/kg and 500mg/kg are considered to be moderately toxic in the rat[20]. The range of toxicity of D. *stramonium* is highly variable and unpredictable as varies from leaf to leaf, plant to plant and season to season[11,21]. This may present danger of misuse since safety range is unpredictable[3,22].

The extract also increased serum urea and serum creatinine levels significantly ($P<0.05$) in the treated rats. This suggests possible kidney damage[16,23,24,25]. Similar findings in rats treated with aqueous seeds extract of the plant have been reported[4,26]. The levels of serum ALT, AST and creatinine which are indicators of liver function were raised. Significant increase in serum ALT, which is a more specific enzymatic indicator of liver function, and creatinine, have been shown to be associated with liver cell necrosis[24].

Qualitative histomorphological study confirmed the quantitative biochemical analysis of the effects of treatment with extracts of *D. stramonium*. Doses of extract at 100-200mg/kg which produced significant increases in the serum levels of ALT (liver marker enzyme) also showed marked necrotic changes in the liver tissue, while similar dose levels which caused significant rise in serum creatinine levels also produced necrotic changes in renal tissues. Both effects support the report of Fernando and Fernando [12] that the plant is poisonous.

Acute and chronic hepatoxic effects of most plants materials have been associated with a particular alkaloids; the pyrrolizidine.[26] Plants containing pyrrolizidine alkaloids are present in all parts of the world, often causing hepatotoxicity in man and grazing livestock.[27, 28] The mechanism and possible constituents of *D. stramonium* causing hepatotoxicity is however not well established but previous studies. [29, 30, 32, 21] revealed the presence of alkaloids and suggested that these alkaloids are likely causing the toxicity.[31, 32, 33]

This study confirmed previous findings[26] that *Datura stramonium* in high doses is toxic. However, further work is required to specifically ascertain the type of alkaloid given the rampant abuse by teenagers in this locality.

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