

Alterations in Marker Enzymes and Kidney Function Indices Following Administration of *Datura metel* Aqueous Seed Extract in Albino Rats

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Abstract

Phytochemical screening and effect of *Datura metel* aqueous seed extract was evaluated in albino rats. The phytochemicals were screen using standard method, enzymes were analyzed using standard method, kidney function indices were also determined using colorimetric method. The study revealed the presence of tannins, flavonoids, alkaloids, glycoside and phenol. The result shows that there was a decreased in body weight at all the doses when compared with the control. The result of the present study revealed a significant increased ($P < 0.05$) in ALP and activity at all the doses in both serum and kidney. Similarly, ALT activity significantly increased ($P < 0.05$) in serum at all the doses, but only at 200 and 300mg/kg bw, whereas no significant different ($P > 0.05$) at 100mg/kg bw in the kidney when compared with control. The result also show significant increased ($P < 0.05$) in AST activity in the kidney at all the doses, but only at 200 and 300mg/kg bw, and no significant difference at 100mg/kg bw in the serum when compared with control. The study revealed a significant increased ($P < 0.05$) in serum urea, uric acid and calcium at all the doses of the extracts treated groups when compared with the control. It also revealed significant increased ($P < 0.05$) in serum creatinine and potassium only at 300mg/kg bw, whereas no significant different at 100 and 200mg/kg bw when compared with the control. Furthermore, the result revealed significant increased ($P < 0.05$) in serum sodium at 200 and 300mg/kg bw, and no significant difference ($P > 0.05$) at 100mg/kg bw when compared with the control. It also revealed significant increased ($P < 0.05$) in serum phosphate at 200 and 300mg/kg bw and show significant decreased ($P < 0.05$) only at 100mg/kg bw of the extract treated groups when compared with the control. However, chloride levels were significantly ($P < 0.05$) decreased at all the doses when compared with the control group. The study therefore, revealed the presence of phytochemicals which attributed the plant to its effects on the functional ability of the kidney as revealed by alterations in the kidney function parameters analyzed.

Keywords

Datura metel, Albino Rats, Kidney Function Indices, Makers Enzymes, Phytochemicals

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1. Introduction

Herbal medicine derived from plant extracts is being increasingly utilized to treat a wide variety of diseases [1] [2]. Plant provides a tremendous reservoir of various chemicals substance with potential therapeutics properties [3] [2]. [4],

defined traditional medicine as the health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illnesses or maintain wellbeing. It's also noted that inappropriate use of traditional medicines or

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practice can have negative or dangerous effects and that further research is needed to ascertain the efficiency and safety of several of the practice and medicinal plants used by traditional medicinal systems [1]. It is therefore become necessary to identify the phytochemical component of local medicinal plants usually employed by herbalist in the treatment of diseases, especially with advocacy for the integration of traditional medicine in health care programs in Nigeria [5]. The World Health Organization (WHO) estimates that four billion people, 80 percent of the world population presently use herbal medicine for some aspects of primary health care, herbal medicine is a major component in all indigenous people traditional medicine and a common element in homeopathic, naturopathic, traditional orient, and Native American Indian medicines [5].

WHO noted that, of the 119 plants derive pharmaceutical medicines, about 74 percent are used in modern medicines in ways that correlated directly with their traditional uses as plant medicine by native culture [6]. Major pharmaceutical companies are currently conducting extensive research on plant materials gathered from the rain forest and other places for their potential medicinal value. The growing interest in herbal medicine demands toxicity risk assessment of the various indigenous preparations used in the treatment of disease [6].

