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RESEARCH ARTICLE

TOXICOLOGICAL EFFECT OF AQUEOUS SEED EXTRACT OF *DATURA STRAMONIUM* ON LIVER OF EXPERIMENTAL RATS

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ABSTRACT

Datura stramonium which is commonly called Jimsonweed in English originated in the Middle East but has since become a cosmopolitan plant that grows in temperate and tropical countries alike. It is often found growing as a weed between rubble in uncultivated spots and on road-sides. The toxicity of *Datura stramonium* has been locally and internationally reported. This is due to the alkaloids produced in the leaves, seeds, roots, and other parts of the plant which are very poisonous. Their ingestion can result in convulsion, coma, and even death. This research paper aimed at finding the potential liver toxicity of the plant's seed as its abuse among youths of this contemporary society is increasing at an alarming rate. The effect of daily administration of aqueous seed extract of *Datura stramonium* for two weeks on the serum activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) as well as serum concentration of albumin was studied using laboratory albino rats. The treatment involved oral administration of the extract at concentrations of 150mg/Kg, 300mg/Kg and 600mg/Kg. The control rats were found to have AST, ALT and ALP activities of 26.00 ± 2.52 19.13 ± 1.63 and 49.17 ± 11.02 IU/L respectively and 3.68 ± 0.45 g/dL albumin serum concentration. The serum levels of AST, ALT, ALP and albumin did not significantly increased for 150mg/Kg, 300mg/Kg and 600mg/Kg at P = 0.05. The result revealed that the aqueous seed extract of *Datura stramonium* did not have significant effect on the serum liver enzymes and albumin. This implies that the plant seed extract does not cause liver damage and therefore is not toxic to the laboratory animal's liver at the doses administered.

Key Words: *Datura stramonium*, Toxicity, Liver, AST, ALT, ALP, Albumin.

INTRODUCTION

1.0 Background

High numbers of indigenous and non-indigenous plants have been in use by man ornamentally, narcotically or for curing different types of ailments without the actual knowledge of their critical toxic effects since time immemorial. One of such plants used by many localities of this society especially for its hallucinating capability is *Datura stramonium.* The plant have some common names like Jimsonweed, locoweed and ditch weed (Amy and Arnett, 1995). The plant is commonly called *Zakami, furen juji*, and *Babba juji* in Hausa.

1.1 Botanical Description

Datura stramonium is a rank, foul smelling annual with large purplish trumpet-shaped flowers and spiny egg-shaped fruits. The plant gets about 3 or 4ft (0.9-1.2m) tall with similar spread. It often falls over from its own weight. The stem is purplish and glabrous (smooth) and the leaves are ovate, irregularly lobed to 8 in (20.3cm) long, and have a foul odour. The flowers however are fragrant and sweet-smelling.

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They open for only one evening, but new ones continue to open throughout the summer and autumn. The flowers are white or pale lavender, shaped like a five-sided funnel, 2-4 in (5.1-10.2cm) long. The green calyx covers about half the length of the corolla. The fruit is about 2 in (5.1cm) long, egg-shaped and covered with spiny prickles. It starts out green and ripens to brown. It is full of kidney-shaped black seeds (Christman and *Datura stramonium*, 2004).

1.2 Toxicity of *Datura stramonium*

All parts of *Datura stramonium* are very poisonous. It contains dangerous levels of poison and may be fatal if ingested by humans or animals, including livestock and pets. Cattle and sheep have died from eating it, and children have been poisoned by sucking nectar from the flower. Even inhaling the sweet fragrance of the flowers can cause headaches and dizziness. Its sap can cause skin rash (Christman, 2004).

The toxins in jimsonweed are tropane belladonna alkaloids which posses strong anticholinergic properties. They include hyoscyamine, hyoscine, atropine and scopolamine. They act as a competitive antagonist to acetylcholine at peripheral and central muscarinic receptors at a common binding site. The peripheral receptors are on exocrine glands which affect sweating, salivation, and smooth and cardiac muscle. Poisoning results in widespread paralysis of parasympathetic

innervated organs. As tertiary amines they also have central nervous system absorption, inhibit CNS receptors and result in a central anticholinergic syndrome of acute psychosis or delirium (Amy and Arnett, 1995).

