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EXCITABILITY EFFECT OF EXTRACT OF *DATURA STRAMONIUM* LEAVES; SERUM BIOCHEMISTRY AND HAEMATOLOGY IN RATS

K. K. Igwe*[1], A. J. Madubuike[1], N. E. Udeh[1], Chika Ikenga[2], C. J. Onyenze[1] and N. S. Nwatu[1]

[1]Department of Vet Biochemistry and Animal Production, Micheal Okpara University of Agriculture Umudike, Nigeria.
[2]Natures Gentle Touch: Anti-Dandruff Unit, 4 Northumberland Ave, TW7 5HU, London, UK.

***Corresponding Author: K. K. Igwe**
Department of Vet Biochemistry and Animal Production, Micheal Okpara University of Agriculture Umudike, Nigeria.

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ABSTRACT

The effect of administration of ethanol extract of the leaves of *Datura stramonium* on excitability, hematology and serum biochemistry parameters was studied in rats. Sixteen (16) experimentally matured male Wistar rats, aged 9 - 10 weeks, with an average weight ranged, 147-150g were distributed into 4 groups of 4 rats each, and group 1 were administered with distilled water (as control) groups 2, 3 and 4 were the test groups and were given 100, 200 and 400 mg/kg body weight of the leaf extract respectively. The treatment period was fourteen (14) days. Excitability scores were recorded daily immediately after administration of extract. End of treatment the rats were sacrificed and blood collected by cardiac puncture for analysis. The result showed that *D. stramonium* demonstrated increased excitability mean score of 2.50±0.28, 3.25±0.25, 3.50±0.29 compared to control group 1.25±0.25 which may suggest nervous system excitation in those treated rats more than the control rats. There were mild changes in haematological parameter not significantly altered but within normal reference range. However, the mild leukocytosis, lymphocytosis, neutrophilia and monocytosis were not significant and elevation was within normal reference range. The result of the serum biomarkers showed significant decreases in the liver and kidney enzymes suggesting that the leaf extract at lower doses possess hepato, nephro and cardio-protective properties It can be concluded that the extract of *Datura stramonium* exhibited excitatory effect, with no toxic effects on the blood profile and internal organs.

Index terms: *Datura stramonium*, Excitability, Haematology, Rats, Serum Biochemistry.

INTRODUCTION

Plants have always played a major role in the treatment of human and animal traumas and diseases worldwide. The demand for medicinal plant is increasing in both developed and developing countries due to growing recognition of natural product. Herbal medicine is an important part of both traditional and modern system of medicines, Kirtikar *et al.*, (1994). Plants contain primary and secondary metabolites. Primary metabolites are those compounds the plants uses for its metabolism. They are protein, carbohydrates and lipids which are used by the plant for their growth and nourishment. Secondary metabolites on the other hand are compounds found in the plants that are used by the plants to protect itself, Ray *et al.*, (2013). Animals in the wild do not have medical care and treat themselves in the wild by identifying plants with medicinal properties, this is what we try to bring home by identifying those medicinal plants for proper documentation and necessary use, Ijeh *et al.*, (2009). *D. stramonium* also known by the common names thorn apple, jimsonweed, devil's snare, devil's trumpet or *Zegemi* in northern and southern Nigeria is one of the widely known folklore medicinal herb of the flowering plant family Solanaceae. It is a wild growing flowering plant and was investigated as a local source for tropane alkaloids which contain a methylated nitrogen atom ($N\text{-}CH_3$) and include the anti-cholinergic drugs atropine, and scopolamine amongst others. The plant is a severely toxic and poisonous plant that has been and is still being used traditionally to treat ailments associated with all kinds of inflammations and all manner of burns and scalds, Glatstein *et al.*, (2016). The leaf although toxic is generally smoked either in a cigarette or a pipe and has been stipulated to have excitatory or hyperactivity effect, Glatstein *et al.*, (2016). There are several ways to ingest *D. Stramonium*, the plants can be smoked, brewed into a tea, or converted into a skin ointment. Since all parts of the plant contain variable amounts of psychedelic compounds, some people have been known to chew the seeds as well, Freye (2010). The