One such plant used for medicinal purposes which may be toxic to biological organs such as liver and kidney is *Datura metel*. The name of *Datura* comes from Sanskrit Dustura [7] or Dahatura. There are many different species in the *Datura* genus. It is commonly known as Thorn apple belonging to the family Solanaceae. It is locally known as zakami or bubajuhji in Hausa, gegemu in Yoruba, Nyaranmuo in Igbo and thorny apple or angel trumpet in English [8]. *Datura metel* (*D. metel*) is a flowering plant. The plant grows up to 3 feet high. Its leaves are covered with short and soft grayish hairs. The leaves are about 10–20 cm long and 5–18 cm broad. It is widely found in Asia, Africa, England, India and other tropical and subtropical regions [9]. The phytoconstituents such as flavonoids, phenols, tannins, saponins and sterols are found in *D. metel* [10]. Some other phytochemicals have also been found in *D. metel* and the main phytoconstituents include alkaloid [11] [10]. Globally it is considered as a poisonous plant when taken in large doses. It can cause delirium, coma, and even death due to high percentage of alkaloids [11] [10]. It should not have more than 0.20% of alkaloids [9]. It is one of the most important medicinal herbs used worldwide due to its anti-inflammatory property [12]. Primarily this plant is used as an intoxicant and hallucinogen [11] [10]. However, there also several reports on the use of the plant for its antibacterial and antioxidant activities [13] [14].

The kidneys are the vital organs situated in the abdomen,

each kidney consists of the nephrons capsules (with blood capillaries), proximal convoluted tube, loop of henle, distal convoluted tubule and collecting duct [15]. The kidneys play several functions among which include; maintenance of homeostasis, excretion creatinine, uric acid sulphate and phosphate [15]. Also kidneys place role in relation of substance vital to body and hormonal function where it play as an endocrine organs by producing hormones [15]. The blood supply to kidney is relatively large, about 1200mls, every minute [16]. From this, about 120-125ml is filtered per minute by the kidney and this referred to as glomerular filtration rate (GER with normal GFR of 120-125ml/min). The glomerular filtration formed on an adult is about 175-180 liters per day, out of which only 1.5 liters are excreted as urine [16]. Thus, more than 90% of the glomerular filtration is reabsorbed by the kidneys [16]. Renal failure, kidney stones and dieresis are considered the major kidney diseases and if treated at the acute stage may lead certain damages at the chronic stage [17] [18] [19].

Therefore, it is important to access the nephrotoxic effect following administration of *Datura metel* aqueous seeds extract in albino rats to ascertain the use of this plant for medicinal and other therapeutic purposes.

2. Materials and Methods

2.1. Plant Material and Authentication

The *Datura metel* plant was collected from the wild in Aliero Town Kebbi State, Nigeria. The plant was identified and authenticated at the Biological Science Department, Kebbi State University of Science and Technology, Aliero, Nigeria with a voucher number of 282. The seeds were clean and air dried at room temperature for a period of two (2) weeks. The seeds sample was then ground to powder form using mortar and pestle. The powdered sample was kept in a dried and air tight container and later used for the extraction.

2.2. Experimental Animals

Twenty five (20) matured (Wistar Strain) albino rats of both sexes weighing 130-232g were used for the study. They were purchased from the animal Research House of Usman Danfodio University, Sokoto and transported in a well ventilated cage to Kebbi State University of Science and Technology Aliero. The animals were kept in cages at room temperature under conditions of natural light and dark schedule and allow free access to feed and water. They were left in the environment for 2 weeks to acclimatize.

2.3. Preparation of Plant Material

The seeds (457.20g) were obtained from the fruits and dried

under shade for 14 days to ease the grinding of the seeds, they were then crushed with a motor and pestle into powder. The aqueous extract was prepared by soaking 400g of the coarse powder in 1600ml of distilled water over a period of 72hrs at room temperature. The extract was filtered using Whatman filter paper (No. 1), the filtrate was oven-dried and weighed. The weight of the aqueous extract was obtained which was 62.90g. A percentage yield of 16.70% was obtained after extraction and evaporation to dryness. The dried extract was then concentrated appropriately to give equivalent doses of 100, 200 and 300 mg/kg body weight using distilled water.

