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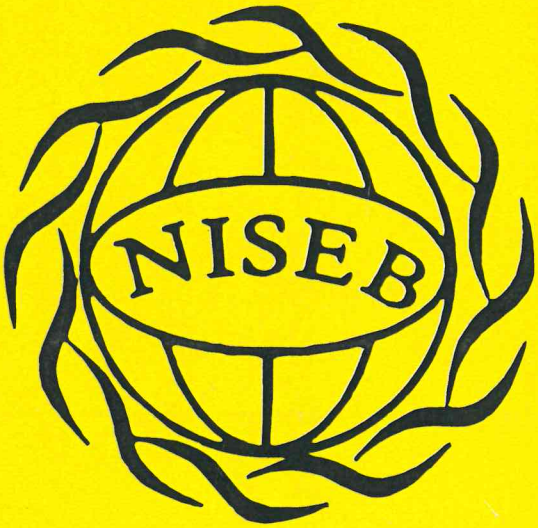

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Toxicological Evaluation of *Datura metel* seed Extracts in Rat

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Abstract
Datura metel seed has been used medicinally for the treatment of different ailments and as a psychoactive agent. Rats in their respective groups were administered 100, 300 and 600mg/kg bodyweights of n-hexane fraction of the seeds of Datura metel for twenty-eight days. Blood samples were collected by carotid puncture into heparinized and non-heparinized bottles for haematological and biochemical analysis respectively. 600 mg/kg bodyweight dose group progressively had reduction in bodyweight (168.00 ± 12.00) at the end of the experimental period. Total haemoglobin (Hb) g/dL, Packed Cell Volume (PCV) %, Red Blood Cells count (RBC) 1012/L, and lymphocytes (L) % all increased in extract fraction treated groups. At doses of 100 and 300mg/kg bodyweight, AST and ALP increased significantly while ALT decreased in all the treated groups. Rats in all treated groups had elevated levels of bilirubin and albumin while only 600mg/kg bodyweight had an elevated level of total protein (10.64 ± 1.02). Cholesterol and triacyglycerol concentrations increased as concentrations increases while HDL decreased in the same manner. At 100 and 600mg/kg bodyweight, sodium (204.01 ± 4.20) and potassium (16.03 ± 0.77) increased respectively. Chloride concentration was however elevated at 300 and 600mg/kg bodyweight. Uric acid, urea and creatinine decreased significantly at 600 mg/kg bodyweight. The fraction has haematinic activity and immunostimulatory effect but with mild toxic effect on nephrocytes.

Keywords: *Datura metel,* haematopoietic system, hepatoprotective, nephroprotective

Introduction
Datura metel is a perennial herbaceous plant that belongs to the family "Solanaceae". It is called datura in Hindi and white apple in English [1]. In Nigeria, the plant is known by different names according to each tribe (Apikan-Yoruba, Zakami-Hausa and Myaramuo-Igbo) [2]. The fractions from seed of the plant are used for alleviating several health conditions such as skin rashes, ulcers, bronchitis and diabetes in traditional folklore. The plant is widely distributed in tropical and subtropical regions of the world and usually found growing in dumpsites or abandoned farmlands. It is native to Asia and Africa [3]. The seed fractions have been reported to possess anti-diabetic [4], contraceptive [5] and insecticidal [6]. Datura metel is abused by adding its decoction or tincture of the seeds, leaves or flower to drinks in order to produce euphoria effect. Some drug addicts use Datura metel leaves as substitute for marijuana because it is readily available and relatively cheap. Apart from enormous pharmacological activities of Datura metel seeds, comprehensive knowledge of its toxicological effects has not been thoroughly examined. The study therefore aims at evaluating the acute and sub chronic toxicity of the n-hexane seed fraction of Datura metel.

Materials and Methods
Plant Collection and Identification
The whole plant parts of *Datura metel* was collected from Sabo area in Bode Sa'adu, Moro, Kwara state, Nigeria, between May and June, 2018. The plant was identified at herbarium and Ethnobotany unit of National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria, where voucher number was deposited as NIPRD/H/6918.
Reagents and Chemicals
All chemicals used were of analytical grades and products of sigma-Aldrich.
Experimental Animals
Twenty-one healthy wistar albino rats were obtained from the animal house of the Department of Biochemistry, Federal University of Technology, Minna, Nigeria. They were allowed to acclimatize to laboratory environment for two weeks with access to feed pellets and water *ad libitum*.

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Ethnics of Laboratory Animals

The principles governing the use of laboratory animals as approved by the ethnical committee of Federal University of Technology, Minna was strictly adhered to. This is also in accordance with the guidelines of the National Research Council Guide for the Care and Use of Laboratory Animals and principles of good laboratory procedures.

Plant sample preparation and fraction

Seeds were collected from fresh fruits of *Datura metel* and air dried at room temperature for three weeks. The dried seeds were then pulverized using electric blender. The powdered sample was fractioned by cold maceration for forty-eight hours using distilled water. The fraction was filtered and concentrated using rotatory evaporator, and the concentrated fraction was dried using lyophilizer (FREEZE-DRYER-LGJ-18).

Partitioning

The partitioning of the extract was carried out according to Abu *et al* [7]. Every one gram of crude aqueous fraction was dissolved in 20ml of water (1:20 (w/v) and then 20 ml of n-hexane was added in a separating funnel (20:20 (v/v)). The separating funnel was shaken for two minutes and allowed to stand at room temperature for two hours. The aqueous phase (lower phase) was collected and hexane phase (upper phase) was decanted into a clean beaker to evaporate. The aqueous was again mixed with 20 ml of hexane, the separating funnel was shaken for further two minutes and allowed to stand for two hours at room temperature. The procedure was repeated five times (till the hexane phase became clear). This way hexane fraction was collected.