incidence of *D. stramonium* poisoning is sporadic with a cluster of poisoning cases in the 1990s and 2000s, the United States media reported some cases occurring mostly among adolescents and young adults dying or becoming seriously ill from ingesting. Some medicinal uses of the plant are its anti-inflammatory property of all parts of the plant, stimulation of the central nervous system, respiratory decongestion, treatment of dental and skin infections, alopecia and in the treatment of toothache amongst other things, Das *et al.*, (2012). Herbal indigenous knowledge possessed by different communities on variety of human and animal medical herds has remained unwritten with the risk of being forgotten and totally lost. Therefore, research on herbs especially medicinal herbs should be documented. Prospects for human and ethno-veterinary research and development should be identified to enable researchers chart new courses of investigation and establish scientific basis of their action and develop potentials of the herb as a medicinal plant, Vijendra and Kumar (2010). *D. stramonium* consumed by domestic animal may have excitability or hyperactivity effects on its consumers and lead to environmental self-pollution, Ikpeazu and Igwe (2023). There is need to have a knowledge on the effects which it could have on liver, kidney and hematology since the use of this plant could be indicated in the treatment of depression or inactiveness on domestic animals especially dogs intended for warfare or security. Dog owners sometimes request for substances that can make their dogs hyperactive or aggressive and this study wants to check if *D. stramonium* can handle the task.

Figure 1: *Datura stramonium* plant showing the leaves, flower and fruit.

The genus name 'Datura' is derived from dhatura, the Bengali name for the plant, while the epithet 'stramonium' combines the Greek word strychnos for nightshade, and makinos meaning mad, referring to the narcotic properties of the plant (Haegi, 1976; Hadkins *et al.*, 1997). Origin is probably the tropical regions of Central and South America, *D. stramonium* has become a cosmopolitan weed in the warm regions of North, Central and South America, Europe, Asia, Africa (especially in Nigeria) and New Zealand and has been used as narcotic by British soldiers, Parsons *et al.*, (1992). *D. stramonium* also known as Jimson weed, devil's snare or apple, thorn Apple or Devil's trumpet is an erect, annual, freely branching herb that forms a bush up to 60 to 150 cm (2 to 5 ft.) tall, Griev,e (1971). Indigenous names of the plant include; *Zakami* or *Zegemi* in Hausa, *Myaramwo* in Igbo, *Zakedi* in Kanuri, *Gegemu* in Yoruba and *Jegemi* in Igala of Nigeria (Gidado *et al.*, 2007; Parker *et al.*, 2021). The root is long, thick, fibrous, and white. The stem is stout, erect, leafy, smooth, and pale yellow-green to reddish purple in color. The stem forks off repeatedly into branches and each fork forms a leaf and a single erect flower, Grieve (1971). The leaves are about 8 to 20 cm (3–8 in) long, smooth, toothed, soft, and irregularly undulated. The upper surface of the leaves is a darker green, and the bottom is a light green. The leaves have a bitter and nauseating taste, which is imparted to extracts of the herb, and remains even after the leaves have been dried, Grieve (1971). The proximate analysis of *Datura stramonium* leaves and contain carbohydrate, protein, ash, lipid, fiber and water, vitamin C, phosphate, phosphorus, nitrate, nitrogen; metallic minerals such as manganese, calcium, sodium, potassium, iron, and trace elements cadmium, copper, zinc and lead in a negligible amount Ibiam *et al.* (2017). GC-MS analysis has also been used to identify compounds in plants. (Igwe *et al.*, 2016; Ikpeazu *et al* 2017). Otuokere *et al.* (2020), in their GC-MS analysis of *D. stramonium* leaves revealed the presence of eighteen bioactive compounds. *D. stramonium* used traditionally to treat asthma, epilepsy, rheumatoid arthritis, Chavhan *et al.*, (2018). Treatment of injuries, wounds, bleeding and management of pains, Njoroge, (2012). Its seeds are used as purgative, in cough, fever and asthma, Aquib *et al.*, (2013). The seeds are smoked due to its narcotic action, Khan *et al*., (2013), used to make somebody unconscious Rahmatullah *et al*., (2009), also used for baldness, Khan *et al*., (2008) Applied to scalp for falling hairs and as antidandruff *et al*., (2009). Shah *et al*., (2006). Paste of leaves is topically applied for skin diseases, Rahmatullah *et al.*, (2010). Pharmacologically it has anti-asthmatic activity and leaves are used in asthma treatment, Savithramma *et al*., (2007). It has anticholinergic activity as reported by Taha *et al*., (1984). The alkaloids found in *D.stramonium* , are organic esters used clinically as anticholinergic agents. The anticholinergic syndrome results from the inhibition of central and peripheral muscarinic neurotransmission, Taha *et al*., (1984). As acaricidal, repellent and oviposition deterrent properties, Kurnal *et al*., (2009) and antimicrobial activity against gram positive bacteria in a dose dependent manner (Sharma *et al*., 2010, Fereshteh *et al*., 2004). Toxicity to environment and body chemistry, Ikpeazu and Igwe (2023). Acute toxicity test (LD_{50}) of *D.stramonium* using up and down method was 3185.25 mg/kg b.w. Khudhair and Abed, (2015)