2.4. Qualitative Phytochemical Analysis

2.4.1. Test for Phenols

0.5ml of the extract, 5ml of Folin Ciocalteu reagent and 4ml of aqueous sodium carbonate were added. Appearance of blue colour indicates the presence of phenols [20].

2.4.2. Test for Saponins

To 2ml of the extract, 2ml of distilled water was added and it was agitated in a test tube for 5minutes. The formation of foams indicates the presence of saponins [21].

2.4.3. Test for Tannins

5 drops of 0.1% ferric chloride was added to 2ml of the extract, a brownish green or blue-black colouration indicated the presence of tannin [21].

2.4.4. Test for Glycosides

2ml of acetic acid was added to 2ml of the extract. The mixture was cooled in cold water bath. 2ml of concentrated H₂SO₄ was then added, color development from blue to bluish green indicates the presence of glycosides [21].

2.4.5. Test for Flavonoids

2ml of 10% Sodium hydroxide was added to 2ml of the extract in a test tube. An intense yellow colour was formed which turned colourless upon addition of 2ml of dilute hydrochloric acid indicating the presence of flavonoids [22].

2.4.6. Test for Phlobatannins

2ml of the extract were boiled with 1% aqueous hydrochloric acid. Formation of red precipitate indicates the presence of Phlobatannins [21].

2.4.7. Test for Terpenoids

5ml of the extract was mixed in 2ml of chloroform and 3ml of concentrated sulphuric acid was carefully added to form a layer. A reddish brown colouration at the interface indicates presence of terpenoids [21].

2.5. Test for Steroids

2ml of extract were dissolved in 10ml of chloroform and then 10ml of concentrated sulphuric acid was added by the side of the test tube. The upper layer turned red whereas the sulphuric acid layer turned yellow with green fluorescence. This indicates the presence of steroids [22].

2.6. Animals Grouping and Extract Administration

Twenty (20) rats were randomly grouped into four (4) groups comprising of five (5) rats per group. Administration was performed orally at 24 hours interval using cannula attached to a 2ml syringe for a period of fourteen (14) days. The administration was done as follows:

GROUP A: Control, received only distilled water

GROUP B: Test, received 1.0ml of 100 mg/kg *Datura metal seed extract*

GROUP C: Test, received 1.0ml of 200 mg/kg *Datura metal seed extract*

GROUP D: Test received 1.0ml of 300 mg/kg *Datura metal seed extract*

24hrs after the last dose, all the rats were sacrificed by anesthesia, using chloroform. Incision was made quickly in the neck region to collect blood into a labeled bottle. The collected blood was centrifuged at 3000 rpm for 10 minutes. The serum was then collected stored under refrigerator and later used for kidney function analysis. The kidney was then removed and placed in a 0.25 M sucrose solution. The kidney was then homogenized using pre-cooled mortar and pestle. The kidney homogenate was then centrifuged at 3000 rpm for 5 minutes. The supernatant was removed and later used for enzyme and kidney function indices analysis.

2.7. Determination of Biochemical Parameters

The activities of the enzymes were assayed from the kidney and serum. Alkaline phosphatase (ALP) activity was determined according to the method described by [23], using reagent kit, while aspartate transaminase (AST) and alanine transaminase (ALT) activities were determined according to the method described by [24].

The serum urea concentration was determined using diacetylmonoxime method as described by [25]. Serum Uric acid concentration was determined using colorimetric method as described by [26]. Serum creatinine concentration was determined using Robbery folin method as described by [27].

The serum calcium ion concentration was determined using colorimetric method described by [28]. Serum phosphate ion

concentration was determined using colorimetric method described by [29]. Serum sodium concentration was determined using colorimetric method as described by [30]. Serum potassium concentration was determined using calorimetric methods as described by [31]. Serum chloride were determined using colorimetric methods as described by [32].