Quantitative determination of secondary metabolites

Total phenols, total flavonoids, tannins, alkaloids and saponins were determined using the methods of [8, 9, 10, 11] respectively.

Acute toxicity study and determination of LD_{50}

LD_{50} of the n-hexane seed fraction of *Datura metel* was determined using the method of Ofem *et al* [12].

Subchronic toxicity study

Twelve rats were randomly allotted to four groups of three rats each. Rats in group one were the control while groups two, three and four were orally administered with 100, 300 and 600 mg/kg bodyweight of the n-hexane seed fraction of *Datura metel* respectively. The rats were allowed to feed on pellets and water *ad libitum*. Feed is withdrawn from the animals two-hours prior fraction administration and two hours post administration [13].

Haematological Analysis

Haematological parameters; Packed Cell Volume (PCV), Mean Cell Volume (MCV), Haemoglobin (Hb), Mean Cell Haemoglobin (MCH), Mean Cell Haemoglobin Concentration (MCHC), Red Blood Cell Count (RBC), Platelet Count (PLC), Total White Blood Cells Count (TWBC), Neutrophils (N) and Lymphocytes (L), were analyzed using Auto-haematological Analyzer (Abacus).

Biochemical Analysis

Randox and Agappe commercial kits were used for the determination of the following biochemical parameters: Aspartate aminotransferase (AST), alanine aminotransferase (ALT), Alkaline phosphatase (ALP), total and direct bilirubin, total protein, albumin, total cholesterol, triacylglycerol, high density lipoprotein-cholesterol (HDL-cholesterol), Low density lipoprotein-Cholesterol (LDL-cholesterol), uric acid, urea, creatinine, sodium, potassium, chloride and bicarbonate are the biochemical parameters analyzed.

Statistical Analysis

The data were analyzed by One-way Analysis of Variance (ANOVA) using Statistical Package for Social Science (SPSS). The results were expressed as mean ± standard error of mean (SEM). The difference in mean of various groups of animals were compared using "Duncan multiple Range Test". *P*-value less than 0.05 was considered significant ($P< 0.05$).

Results

Quantitative phytochemicals composition of aqueous extract of *Datura metel* seeds

Table 1 shows the quantitative phytochemicals composition of aqueous extract of *Datura metel* seeds. Phenols are the highest in composition (252.80 ± 3.58mg/100g) compared to other photochemicals.

Table 1. Quantitative phytochemical composition of *Datura metel* seeds

Phytochemical	Amount(mg/100g)
Phenol	252.80±3.58
Flavonoids	30.11±2.36
Alkaloids	44.54±2.62
Tannins	39.80±2.13
Saponins	185.64±4.69

Values are mean ± standard error of mean, (n= 3)

Acute toxicity (LD_{50})

Table 2 shows the result of acute toxicity study. No mortality or sign of toxicity is recorded in phase 1 and 2 of Lorke's method of acute toxicity study.

Table 2: LD_{50} of n-hexane seed fraction of *Datura metel*

Phase 1		Phase 2	
Dose (mg/kg bodyweight)	Mortality	Dose (mg/kg bodyweight)	Mortality
10	0/3	1600	0/1
100	0/3	2900	0/1
1000	0/3	5000	0/1

Bodyweight of experimental rats

Table 3 shows weekly variation in bodyweight of experimental rats during the experimental days. Increased bodyweight was observed in groups 1, 2 and 3 while a decrease in bodyweight was observed in group 4 throughout the experimental weeks.

Table 3: Weekly variation in bodyweight of rats administered various doses of n-hexane seed fraction of *Datura metel*.

	Weight (g)			
Group	Week 1	Week 2	Week 3	Week 4
Group 1	172.00±18.00	174.50±18.50	178.67±15.50	179.67±14.50
Group 2	155.33±8.50	160.50±7.50	162.50±7.00	169.67±8.50
Group 3	190.00±22.50	190.67±27.50	195.67±23.50	197.67±27.50
Group 4	214.33±37.50	192.00±25.00	175.00±12.00	168.00±12.00

Values are mean ± standard error of mean (n= 3)
Group 1= control group administered with 0.5mL of normal saline
Group 2= Group administered with 100mg/kg bodyweight of the fraction
Group 3= Group administered with 300mg/kg bodyweight of the fraction
Group 4= Group administered with 600mg/kg bodyweight of the fraction

Haematological Analysis

Table 4 shows the effect of aqueous extract of *Datura metel* on haematological parameters of experimental animals. The concentrations of Red blood cells indices in the experimental animals administered with the doses of 100 and 300mg/kg bodyweight except PCV and RBCs are not significantly different ($P > 0.05$) from the control group. PCV increased significantly ($P< 0.05$) at dose of 100mg/kg bodyweight and no significant different ($P > 0.05$) was found at dose of 300mg/kg bodyweight when compared to the control group. At dose of 600mg/kg bodyweight, concentrations of red blood cells indices increased significantly ($P< 0.05$) except for MCV and MCH which decreased significantly ($P< 0.05$) with no significant difference ($P > 0.05$) observed when compared to the control group. There was no significant difference ($P > 0.05$) in platelets count (PLC) at all the tested doses when compared to the control group. No significant difference ($P > 0.05$) in Total white blood cells count and neutrophils was observed at dose of 100mg/kg bodyweight whereas a significant increase ($P< 0.05$) was observed in lymphocytes concentration when compared to the control group. Significant increase ($P< 0.05$) in total white blood cells count was found at doses of 300 and 600mg/kg bodyweight. At doses of 300 and 600mg/kg bodyweight, neutrophils concentration was significantly increased ($P< 0.05$) and no significant difference ($P > 0.05$) was observed when both groups were compared to the control group.