Laboratory reports on excitability studies in animal models

Medically excitability refers to a state of being alert and hyperactive. An animal can be said to hyperactive if it

shows the following; Alert and awake (absence of sleep), a state of panic (ie. increased pacing), easily aggrieved, raised blood pressure, an increased feeling of energy, may tend to have an increased libido and may have a tendency to get into a fight with other herd mates Ziad *et al*., (2019). Excitability at cellular level describes the ease with which cells respond to stimulus with a regenerative action potential depending on the passive and active properties of the cell membrane, Ziad *et al*., (2019). Adenkola and Oluremi (2014) reported excitability scores of rabbits fed graded level of *Hibiscus sabdariffa* calyx (HSC), showed increasing excitability scores as the inclusion levels were increased.

MATERIALS AND METHODS

Plant Material

Fresh leaves of *Datura stramonium* were collected at Ubakala in Umuahia, Abia State, Nigeria. The plant was identified using Google Plant Identifier and confirmed by the Forestry Department of Michael Okpara University of Agriculture Umudike, Nigeria.

Preparation of Extract

Cold masceration technique was used in this study as described by Igwe *et al* (2020). The leaves were dried under room temperature for 10 days and were grounded using manual grinder model (Corona-Landers C 1A SA). The grounded leaves were soaked in ethanol for 48 hours and was filtered using filter paper. The extract was concentrated using hot air at 30 ^{0}C and the dried extract weighed and kept in labelled sterile specimen bottle for the experiment.

Experimental Design

Sixteen (16) adult male albino rats were used for the study. The rats were acclimatized for one week and fed *ad libitum* with standard pelleted rat feed. The Wistar rats were randomly distributed into 4 groups; (1- 4). Rats in group 2, 3 and 4 were given 100, 200 and 400mg/kg graded doses of the extract respectively, via oral gavage, while group 1 received distilled water at 5ml/kg body weight, and served as the normal control. The treatment lasted for fourteen (14) days.

Measurement of Excitability Scores in rats

Excitability scores were recorded daily immediately, five (5) minutes after administration. They were measured as described by Adenkola and Oluremi (2014)**),** while weighing them. A score of one to four was allocated to each rat by a single observer; a higher score representing a greater level of excitability. A score of one was allocated to a rat that was calm, and made little movement during the handling. Two was allocated to a rat that occasionally shook itself in an attempt to escape from the weighing pan, while three was assigned to a rat that continuously attempted to free itself. A score of four was given to a rat that struggled violently the entire weighing period.

Excitability Scores in rats

Scores	Degree of excitability
1	No struggling, calm, and make little movement during the handling/weighing
2	No struggling, occasionally shake itself in an attempt to escape from the weighing pan
3	No struggling, continuously attempt to free itself from the weighing pan
4	Struggling violently throughout the entire weighing period.

Source : Adenkola and Oluremi (2014)

$$\text{MCV } (\mu m^3) = \frac{\text{PCV} \times 10}{\text{Number of erythrocytes per } \mu L \text{ blood} \times 10^{-6}}$$

$$\text{MCH } (\mu g) = \frac{\text{Haemoglobin (g/dL)} \times 10}{\text{Number of erythrocytes per } \mu L \text{ blood} \times 10^{-6}}$$

$$\text{MCHC (g/dL)} = \frac{\text{Haemoglobin (g/dL)} \times 100}{\text{PCV (mL / dL)}}$$

Serum Clinical Biochemistry Determination Procedures

The serum ALT activity was determined by the Reitman-Frankel colorimetric method, Reitman and Frankel, (1957). The serum AST activity was determined by the Reitman-Frankel colorimetric method, Reitman and Frankel, (1957). The serum ALP activity was determined by phenolphthalein monophosphate method (Klein *et al.*, 1960; Babson *et al.*, 1966). The serum total protein levels were assayed by the direct Biuret method, (Lubran, 1978; Johnson, 2008). The serum total bilirubin levels were determined by Jendrassik and Grof method (Doumas *et al*., 1973; Higgins *et al*., 2008). The serum total cholesterol was determined by enzymatic colorimetric method, Allain *et al*., (1974).