3. Statistical Analysis

Data were expressed as mean \pm S.D of five (5) replicates. Significant differences were determined using one-way analysis of variance (ANOVA) and Duncan Multiple Range Test (DMRT) at 5% confidence limit using a software SPSS version 17.0.

4. Results

Table 1. Result for phytochemical screening.

PHYTOCHEMICALS	RESULT
Alkaloids	+++
Flavonoids	++
Tannins	++
Cardiac glycosides	++
Steroids	-
Phlobatinins	-
Saponin	-
Terpenoids	-
Phenol	++

KEY

= absent,

+ = Slightly Present,

++ = Moderately Present,

+++ = Highly Present

Table 2. Alkaline Phosphatase (IU/L) activity in Kidney and Serum of Albino Rats.

Organs/serum	Control	100mg/kgbw	200mg/kgbw	300mg/kgbw
Kidney	98.65 \pm 7.79 ^a	337.77 \pm 15.43 ^b	472.90 \pm 10.85 ^c	553.15 \pm 19.68 ^d
Serum	94.50 \pm 7.50 ^a	325.25 \pm 5.60 ^b	469.60 \pm 10.96 ^c	593.35 \pm 27.65 ^d

Values are presented as mean \pm SD of 5 replicate.

^{a-d} values carrying different superscript from the control are statistically different at (P <0.05)

4.3. Effect of *D. metel* Seed Aqueous Extract on Alanine Amino Transaminase (ALT)

The activity of ALT significantly increased in the serum at all the doses of extract treated groups when compared with

Table 3. Alanine amino transaminase (IU/L) activity of kidney and serum in albino rats.

Organs/serum	Control	100mg/kgbw	200mg/kgbw	300mg/kgbw
Kidney	55.15 \pm 4.56 ^a	55.60 \pm 2.07 ^a	65.55 \pm 3.42 ^b	98.10 \pm 1.50 ^c
Serum	85.50 \pm 6.35 ^a	195.50 \pm 4.04 ^b	395.00 \pm 4.61 ^c	554.00 \pm 8.08 ^d

Values are presented as mean \pm SD of 5 replicate.

^{a-d} values carrying different superscript from the control are statistically different at (P<0.05)

Phytochemical constituents of *Datura metel* aqueous seeds extract revealed the presence of some phytoconstituents (Table 1).

4.1. Effect of *D. metel* Aqueous Seeds Extract on the Organ-Body Weight Ratio

Administration of *D. metel* seeds aqueous extract at 100, 200 and 300 mg/kg bw significantly decreased the organ-body weight ratio at all the doses of the extract treated groups when compared with control (Figure 1).

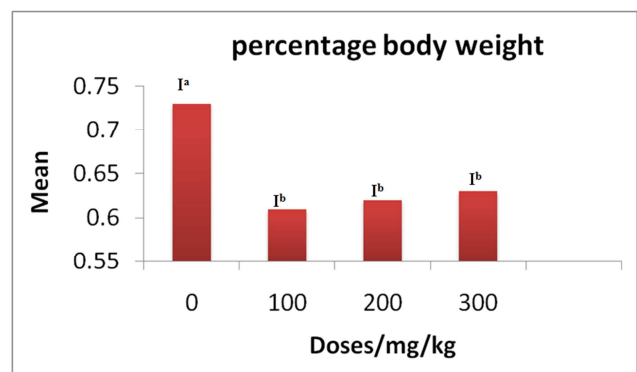


Figure 1. Effect of *Datura metel* aqueous seed extract on the organ-body weight ratio of albino Wistar rats.

4.2. Effect of *D. metel* Seed Aqueous Seed Extract on Alkaline Phosphatase (ALP)

Administration of the aqueous seed extract of *D. metel* at 100, 200 and 300 mg/kg bw significantly increased the activity of ALP in both kidney and serum at all the doses of extract treated groups when compared with the control (Table 2).

control group, following administration of the aqueous seeds extract of *D. metel*. Similarly, the enzyme activity increased significantly at 200 and 300 mg/kg body weight when compared with control, and no significance difference at 100 mg/kg body weight when compared with control (Table 3).