Table 4: Haematological indices of rats administered various doses of n-hexane seed fraction of *Datura metel*

Parameter	Dosage (mg/kg bodyweight)			
	Group 1 Control	Group 2 100	Group 3 300	Group 4 600
Hb (g/dL)	11.70 ± 0.80^{a}	13.00 ± 0.78^{a}	12.90 ± 0.90^{a}	14.60 ± 0.50^{b}
PCV (%)	34.50 ± 2.50^{a}	39.00 ± 2.0^{b}	38.00 ± 2.0^{ab}	45.00 ± 1.70^{c}
MCV (fi)	51.50 ± 1.50^{b}	49.00 ± 3.0^{ab}	54.00 ± 4.0^{b}	46.00 ± 2.00^{a}
MCH (pg)	20.50 ± 0.50^{b}	18.00 ± 0.70^{a}	21.20 ± 2.00^{b}	19.00 ± 0.50^{ab}
MCHC (g/dL)	40.00 ± 0.00^{a}	38.00 ± 1.30^{a}	39.00 ± 1.00^{a}	43.00 ± 2.00^{b}
RBC (10^{12}/L)	5.65 ± 0.15^{a}	6.50 ± 0.21^{b}	6.65 ± 0.75^{b}	7.50 ± 0.12^{c}
PLC (10^{6}/L)	170.00 ± 5.00^{a}	160.00 ± 6.00^{a}	161.00 ± 6.00^{a}	168.00 ± 3.00^{a}
TWBC (10^{12}/L)	5.20 ± 0.04^{a}	5.10 ± 0.03^{a}	6.35 ± 0.05^{c}	6.10 ± 0.13^{b}
N (%)	44.00 ± 2.00^{a}	40.00 ± 1.00^{a}	54.00 ± 4.00^{b}	44.00 ± 1.88^{a}
L (%)	28.00 ± 0.85^{a}	35.00 ± 1.50^{b}	42.00 ± 2.00^{c}	40.00 ± 1.50^{c}

Values are mean ± standard error of mean (n=3)
Values with different superscript in a row were significantly different from each other ($P<0.05$)
Hb= Haemoglobin, PCV= Packed Cell Volume, Mean Cell Volume, MCH= Mean Cell Haemoglobin, MCHC= Mean Cell Haemoglobin Concentration, RBC= Red Blood Cell Count, PLC= Platelet Count, TWBC= Total White Blood Cell Count, N= Neutrophils= Lymphocytes, E= Eosinophils, B= Basophils, M= Monocytes, RDWC= Red Blood Cell Distribution Width Count

Biochemical Analysis

Total protein and Albumin: Figure 1 shows the effects of sub-chronic oral administration of n-hexane seed fraction of *Datura* metel on serum total protein and albumin of experimental rats. Serum total protein concentration at dose of 100 and 600mg/kg bodyweight shows a significant difference ($P > 0.05$) from the control group, while significant increase ($P < 0.05$) in total protein was observed at doses of 300 and 600mg/kg bodyweight when compared to the

total protein concentration at dose of 100mg/kg bodyweight. At all the tested doses, albumin concentration showed no significant difference ($P > 0.05$) from the control group.

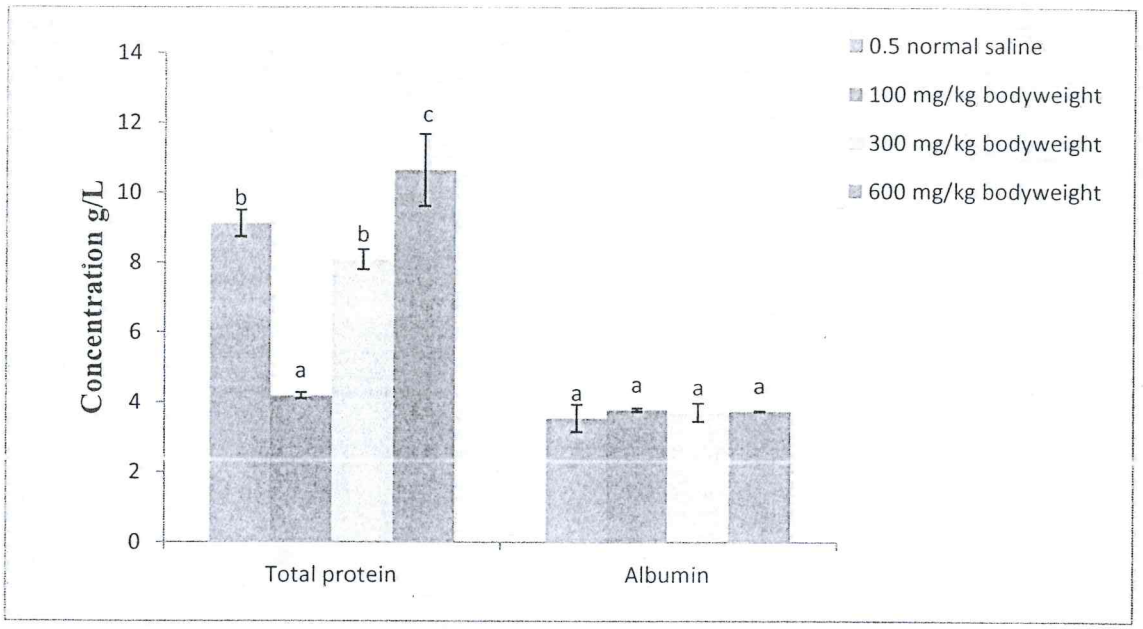

Figure 1: Effect of n-hexane seed fraction of *Datura metel* on serum total protein and albumin of rats.