STATISTICS

The data will be analyzed using statistical package of social sciences (SPSS) version 23. Data will be expressed as Mean ± Standard Error of mean. The data will be subject to One-way analysis of varience (ANOVA). The different doses will be compared and

seperated using posthoc analysis (Duncan test) to check mean that is significant. The statistical confidence will be placed at 95% ($p<0.05$)

RESULTS

EFFECT OF *Datura Stramonium* ON EXCITABILITY IN WISTAR RATS

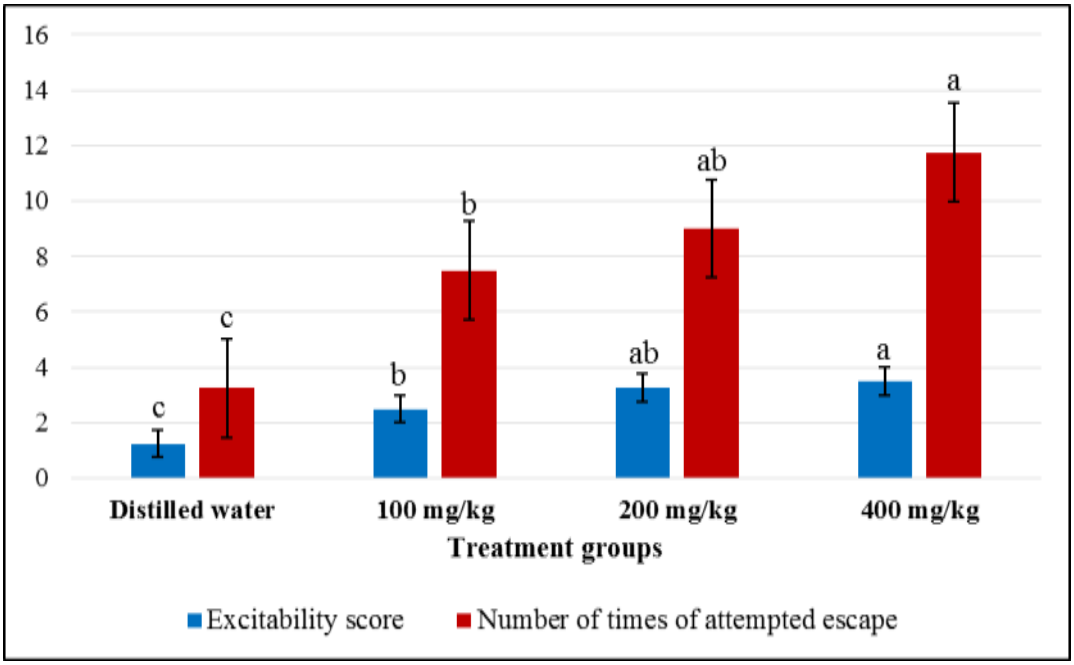

Figure 2 shows excitability effect of *D. stramonium* on Wistar rats.

Excitability of animals depends on their temperament, and temperament is a trait that seems to be stable over time. The excitability score in the experimental rats treated with the graded doses of the extract were increased as the doses were increased in a dose dependent manner. On a scale of 4, the 100, 200 and 400 mg/kg graded doses of the extract recorded mean scores of 2.50±0.28, 3.25±0.25 and 3.50±0.29, respectively, compared to 1.25±0.25 excitability mean score of the control rats. Similarly, the number of times the individual rat from each experimental group attempted to jump out from the weighing trough were higher in the extract groups as the doses were increased and compared to control.