The clinical effects of typical atropine poisoning includes the following symptoms; mydriasis (extreme dilation of the pupil), cycloplegia, flushed, warm, dry skin, dry mouth, ileus (slowing or stopping of intestinal movement), urinary retention; tachycardia; hyper or hypotension; delirium with hallucinations (Amy and Arnett, 1995).

According to the research carried out by (El Dirdiri *et al.,* 1981), the effect of oral administration of *Datura stramonium* to Desert sheep and Nubian goats revealed the main clinical signs to be disturbances in locomotion, fasciculation, hyperesthesia, rapid respiration, and reduced water intake. There were increases in the concentration of aspartate aminotransferase and ammonia and decreases of total protein and magnesium in the serum of the animals.

1.3 Chemical Composition

The alkaloids are distributed all over the leaves, roots and seeds of the plant. They are also found even in the nectar and can contaminate honey (Amy and Arnett, 1995) (Holoweb, 2009)

1.4 Liver Function Tests

Liver function tests are normally based on assessment of the synthetic, secretory or excretory functions of the liver (Sucker *et al.,* 2002). Several serum enzymes are measured in these widely available biochemical tests (Finlayson *et al*., 1995). The activities of one or two transaminase enzymes, alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) are often measured. The activities of alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), serum guanase and 5-nucleotidase (5' NT) as well as serum albumin concentration are also measured.

MATERIALS AND METHODS

All the apparatus and equipment utilised in the research work were of standard quality and in good working condition.

2.1.1 Chemical Reagents

All the chemicals and reagents utilized in the assay of the liver enzymes: aspartate aminotransferase (AST) (EC 2.6.1.1), alanine aminotransferase (ALT) (EC 2.6.1.2) and alkaline phosphatase (ALP) (EC 3.1.3.1) are of analytical grade. AST and ALT have been analysed using Randox (brand name) commercially prepared kit, while ALP using TECO (brand name) commercially prepared kit. Serum albumin using Bromocresol green (BCG) reagent.

2.1.2 Plant Sample Collection and Extraction

The fruits of *Datura stramonium* were collected from *Kurmi market* of Kano Municipal Local Government of Kano State (Nigeria) and were identified at the Botanical Garden, Department of Biological Sciences, Bayero University, Kano, (Nigeria). The tiny, brownish kidney-shaped seeds were exposed out of the pods and allowed to dry. The dried seeds were ground to a fine powder using pestle and mortar and then stored in a clean container at room temperature. The water extract of the powdered seeds was prepared by soaking 20g of the powder in 60ml of distilled water in a clean and well-dried beaker. The preparation was kept at room temperature for 24 Hours. The mixture was then filtered using 110mm whatman filter paper into a clean conical flask. The residue was dried and weighed. The extract was diluted with distilled water to required concentrations corresponding to the dosage of each group i.e. Group II (150mg/Kg), Group III (300mg/Kg) and Group IV (600mg/Kg). The diluted extracts were stored in good laboratory conditions.

2.1.3 Treatment of Experimental Animals

Sixteen (16) male white albino rats of an inbred Novergicus Strain (*Rattus novergicus*) weighing between 160g to 210g were obtained from the Animal Holding Unit of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria (Nigeria). The rats were kept in a well ventilated house conditions (Temperature: 25-30^{o}; Photoperiod: 12 Hours natural light and 12 Hours dark), with free access to both food and water. They were housed in aluminium cages (Dimensions 39.50cm by 25.30cm by 14.80cm) with saw dust at the bottom of the cages. The rats were distributed into four (4) groups of four (4) rats each.

Rats in Group I were administered orally with 1ml of distilled water daily for a period of two weeks. This group served as control. All the animals in the other groups were orally administered with 1ml of extract for a period of two weeks. Group II, III and IV have doses of 150mg/kg, 300mg/kg, and 600mg/kg respectively. All the rats were sacrificed on the fifteenth day (15th) by carefully cutting their jugular vein with a very sharp razor. Blood samples were collected into dried and clean centrifuge tubes and were separated. The sera were collected into plain serum bottles for laboratory investigations.