Lipid profile: Figure 2 shows the effects of sub-chronic oral administration of n-hexane seed fraction of *Datura* metel on lipid profile of experimental rats. Cholesterol and triglycerides levels increased significantly ($P < 0.05$) at all the tested doses. Significant decrease ($P < 0.05$) was observed in LDL-cholesterol concentrations at all the tested doses whereas the concentration of HDL-cholesterol increased significantly ($P < 0.05$) at 100mg/kg bodyweight and decrease significantly at doses of 300 and 600mg/kg bodyweight compared to control group.

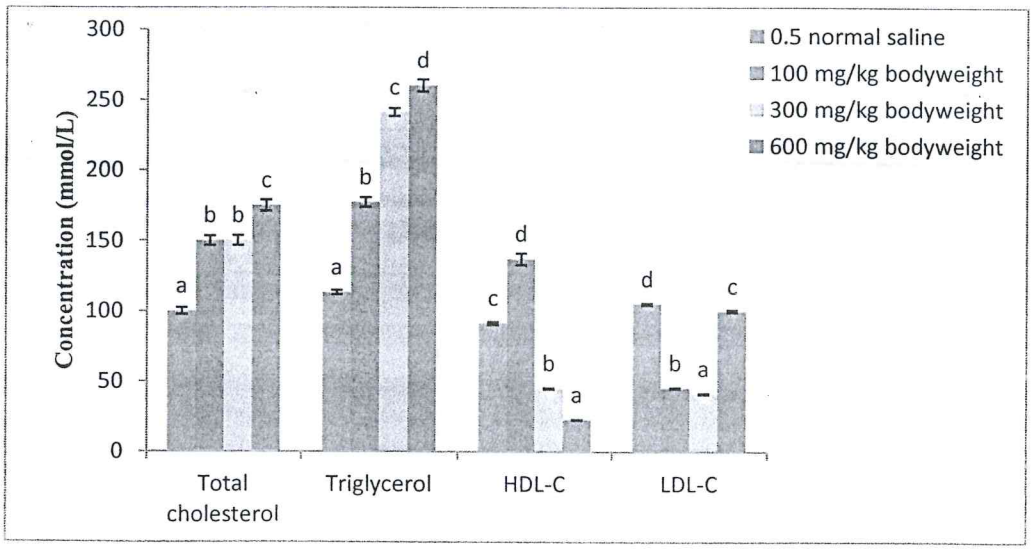

Figure 2: Effect of n-hexane seed fraction of *Datura metel* on lipid profile of rats.

Liver Enzymes: Figure 3 shows the effects of sub-chronic oral administration of n-hexane seed fraction of *Datura metel* on liver enzymes of experimental rats. ALT activity decreased significantly ($P < 0.05$) at all the tested doses. AST and ALP activity were increased significantly ($P < 0.05$) at doses 100 and 300mg/kg bodyweight whereas dose of 600mg/kg bodyweight caused no significant difference ($P > 0.05$) in AST and ALP activity when compared to the control group.

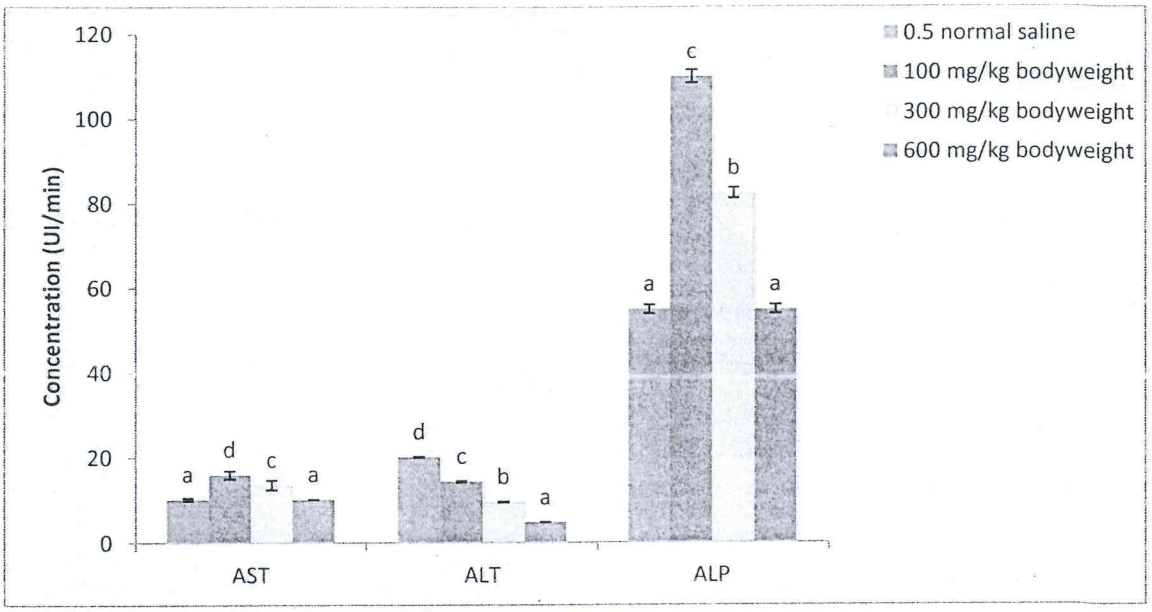

Figure 3: Effect of n-hexane seed fraction of *Datura metel* on AST, ALT and ALP of rats.