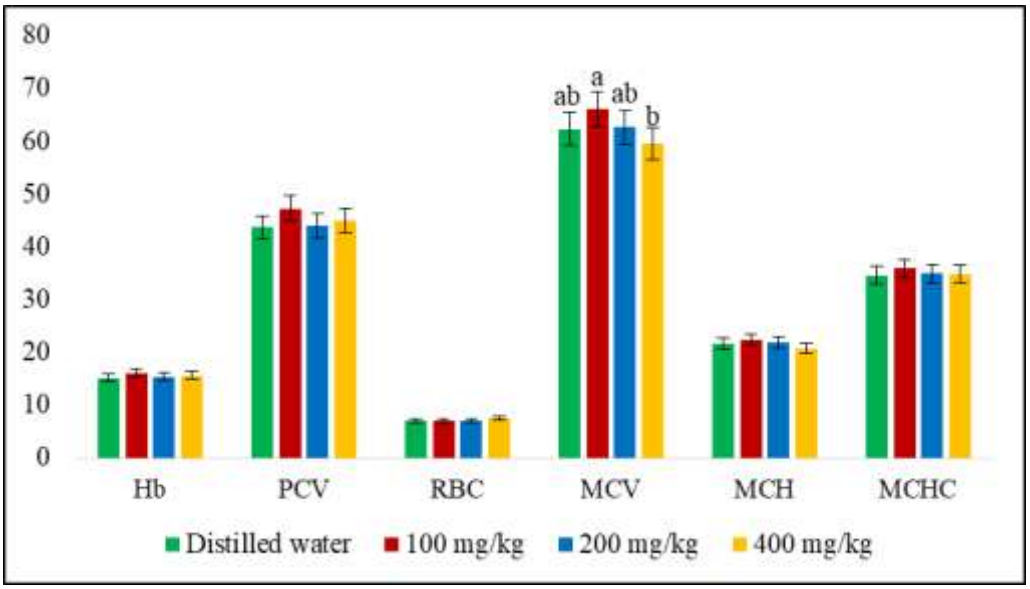

Figure 3 shows the effect of *D. stramonium* extract on the erythrogram of Wistar albino rats.

Values presented as mean ± S.E.M (Standard Error of Mean) at ($p<0.05$).

Hb (g/dl) 16.00±0.64, 15.37±0.13, 15.65±0.86 compared to control 15.15±0.02;

PCV (%) 47.25±1.79, 44.00±0.70, 45.00±2.27, compared to control 43.75±0.47;
RBC ($\times 10^{12}$) 7.15±0.25, 7.02±0.06, 7.57±0.44, compared to control 7.01±0.01;
MCV (fl) 66.08±1.84, 62.69±1.22, 59.56±1.68 compared to control 62.34±0.67;
MCH (pg) 22.35±0.16, 21.90±0.10, 20.78±1.22, compared to control 21.58±0.03;
MCHC (g/dl), 35.89±0.93, 34.96±0.57, 34.81±1.07 compared to control 34.63±0.31.

The result shows no significant difference between the treated and control groups at ($p<0.05$) on the erythrogram.

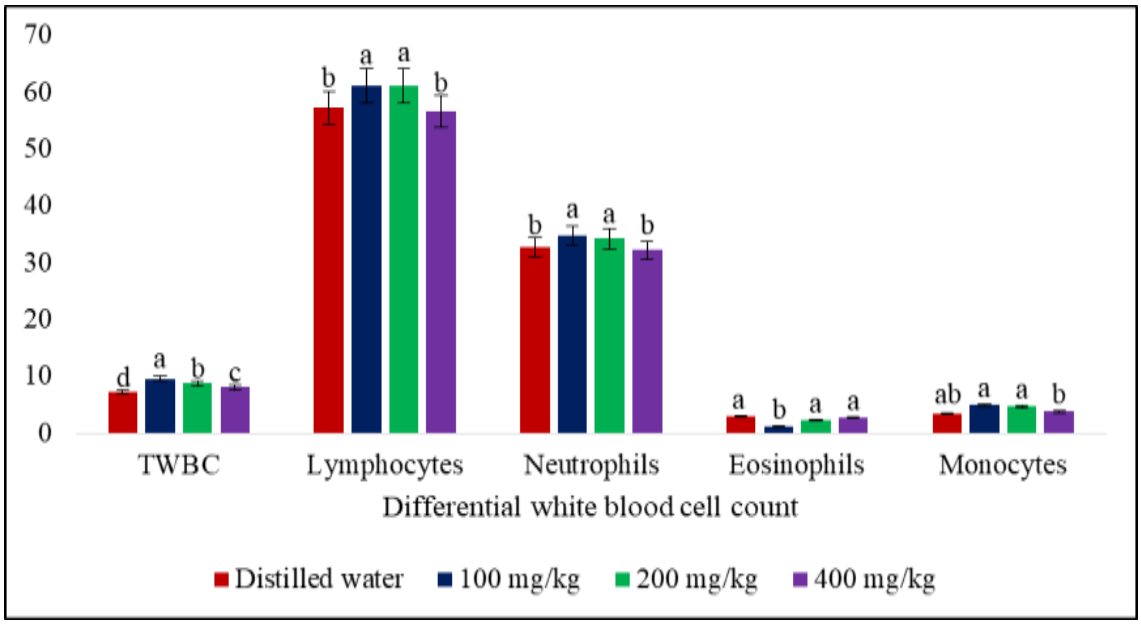

Figure 4: graph shows the effect of *D stramonium* extract on leukogram of Wistar albino rats.