4.4. Effect of *D. metel* Aqueous Seed Extract on Aspartate Amino Transaminase (AST)

The activity of AST in the kidney significantly increased at all the doses of extract treated groups following

administration of the aqueous seeds extracts of *D. metel* when compared with the control. Similarly, the enzyme activity significantly increased at 200 and 300 mg/kg body weight doses of the extract treated groups when compared with control, and no significant difference between 100 mg/kg body weight when compared with control (Table 4).

Table 4. Aspartate amino transaminase (U/L) activity of kidney and serum in albino rats.

Organs/serum	Control	100mg/kgbw	200mg/kgbw	300mg/kgbw
Kidney	90.00±9.23 ^a	201.50±4.04 ^b	403.50±5.19 ^c	571.25±15.92 ^d
Serum	51.75±1.90 ^a	51.50±1.15 ^a	59.60±1.50 ^b	91.95±0.17 ^c

Values are presented as mean ±SD of 5 replicate.

^{a-d} values carrying different superscript from the control are statistically different at (P<0.05)

4.5. Effect of *D. metel* Aqueous Seed Extract on Some Kidney Function Indices

Administration of the aqueous seed extracts of *D. metel* at 100, 200 and 300 mg/kg bw significantly increased the concentration of serum urea, uric acid and calcium ions at all the doses of extract treated groups when compared with

control, serum sodium and phosphate ions concentration also increased at 200 and 300 mg/kg bw. Similarly, serum potassium and creatinine levels increased but only at 300 mg/Kg bw. In contrast, the serum concentration of chloride ion significantly decreased at all the doses of extract treated groups when compared with the control (Table 5).

Table 5. Serum biochemical changes in the Kidney Function Indices.

Groups	Control	100mg/kg bw	200mg/kg bw	300mg/kg bw
Urea (mmol/L)	3.55±0.19 ^a	4.60±0.11 ^b	5.60±0.11 ^c	6.55±0.05 ^d
Uric acid (mg/dl)	3.60±0.11 ^a	4.65±0.05 ^b	5.35±0.17 ^c	6.05±0.57 ^d
Creatinine (mg/dl)	1.00±0.11 ^a	0.95±0.05 ^a	1.05±0.05 ^a	1.50±0.11 ^b
Sodium (mmol/L)	123.00±2.30 ^a	127.00±4.61 ^a	137.00±2.30 ^b	138.00±2.30 ^b
Potassium (mmol/L)	5.59±0.05 ^a	6.02±0.151 ^a	6.05±0.25 ^a	6.85±0.05 ^b
Calcium (mmol/L)	1.60±0.01 ^a	1.98±0.01 ^b	2.05±0.05 ^b	3.10±0.08 ^c
Phosphate (mmol/L)	1.28±0.01 ^a	1.11±0.06 ^b	1.47±0.01 ^c	1.45±0.01 ^c
Chloride (mmol/L)	105.50±0.57 ^a	93.50±1.73 ^b	99.50±0.57 ^c	97.00±3.46 ^c

Values are presented as mean ±SD of 5 replicate.

^{a-d} values carrying different superscript from the control are statistically different at (P<0.05)

5. Discussion

This study revealed the presence of alkaloids, flavonoids, tannins, glycoside and phenol. These compound are biologically active and responsible for different activities such as antioxidant, antimicrobial, antifungal and anticancer [33] [14]. However, the toxic effect of this medicinal plant may be due to the presence of these secondary metabolites. Typical alkaloids often have marked pharmacological effects when administered to man and other animals, thus their presence is of particular interest [34].

The presence of these constituents may also be responsible for part of the nephrotoxic properties shown by *D. metel*. Hence their presence plays an important role in the reduction of body weight in Wister albino rats (Figure 1).