Total, indirect and direct bilirubin: The fraction caused significant increase ($P < 0.05$) in total bilirubin, direct bilirubin and indirect bilirubin at doses of 100 and 300mg/kg bodyweight. Total bilirubin and indirect bilirubin concentrations also increased significantly ($P < 0.05$) at 600mg/kg bodyweight; however, direct bilirubin concentration showed no significant difference ($P > 0.05$) from the control group (figure 4).

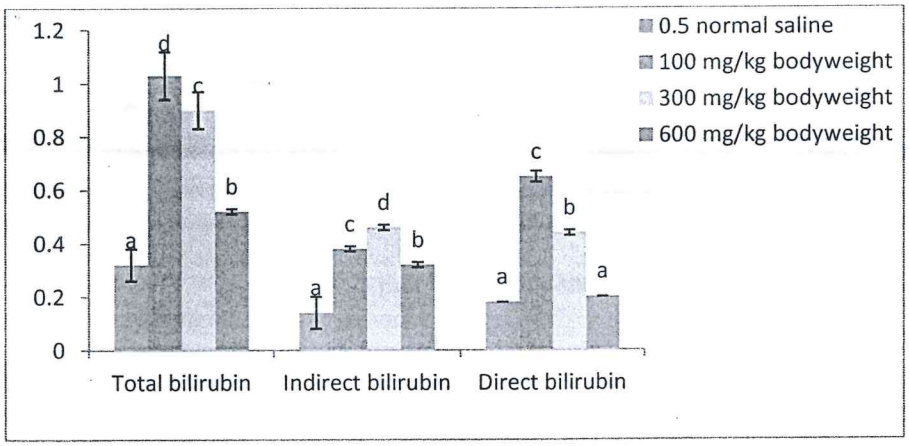

Figure 4: Effect of n-hexane seed fraction of *Datura metel* on serum total bilirubin, indirect bilirubin and direct bilirubin of rats.

Serum Electrolytes: The effects of sub-chronic oral administration of n-hexane seed fraction of *Datura* metel on total, indirect and direct bilirubin of experimental rats is presented in **figure 5**. Sodium concentration was

significantly increased ($P < 0.05$) at dose of 100mg/kg bodyweight and decrease significantly ($P < 0.05$) at 300 and 600mg/kg bodyweight compared to the control group. No significant difference ($P < 0.05$) was observed in the concentrations in the potassium concentration at 100 and 300mg bodyweight, but significant increase ($P < 0.05$) in potassium concentration at dose of 600mg/kg bodyweight was observed when compared to the control group. Chloride level at dose of 100mg/kg bodyweight showed no difference from the control group but significant increase was found at doses of 300mg/kg bodyweight. No significant difference was seen in the bicarbonate concentration at all tested doses.

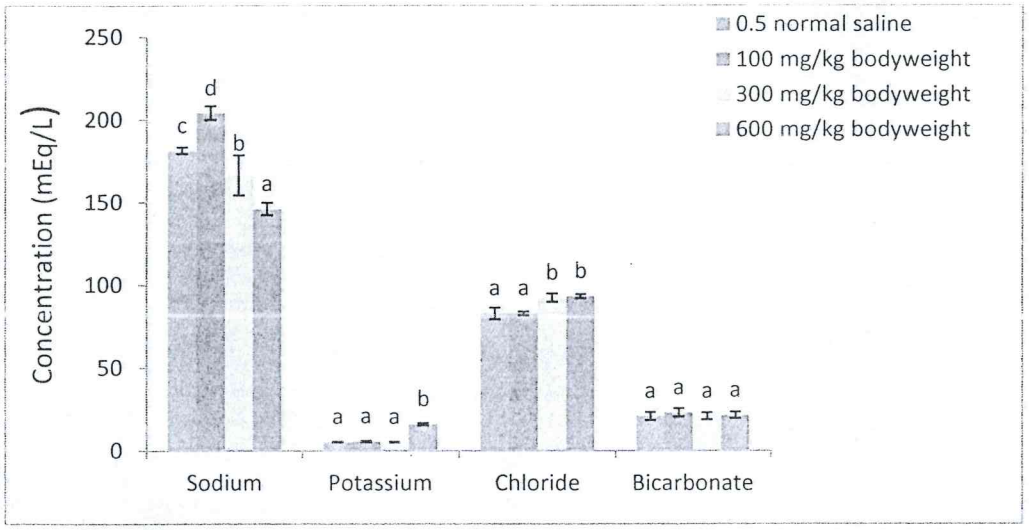

Figure 5: Effect of n-hexane seed fraction of *Datura metel* on serum electrolytes of rats.

Uric acid, Urea and Creatinine: Figure 6 shows the effects of sub-chronic oral administration of n-hexane seed fraction of *Datura* metel on uric acid, urea and creatinine of experimental rats. Uric acid concentrations at all tested doses showed no significant difference ($P > 0.05$) from the control group. A significant decrease in urea level was observed at 100 and 600mg/kg bodyweight while no significant decrease was found at 300mg/kg bodyweight compared to control group. Creatinine concentration significantly decreased at all tested doses ($P < 0.05$) compared to the control group.