Values are presented as mean ± S.E.M (Standard Error of Mean) significant difference at ($p<0.05$).
TWBC ($\times 10^9$/L), 9.62±0l.25^a, 8.80±0.14^b, 8.13±0.04^c compared to control 7.23±0.02^d**;**
Lymphocytes (%) 61.25±0.47^a, 61.25±0.25^a, 56.75±2.28^b compared to control 57.25±0.25^b**;**
Neutrophils (%) 34.75±0.94^a, 34.25±0.25^a, 32.25±0.25^b compared to control 32.75±0.62^b**;**
Eosinophils (%) 1.25±0.25^b, 2.25±0.25^a, 2.75±0.25^a compared to control 3.00±0.40^a**;**
Monocytes (%) 5.00±0.40^a 4.75±0.47^a 3.80±0.28^b compared to control 3.50±0.28^{ab}**;**
Basophils (%) 0.00±0.00 0.00±0.00 0.00±0.00 compared to control 0.00±0.00
The result showed no significant difference between the treated and control groups at ($p<0.05$) on the leukogram. There was mild eosinopaenia and monocytosis.

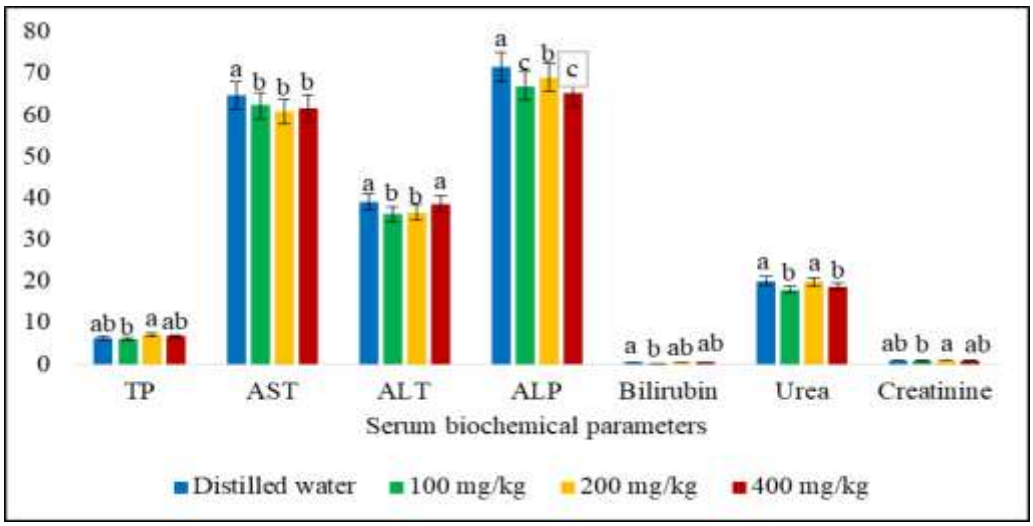

Figure 5: graph shows the effect of *D. stramonium* extract on serum biochemical parameter of Wistar albino rats.

Values are presented as mean ± S.E.M (Standard Error of Mean) significant difference at ($p<0.05$).
TP (g/dl): 6.06 ± 0.28^{b}, 7.08 ± 0.35^{a}, 6.77 ± 0.19^{ab} compared to control 6.40 ± 0.03^{ab}**;**
AST (IU/L): 62.30 ± 1.03^{b}, 60.94 ± 0.15^{b}, 61.76 ± 0.26^{b} compared to control 64.78 ± 0.26^{a}**;**
ALT (IU/L): 36.11 ± 0.59^{b}, 36.54 ± 0.70^{b}, 36.54 ± 0.70^{b} compared to control 39.02 ± 0.27^{a}**;**
ALP (IU/L): 66.98 ± 0.56^{c}, 69.02 ± 0.84^{b}, 65.33 ± 0.31^{c} compared to control 71.56 ± 0.15^{a}**;**
Bilirubin (mg/dl): 0.31 ± 0.10^{b}, 0.48 ± 0.02^{ab}, 0.46 ± 0.01^{ab} compared to control 0.55 ± 0.00^{a}**;**
Urea (mg/dl): 18.01 ± 0.49^{b}, 19.89 ± 0.21^{a}, 18.67 ± 0.29^{b} compared to control 20.06 ± 0.05^{a}**;**
Creatinine (mg/dl): 0.85 ± 0.01^{b}, 0.96 ± 0.05^{a}, 0.87 ± 0.01^{ab} compared to control 0.93 ± 0.01^{ab}.