2.2 Methods

2.2.1 Determination of Serum Aspartate aminotransferase (AST)

Aspartate aminotransferase (AST) (EC 2.6.1.1) activity was assayed using the procedure described by (Reitman and Frankel, 1957). The colorimetric measurement was done using Corning 253 colorimeter.

Principle

The Aspartate aminotransferase catalyzes the transamination of Aspartate in the presence of Alpha-ketoglutarate to give Oxaloacetate and Glutamate.

$$\text{L-aspartate} + \alpha\text{-ketoglutarate} \xrightleftharpoons{\text{AST}} \text{oxaloacetate} + \text{L-glutamate}$$

AST was measured by monitoring the concentration of Oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine calorimetrically at 540nm.

Procedure

To each of *Sample* labelled test tubes, 100µL of sample was dispensed. 0.5ml of Reagent-**1** (buffer) was added to both *Sample* and *Blank* test tubes and followed by 100µL of distilled water to the Blank only. The solutions were mixed and incubated for 30min at 37^{o}C. After the incubation, 0.5ml of Reagent-**2** was added to each of *Sample* and *Blank.* The solutions were mixed and allowed to stand for exactly 20min at 20 to 25^{o}C. To each of *Sample* and *Blank*, 5ml of 0.4N NaOH (Sodium Hydroxide) was then added. They were mixed and allowed to stand for 5min at room temperature after which the absorbance of *Sample* was read against the absorbance of *Blank* at 540nm.

Activity

The activity of AST was obtained by comparing absorbance on the table of values on the Reagent's manual

2.2.2 Determination of Serum Alanine aminotransferase (ALT)

Alanine aminotransferase (ALT) (EC 2.6.1.2) activity was assayed using the procedure described by (Reitman and Frankel, 1957). The colorimetric measurement was done using Corning 253 colorimeter.

Principle

The Alanine aminotransferase catalyzes the transamination reaction between Alanine and Alpha-ketoglutarate to give Pyruvate and Glutamate.

$$\text{L-alanine} + \alpha\text{-Ketoglutarate} \xrightleftharpoons{\text{ALT}} \text{pyruvate} + \text{L-glutamate}$$

ALT was measured by monitoring the concentration of Pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine calorimetrically at 540nm.

Procedure

To each of *Sample* labelled test tubes, 100µL of sample was dispensed. 0.5ml of Reagent-**1** (buffer) was added to both *Sample* and *Blank* test tubes and followed by 100µL of distilled water to the *Blank* only. The solutions were mixed and incubated for 30min at 37^{o}C. After the incubation, 0.5ml of Reagent-**2** was added to each of *Sample* and *Blank.* The solutions were mixed and allowed to stand for exactly 20min at 20 to 25^{o}C. To each of *Sample* and *Blank*, 5ml of 0.4N NaOH was then added. They were mixed and allowed to stand for 5min at room temperature after which the absorbance of *Sample* was read against the absorbance of *Blank* at 540nm.

Activity

The activity of ALT was obtained by comparing absorbance on the table of values on the Reagent's manual**.**

2.2.3 Determination of Serum Alkaline Phosphatase (ALP)

Alkaline Phosphatase (ALP) (EC 3.1.3.1) activity was assayed using the method developed by (Roy, 1970) . The colorimetric measurement was done using Corning 253 colorimeter.

Principle

The ALP acts upon the AMP-buffered sodium thymolphthalein monophosphate. The addition of alkaline reagent stops enzyme activity and simultaneously develops a blue chromogen, which is measured calorimetrically at 590nm.

Procedure

To each *Sample* labelled test tube, 0.5ml of ALP substrate was dispensed and equilibrated to 37^{o}C for 3min. At timed intervals, 50µL of each standard and sample were added to their respective test tubes. They were mixed gently. Deionised water was used as sample for Reagent Blank. They were incubated for exactly 10min at 37^{o}C. After the incubation, 2.5ml ALP Colour Developer was added at the same timed intervals as above and they were mixed well. Then absorbance of *Sample* and *Standard* were read at 590nm after the colorimeter was set to zero with Reagent Blank.

Activity

The activity of ALP was obtained by dividing the absorbance of *Sample* with that of standard and multiplying the result with concentration of standard.