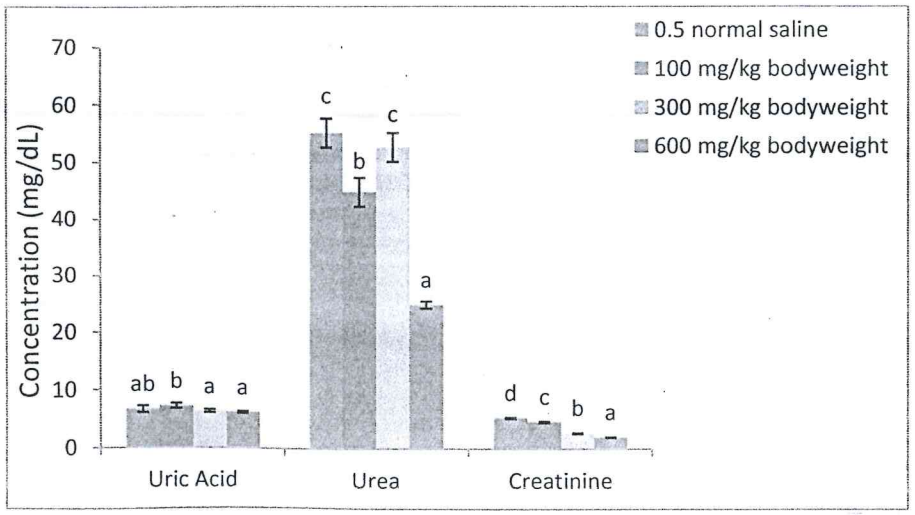

Figure 6: Effect of n-hexane seed fraction of *Datura metel* on uric acid, urea and creatinine of rats

Discussion

Quantitative phytochemical analysis confirmed the presence of phenols, flavonoids, alkaloids, tannins and saponins. Phenolic compounds such as phenols, flavonoids and tannins among others possess hypoglycemic, antidiabetic effects [14, 15, 16 and 17]. Other pharmacological activities exhibited by this group of compounds include anti-inflammatory, antimicrobial and antioxidant activities [18]. Alkaloids have wide range of pharmacological activities which include antimalarial, antiasthma, anticancer [19], vasodilatory, antiarrhythmic, analgesic [20] activities etc. Saponins exhibit enormous biological effects, such as cell membrane permeability enhancing, lowering of serum cholesterol level, immunomodulatory and antitumor activities [21]. Therefore, presence of these phytochemicals is responsible for the biological activities exhibited by the seeds of *Datura metel* plant.

Determination of LD_{50} is usually the first step in the toxicological evaluation of medicinal plants [22]. The absence of mortality at the dose of 5000mg/kg bodyweight of n-hexane seed fraction of *Datura metel* in rats showed that the fraction is non-toxic acutely. Plant fractions or drugs with LD_{50} > 5000mg/kg bodyweight, are placed in class 5 which is the lowest toxicity class by Guidance Document on Acute Oral Toxicity Testing based on oral LD_{50} value recommended by Organization for Economic Cooperation and Development [23]. Therefore, n-hexane seed fraction of *Datura metel* with LD_{50} > 5000mg/kg bodyweight is placed in class 5 of oral acute toxicity testing put forward by OECD.

Loss of bodyweight is as a result of loss of muscle and adipose tissue resulting from excessive breakdown of tissue protein and fatty acids [24]. Sub-chronic oral administration of n-hexane seed fraction of *Datura metel* caused no change in bodyweight of the experimental rats administered doses of 100 and 300mg/kg bodyweight, there was however, a significant decrease ($P > 0.05$) in bodyweight of rats administered dose of 600mg/kg bodyweight. This implies that the fraction at higher doses causes loss of muscle and adipose tissue.

Effect of fraction on haematological indices is presented in Table 4. These parameters reflect the effects of foreign compounds contained in medicinal plants that may lead to some physiological changes [25, 26]. This may be useful in determining the haematological relating function of plant products [27]. Evaluation of the toxicity of medicinal plant extracts aids in assessing the changes in hematological status of the body that will predict the integrity of the hemapoeitic system [28]. Thus, haematological parameters reflect the physiological state of an animal [29].

Packed Cell Volume (PCV) also known as hematocrit (Ht or Hct) or Erythrocyte Volume Fraction (EVF) is the percentage of red blood cells in the total blood volume. Some investigators [30, 31] reported that PCV measures the percentage volume of red blood cells in the blood; low production of red blood cells or increased hemolysis is associated with anemia. Oxygen and absorbed nutrients transport are also measured by PCV. Increased PCV level is an indication of better transportation and thus result in primary and secondary polycythemia [32]. Therefore, significant increase in PCV level at all tested doses indicates that the fraction was able to stimulate the production of red blood cells in the bone marrow. This suggests that the fraction contain certain phytochemicals that cause the release of erythropoietin which stimulate the rate of red blood cells production. Hence, the fraction is said to have hematinic activity and can be used in ameliorating anemia. Increase in MCH and MCV is indicative of macrocytic anemia in which there is reticulocytosis; an initial response when erythropoiesis is stimulated. Previous researchers [33, 34] also reported that RBCs, Hb, MHC, MCHC, and Platelets are associated with erythropoiesis and osmotic fragility of red blood cells. Significant increase in RBCs and MCHC at all tested doses further attest the hematinic property of the fraction. No change in MCH and MCV levels at all tested doses when compared to the control group implies that the fraction unaltered haemoglobin synthesis in individual RBCs and at the same time unaltered the size of RBCs.