There were no significant changes in the levels of serum total protein (TP), Aspartate Transaminase (AST), Alanine Aminotransferase (ALT) and Alkaline Phosphatase (ALP) evaluated. All values were within normal reference range across the treatment groups compared to the control. Similarly, the kidney enzymes; urea, creatinine and the serum bilirubin were also within normal reference range compared to control indicating liver and kidney safety.

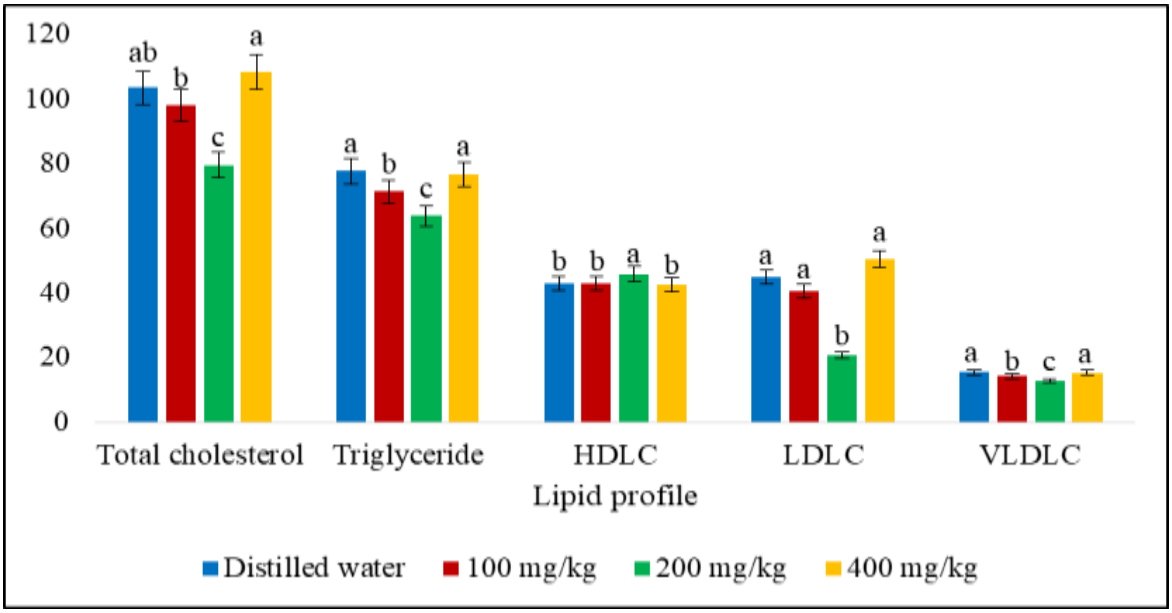

Figure 6: Effect of *D. stramonium* extract on lipid profile of Wistar albino rats.

Values are presented as mean ± S.E.M (Standard Error of Mean) significant level at ($p<0.05$).

Total cholesterol (mg/dL): 98.03 ± 5.75^{b}, 79.67 ± 0.24^{c}, 108.40 ± 1.44^{a} compared to control 103.52 ± 0.50^{ab}**;**
Triglyceride (mg/dL): 71.55 ± 1.09^{b}, 64.00 ± 0.45^{c}, 76.80 ± 1.54^{a} compared to control 77.74 ± 0.28^{a}**; HDLC (mg/dL):** 43.05 ± 0.38^{b}, 45.95 ± 0.24^{a}, 42.61 ± 0.55^{b} compared to control 42.98 ± 0.26^{b}**; LDLC (mg/dL):** 40.67 ± 5.86^{a}, 20.91 ± 0.17^{b}, 50.43 ± 1.68^{a} compared to control 44.98 ± 0.50^{a}**; VLDLC (mg/dL):** 14.31 ± 0.21^{b}, 12.80 ± 0.09^{c}, 15.36 ± 0.30^{a} compared to control 15.54 ± 0.05^{a}**.** Triglyceride (TG) level and Very Low Density Lipoprotein Cholesterol (VLDLC) were reduced compared to the control. The High Density Lipoprotein Cholesterol (HDLC) was mildly elevated within normal reference range. These indicate safety and could prevent cardiac associated diseases like arthereosclerosis.

DISCUSSION

Most of the natural herbs used in traditional medicine are believed to be safe, compared to synthetic drugs, even when there are no toxicological records or scientific evidence to this believe.