2.2.4 Determination of Serum Albumin

The determination of Albumin was carried out using *Dye Binding Method* in which Bromocresol green (BCG) was used as a dye. The colorimetric measurement was done using Corning 253 colorimeter.

Principle

Albumin binds with bromocresol green at pH 4.2 to give a related colour. The reaction is referred to as *Protein Error Indicator*. Brij-35 as a surfactant decreases the colour of the unreacted dye and helps prevent turbidity.

Procedure

To each *Test*, *Standard* and *Blank* well labelled test tubes, 4ml of Bromocresol green was dispensed. 20µL of Serum, 20µL of standard and 20µL of distilled water were added to *Test*, *Standard* and *Blank* respectively. The solutions were mixed and allowed to stand for 10min at room temperature. The Absorbance of *Test* and *Standard* were read against absorbance of *Blank*.

Serum Concentration

The serum concentration of Albumin was obtained by dividing the absorbance of *Test* with that of *Standard* and multiplying the result with concentration of standard.

RESULTS AND DISCUSSION

3.1 Results

The effects of administration of aqueous seed extract of *Datura stramonium* to rats on mean weight the animals were shown on Table 1.

Table 1. Mean weight of the experimental rats in grammes (g) for the first day and the last day of experimental process

Group/Date	First Day (Day One)	Last Day (Day Fourteen)
Group I (Control) n= 4	160.00	160.00
Group II (150mg/Kg) n =4	177.50	175.00
Group III(300mg/Kg) n= 4	180.00	181.25
Group IV (600mg/Kg) n=4	200.00	*190.00

{*} = Decrease in weight after administration of extract for two weeks

Table 2. Serum Enzyme Activities of AST, ALT and ALP and Albumin Serum Concentration of Rats Orally Administered with 150mg/Kg, 300mg/Kg and 600mg/Kg Aqueous Seed Extract of *Datura stramonium* for Two Weeks.

Treatment	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Albumin (g/dL)
Control (n = 4)	26.00 ± 2.52	19.13 ± 1.63	49.17 ± 11.02	3.68 ± 0.45
150mg/Kg(n=4)	28.25 ± 6.40	21.84 ± 3.48	40.00 ± 9.81	3.59 ± 0.53
300mg/Kg(n=4)	24.00 ± 2.00	17.56 ± 3.11	52.50 ± 4.19	4.11 ± 0.60
600mg/Kg(n=4)	25.00 ± 4.00	18.28 ± 0.85	40.00 ±12.47	3.94 ± 0.53

The effects of administration of aqueous seed extract of *Datura stramonium* to rats on the activities of aspartate amonitransferase (AST), alanine amonitransferase (ALT) and alkaline phosphatase (ALP), and on serum concentration of albumin were shown on Table 2.

From the table-2 values, statistical comparisons between the results were done using student's t-test at 5% level of significance as described by (Mukhtar, 2003).

The AST results of doses: 150mg/Kg, 300mg/Kg and 600mg/Kg were compared with that of control. The result of 150mg/Kg was also compared with that of 300mg/Kg and 600mg/Kg. A comparison between results of 300mg/Kg and 600mg/Kg was also made. All the comparison calculated values were lower than the table value of 2.447 which means that there is no significant difference between the means. But the calculated values between control and 300mg/Kg; and that of 150mg/Kg and 300mg/Kg were a little higher than the rest. The results of ALT were also compared as it was done with AST results and did not show any significant difference. But the calculated values between control and 150mg/Kg; 150mg/Kg and 300mg/Kg; and 150mg/Kg and 600mg/Kg are those that appears higher than the others.

The ALP results were also compared. They did not show any statistical significant difference. The values of comparison between 150mg/Kg and 300mg/Kg; and 300mg/Kg and 600mg/Kg are higher than the rest.

Albumin results were also compared as done with AST, ALT and ALP, and there is no significant difference between them. The only pairs that their calculated values appear to be a little bit higher than their counterparts are: control and 300mg/Kg; and 150mg/Kg and 300mg/Kg.

A decrease in average weight has been observed in the animals administered with 150mg/Kg and 600mg/Kg at the end of the administration.