The observed decrease in body weight of the animals in the present study may suggest atrophy as reflected in the alteration of biochemical indices analyzed following administration of

the aqueous seed extracts of *D. metel* (Figure 1).

Evaluation of biochemical indices in serum and organs of animals has become the most valuable tools for assessing the integrity and functionality of organs as well as risk assessment, pathological condition and general health status of the body. Defeat in the activities of these biomarkers in serum and body tissues are to a sensible degree the detrimental effect of a drug or extract/compound under investigation [35]. The result in the present study shows that there is significant difference in both the level of kidney and serum diagnostic enzymes alkaline phosphatase (ALP) on administration of the aqueous seed extract of *Datura metel* at all the doses when compared with the control group. These show that there is an increase in the activity of ALP in both serum and kidney. Alkaline Phosphatase which is localize in the microvilli of canaliculi located in the plasma membrane is the most widely used biochemical test to put in evidence for cholestasis of both intra and extra hepatic origin.

The significant increase in ALP activity following

administration of the aqueous seed extract of *Datura metel* as compared with the control (Table 2) may suggest possible occurrence of cholestasis. The increase in the level of ALP in both the serum and kidney might be associated by the liver damage since the liver has detoxification ability, so when it cannot overcome the dosage it may be damage which lead to increase of ALP in serum and kidney. This is in contrast with the study report by [36].

AST and ALT are two of the most reliable markers of hepatocellular injury or necrosis whose levels are elevated in a variety of hepatic disorders or dysfunction of these transaminases, ALT is more specific for hepatic injury because it is present mainly in the cytosol of the liver and low concentrations elsewhere [37].

The significant increase in ALT and AST activities for both kidney and serum following administration of the aqueous seed extract of *Datura metel* as compared with the control may be due to default of the liver since these enzymes are predominant in the liver but not in the kidney (Table 3). However, Kidney cell destruction is characterized by increase in the level of serum enzymes (ALT and AST). The observed alteration in these enzymes for both kidney and serum may suggest malfunctions from other tissues. Furthermore, about 80% of AST is found in mitochondria where as ALT is located in the cytosol. Thus AST appear in other tissue like heart, liver and spleen aqueous. Therefore, aqueous seed extract of *Datura metel* may suggest that there may be possible kidney damage that has occurred.

Kidney function parameters are valuable tools for assessing the integrity of various parts of the kidney [38]. The level of creatinine, electrolytes, urea, and serum total protein could also provide significant information regarding the influence of a drug/compound/extract on the glomerular and tubular region of the kidney [36].

The increase in the level of urea, uric acid, sodium and calcium may be due to kidney malfunction which implies dysfunction at tubular levels. The serum urea concentration is elevated in renal disease especially glomerular damage. The significant increase in serum urea levels may be possible due to some regenerative mechanism by the kidney in response to the effect of *Datura metel* and studies have shown equally that before any makers (urea or creatinine) become significantly increased in the blood, about 75% of the nephron must have been damage [39].

The significant increase in the concentration of serum creatinine, potassium at 300mg/kg bw may indicate impaired glomerular filtration caused by aqueous seed extract of *Datura metel* as well as enhanced phosphate ion. The significant reduction in the levels of serum creatinine in the experimental rats by the end of the administration period may

be an indication of compromised renal function [36] [40].

The observed decreased in the serum concentration of chloride ion in this study may also supported the glomerular and tubular renal dysfunction caused by the extract.

The study therefore, revealed possible compromised in tubular and glomerular function leading to renal dysfunction following administration of *Datura metel* aqueous seed extract in rats.

6. Conclusion

The result of the present study suggest that the aqueous seed extract of *Datura metel* seeds extract may have some nephrotoxic effect on the basic functions of kidney investigated in Wister albino rats as revealed in the alterations of the kidney functions parameters analyzed. These findings are therefore of clinical importance considering the various reported medicinal potentials of the plants.

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