White blood cells (WBCs) and its differentials function exclusively to fight diseases, phagocyte foreign materials, produce or transport and distribute antibodies in immune responses. Thus, WBCs and its differentials represent the body's immunomachineries. Animals with low WBCs count are susceptible to disease infections, whereas those with high WBCs have high degree of disease resistance to infections [35]. Adaptability to local environmental and disease prevalence conditions are enhanced by high WBC count. [36, 37] reported low levels of WBCs differentials especially low eosinophils and lymphocytes in patient with heart failure. There was a significant increase ($P>0.05$) in WBC count, neutrophils and lymphocytes at doses of 300 and 600mg/kg bodyweight of the n-hexane seed fraction of *Datura metel.* Lymphocytes also increased significantly ($P>0.05$) at 100mg/kg bodyweight. This give a scientific basis for the use of this plant seeds for immune boosting in patients with weak immune system.

Assessment of liver and kidneys function tests is an important factor when evaluating toxicity of drugs and plant fractions since they are both necessary for the animal survival [38]. Serum protein level is an important marker relating to liver synthetic functions and it is a very helpful guide in assessing the degree of liver damage. [39] also reported decrease in the level of serum total protein in hepatotoxicity state to simply indicate the presence of para proteins or deficient production of antibodies. Therefore, significant increase in serum total protein level at dose of 600mg/kg bodyweight showed that the fraction improved total protein level and further attest the

immunostimulatory effect of the fraction at higher doses (Figure 1). However, insignificant decrease in serum total protein level at dose of 300mg/kg bodyweight suggests that the fraction did not alter protein synthesis whereas significant decrease in serum total protein at dose of 100mg/kg bodyweight showed that the fraction inhibit protein synthesis at lower doses. Albumin is a serum protein which functions in the maintenance of osmotic pressure of the body fluids and transport hormones, fatty acids and drugs[39].A non-significant difference($P>0.05$) in albumin levels at all doses is an evidence that the fraction does not affect albumin synthesis and thus unaltered the transport and metabolism of the materials transported by albumin (Figure 1). This observation is in agreement with the research carried out by [40] who worked on effect of ethanolic seeds fraction of *Datura metel* and found no significant difference in albumin concentration at doses of 300 and 600mg/kg bodyweight when compared to the control group.

Lipid profile of an animal comprise important indices; total cholesterol, Triacylglycerol, high density lipoprotein-cholesterol (HDL-cholesterol) and low-density lipoprotein (LDL-cholesterol). Low serum HDL-cholesterol level reflects poor cholesterol transport by HDL particles from peripheral tissues to liver for its metabolism.[41] reported that increased levels of total cholesterol, triacylglycerol and LDL-cholesterol and decrease in the level of HDL-cholesterol contribute to the increased risk for the development of cardiovascular diseases in patients with diabetes. Figure 2 revealed that the fraction might have caused a significant increase ($P>0.05$) in the levels of triacylglycerol and total cholesterol at all tested doses. These effects on triacylglycerol and total cholesterol is seen to be dose dependent. The concentrations of triacylglycerol and cholesterol increase with increase in the concentration of the fraction. However, significant decrease($P<0.05$) in HDL-cholesterol level at doses of 300 and 600mg/kg bodyweight and significant increase($P>0.05$) in HDL-cholesterol at doses of 100mg/kg bodyweight, and significant decrease in LDL-cholesterol level at all doses but with higher value at dose of 600mg/kg bodyweight suggest that, this fraction at higher doses may predispose individual to the development of cardiovascular diseases. Though, the levels of triacylglycerol and total cholesterol increased at dose of 100mg/kg bodyweight, but was compensated with significant increase in HDL-cholesterol. The findings inferred that as cholesterol synthesis increased, the transport rate to liver also increased preventing the accumulation of cholesterol. The potential effect of this fraction to increase the levels of triacylglycerol and cholesterol may largely due to its stimulation of the rate-limiting enzyme, HMG-CoA reductase of cholesterol synthesis and reduction in lipolysis by inhibiting the activity of hormone sensitive lipase.

Increased levels of liver enzymes have been attributed to the leakage and malfunctioning of the liver cell membrane. AST and ALT are transaminases found in the liver, but ALT is more liver specific than AST as AST can be found in other tissues like muscles, kidney, brain etc. This however means that elevated level of AST may not be due to the liver damage, but elevated level of ALT is mostly due to the liver damage. Therefore, significant increase ($P>0.05$) in AST activity at doses of 100 and 300mg/kg bodyweight may and may not be as a result of damage caused by this fraction on the liver, but significant increase in ALP at doses of 100 and 300mg/kg bodyweight further support the hepatotoxicity of this fraction at lower doses. However, non-significant difference ($P<0.05$) in the activity of AST and ALP, and significant decrease ($P<0.05$) in ALT activity showed hepatoprotective effect of the fraction at higher doses.

Elevation in the level of indirect bilirubin majorly indicates liver damage and haemolysis of RBCs. In this condition, liver can no longer conjugate bilirubin with glucoronic acid and therefore indirect bilirubin re-enters the circulation. This happens in conditions like severe hemolytic anemia, where excessive indirect bilirubin overwhelms the liver conjugating mechanisms. Increased direct bilirubin level on the other hand indicates biliary obstruction [42,43]. Elevated levels of total bilirubin, indirect bilirubin and direct bilirubin can also be caused by increased rate of RBCs formation (polycythemia), hemolysis, as seen in ineffective erythropoiesis, or from deficient bilirubin transport across the liver as presented in Gilbert's syndrome[44].Significant increase ($P>0.05$) in total bilirubin, indirect bilirubin and direct bilirubin was observed at all the tested doses (Figure 4). This observation also correlates with the research carried out by [45] who found increase in the levels of total bilirubin, indirect bilirubin and direct bilirubin. Therefore, significant increase ($P>0.05$) in the levels of total bilirubin, indirect bilirubin and direct bilirubin may be due to the hematinic effect of this fraction and not due to the liver damage.