3.2 Discussion

Liver function tests are normally based on assessment of the synthetic, secretary or excretory functions of liver (Sucker, 2002). The clinical suspicion of liver diseases usually leads to the conduct of the liver function tests. Several liver enzymes found in the serum are measured in these widely available biochemical tests (Finlayson, 1995). The measurement of the activities of marker or diagnostic enzymes in the serum plays a significant role in diagnosis of disease and in the assessment of drugs or plant extract for safety or toxicity risk. The enzymes considered in this study are useful marker enzymes of the liver cells (Schmidt and Schmidt, 1979). AST and ALT are normally localized within the cells of the liver, heart, gill, kidney, muscle and other organs in which the enzymes are of major importance in assaying and monitoring liver cytolysis (Wada and Shell, 1962). Their presence in the serum may give information on organ dysfunction (Wells *et al*., 1986). It was found from this research that the control group rats have serum AST, ALT, ALP and Albumin levels of 26.00 ± 2.52, 19.13 ± 1.63, 49.17 ± 11.02 and 3.68 ± 0.45 respectively. The rats that were administered with 150mg/Kg of the extract have serum AST, ALT, ALP and Albumin levels of 28.25 ± 6.40, 21.84 ± 3.48, 40.00 ± 9.81 IU/L and 3.59 ± 0.53 g/dL respectively. All these values did not have significant difference at P = 0.05 compared to control rats.

The experimental rats treated with 300mg/Kg of the extract have serum AST, ALT, ALP and Albumin levels of 24.00 ± 2.00, 17.56 ± 3.11, 52.50 ± 4.19 IU/L and 4.11 ± 0.60 g/dL respectively. All these values also have no any significant difference from control rats' values when compared at P = 0.05. The laboratory rats treated with 600mg/Kg of the extract revealed serum AST, ALT, ALP and Albumin results to be 25.00 ± 4.00, 18.28 ± 0.85, 40.00 ± 12.47 IU/L and 3.94 ± 0.53 g/dL respectively. These rats also like their counterparts in the above doses did not show any statistically significant difference when compared with control values at 5% significance level. The pattern of AST, ALT, ALP activities and albumin serum concentration observed in this study on the animals administered with the extract displayed no significant difference with AST, ALT and ALP activities and albumin serum concentration of the control animals. But, this result is in contrast with the findings of (Amy and Arnett, 1995) and (El Dirdiri, 1981), in which both reported increase in AST activity. Reference Amy and Arnett (1995) attributed it possibly secondary to muscle breakdown from seizures, tone and hyperthermia as mentioned earlier. AST is found in the liver, cardiac muscle, kidney and erythrocytes, whereas ALT is found primarily in the liver.

As such having a high level of AST does not always indicate that there is a liver damage. For example, even vigorous exercise may elevate AST level in the body. Whereas, high levels of ALT almost always indicate there is a problem with the liver. Therefore, ALT is more specific to the liver than AST (Palmer, 2009). This means that the report of increase in serum AST level (Amy and Arnett, 1995) and (El Dirdiri, 1981) might not probably be as a result of liver cytolysis. All the statistical comparisons done between the AST, ALT, ALP and albumin of control animals and those of animals exposed to the plant extract did not show any significant difference at 5% level of significance. This implies that the seed extract have no toxic effect on the liver of the experimental animals at the administered doses or it could be attributed to adaptation by the animals to the doses exposed.

4.0 Conclusion

The results of this study have shown that daily administration of aqueous seed extract of *Datura stramonium* for two weeks have no significant effect on the liver of experimental animals at the doses exposed. This implies that, within the limit of experimental error, the plant seed extract does not cause liver damage and therefore is not toxic to the liver at the administered doses.

5.0 Recommendations

This research work is an attempt made at finding out the effect of *Datura stramonium* on liver. Further work on the plant should involve the use of large number of animals so that larger data could be obtained and more information could be driven from it. Longer period of administration could also lead to effective manifestation of effect of the extract. Another approach that could be fruitful is the involvement of other liver related biochemical parameters that can reveal more information about the state of the liver. These parameters include bilirubin (both conjugated and unconjugated), gamma glutamyl transaminase (GGT), total protein and prothrombin. In addition, histopathological studies of the liver should be conducted after administration of the extract. This will help to further characterise the effect of the extract.

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