The primary roles played by renal system include electrolyte/fluid regulation, buffering effect and in the elimination of waste products[48]. Sodium is the major extracellular cation which serves to regulate the total amount of water into and out of individual cells, it also plays role in critical body function [45]. Hyponatremia is reported to be due to an inappropriate production of anti-diuretic hormone (vasopressin) by the leukemic cells [46]. According to the Centers for Disease Control and Prevention (CDCP), increased sodium level can cause increase blood pressure and the risk for heart diseases and stroke in some individual [47]. Hence, significant increase ($P>0.05$) in sodium concentration at dose of 100mg/kg bodyweight showed that the fraction was able to increase water resumption in the experimental rats but increased sodium level has been linked to elevated blood pressure which suggest that the

fraction at this dose may predispose individuals to heart diseases and stroke. However, this effect may be useful in hyponatremia condition. Significant decrease ($P>0.05$) in the level of sodium at doses 300 and 600mg/kg bodyweight shows the fraction at these doses causes dehydration in the experimental rats. This, however, prevents increase blood pressure further preventing the risk for the development of heart diseases and stroke. This suggests that the fraction can be used at higher doses to alleviate hypernatremia.

Potassium ion is the major intracellular cation which protects against hypertension [48]. The fraction was able to induce an increase in the level of potassium at dose of 600mg/kg bodyweight. However, the fraction did not cause alteration in the level of potassium at doses of 100 and 300mg/kg bodyweight. Bicarbonate acts as a buffering system that prevents significant change in physiological pH [49]. Bicarbonate test helps to evaluate and keep track of the conditions that affect blood bicarbonate level including liver, kidney and metabolic conditions [50]. Therefore, non-significant difference($P<0.05$) in bicarbonate level at all doses indicated that the fraction did not alter the physiological pH and thus can prevent metabolic alkalosis and acidosis.

The body's acid-base balance is kept by serum chloride whose amount is carefully regulated by the kidneys. Significant increase ($P>0.05$) in chloride concentration at doses of 300 and 600mg/kg bodyweight showed that the fraction caused disturbance in acid-base balance of the body which may result in acidosis or alkalosis indicating kidneys dysfunction. However, at dose of 100mg/kg bodyweight, acid-base was kept in check, showing preventive effect of this fraction at that dose against alkalosis and acidosis. Uric acid is the end product of both exogenous and endogenous purine metabolism. Kidneys eliminate about two-third of uric acid while about one-third is eliminated by gastrointestinal tract. Hyperuricemia is a key risk factor for the development of gout, renal dysfunction, hypertension, hyperlipidemia, diabetes and obesity. Hyperuricemia occurs as a result of the increased uric acid production, impaired renal uric acid excretion, or a combination of the two [51]. This condition is characterized by high uric acid load in the blood causing deposition of urate crystals in the joints and kidneys [52]. Therefore, non-significant difference($P<0.05$) in uric acid level at all tested doses proved that the fraction unaltered renal uric acid excretion.

Serum creatinine being an easily measured byproduct of muscle metabolism that is excreted unchanged by the kidneys is regarded as an important indicator of renal health. Biological system involving creatine, phosphocreatine and adenosine triphosphate (ATP) gives rise to creatinine. Creatinine is chiefly removed from the blood by the kidneys, primarily by glomerular filtration, but by proximal tubular secretion. There is little or no tubular reabsorption of creatinine. In kidney dysfunction, there is surge in the level of serum creatinine. Hence, creatinine level in the blood and urine may be used to calculate creatinine clearance time, which is direct proportional approximately to glomerular filtration rate (GFR). Blood creatinine may also be used to calculate estimated GFR (eGFR). Therefore, significant decrease ($P>0.05$) in creatinine levels at all tested doses is an evidence that the fraction increases creatinine clearance time and thus increases GFR.

Urea is formed by the liver by combining two molecules of ammonia and a molecule of carbon (IV) oxide in the urea cycle. Urea is a highly soluble compound and plays an important role in the metabolism of nitrogen-containing compounds in animals. It is also the chief nitrogen-containing compound in the urine of mammals. Body uses urea for nitrogen excretion preventing physiological toxicity of ammonia resulting from deamination of amino acids. Hence, significant decrease ($P<0.05$) in the urea level at doses of 100 and 600mg/kg bodyweight is indicative of the fraction at lower and higher doses not affecting the normal renal function.

Conclusion

From acute toxicity study, *Datura metel* seeds is not acutely toxic. Haematological analysis reveals that n-hexane seed fraction can be used in the treatment of anaemia due to its hematinic property. The fraction can also be used for immunostimulatory. Higher doses of this fraction, however, could pose risk of kidney dysfunction, due to its effect on serum electrolytes concentrations. The n-hexane seed fraction of *Datura metel* therefore has both positive and negative effect on nephrocytes. The higher doses cause elevated levels of total bilirubin, direct and indirect Bilirubin which may result in health disorder (e.g. jaundice). Using higher doses of the fraction also can predispose individual to the development of atherosclerosis.

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