Excitability of animals depends on their temperament and temperament, and such animal seems to be stable over time. The excitability score and the number of times each experimental rat attempted to escape from the weighing trough in the group treated with the graded doses of the extract were increased ($p<0.05$) as the dose of the extract were increased in a dose dependent manner, higher than the control rats. It has been reported that the primary biologically active substances in *D. stramonium* are the alkaloids atropine and scopolamine (Otuokere *et al.*, 2020). *D. stramonium* has been documented to contain vitamin C (Wang *et al.*, 2000; Essas *et al.*, 2006) and other antioxidant principles (Tee *et al.*, 2002; Ologundudu *et al.*, 2009), and has been shown to increase excitability scores in animals, Ayo *et al.*, (2006) possibly because it plays a significant role in the synthesis of vitaminergic neurotransmitters in the brain. This could possibly explain the higher excitability score observed in rats administered with the extract especially, with higher doses of *D. stramonium*. This

may suggest the ability of *D. stramonium* leaf extract to activate the nervous system in those treated rats more than the control group. The excitability effect of the leaf extract as observed in this study could also could be due to the phytochemical compound; 2-Phenanthrenol, 1,2,3,4,4a,9,10,10a-octahydro-7-methoxy-1,1,4a-trimethyl- as reported by Otuokere *et al.* (2020), which is 5-alpha-reductase-inhibitor, and acetyl-CoA-carboxylase-inhibitor known to elicit excitability in animals.

Haematological and serum biochemical indices are used in monitoring feed, drug toxicity and health status of animals, Oyawoye and Ogunkunle, (2004). No significant change in erythrogram values as recorded in this study (Fig 3), might be attributed to the non-toxic effect of *Datura* on the haematological system Bouzidi *et al.* (2011). According to Fatoba *et al*. (2013) the determination of total leucocyte and differential count are important markers of immune function. In this study, the total leukocyte counts of the *D. stramonium* extract treated groups significantly ($p<0.05$) increased relative to the control (Fig 4) but within normal reference range. *D. stramonium* caused mild leukocytosis in this research. This result is consistent with the findings of Fatoba *et al*. (2013), who stated that rabbits treated with aqueous seed extract of *D. stramonium* recorded higher white blood cell counts than the control. The result of the TWBC appears to validate the indigenous claim that the leaves of this plant can boost the body immunity, and assist the body system to fight against mixed infections (Iwuji and Herbert, 2012; Soetan *et al*., 2013; Isaac *et al.*, 2013). The lymphocyte counts of low and medium doses were observed to be significantly ($p<0.05$) higher when compared with the highest dose (Fig 4). This implies that extract at low dose was enough to stimulate very high lymphocytes production and has capacity to boost both antibody-dependent and cell-mediated immune responses since lymphocytes play major role in immune responses. This observation strongly supports the findings of Fatoba *et al.* (2013) who reported increase in lymphocyte counts with decreasing extract dosage. The neutrophil counts also increased significantly ($p<0.05$) in low and medium doses compared with the control and the highest dose groups; suggesting that the extract at lower doses can stimulate cell mediated elimination of bacterial pathogens because major phagocytes mobilized during bacterial invasion are neutrophils. As observed in the lymphocytes count, the effect had an inverse dose dependence effect, meaning that lower doses of the extract appeared to stimulate more synthesis of neutrophils than the highest dose. This finding strongly affirm the study done by Ogunmoyole *et al.* (2019), who reported that *D. stramonium* extract at higher dose of 400 mg/kg generally caused a significant decrease in neutrophils count. Monocyte counts significantly ($p<0.05$) followed the same trend as leucocytes and lymphocytes compared to all control groups. The eosinophil is specialized in producing peroxidase enzymes and proteins that are toxic to invading elements and is seen to increase in the presence of parasites, toxin or allerge (Faghani *et al.,* 2014). The eosinophils count decreased significantly ($p<0.05$) in the 100 mg/kg dose relative to 200, 400 mg/kg doses and the normal control respectively. No change in Basophil count.

AST and ALT elevation is seen in condition of hepatocyte damage, in inflammatory condition of liver, hepatotoxicity by toxicants, trauma and some plant extracts, Reitman and Frankel, (1957). Liver ALP elevation is seen in hepatocyte and biliary epithelial damage. In kidney, elevated creatinine occurs in pathological condition of decrease in glomerular filtration rate which could be pre-renal, renal or post renal, Bouzidi *et al.* (2011). An obvious sign of hepatic injury is leakage of cellular enzyme into plasma Udem *et al.,* (2009). When the liver cell membrane is damaged, a variety of enzymes normally located in the cytosol are released into blood stream accounting for their raised levels in the serum (Kumar *et al.,* 2004; Batool *et al.*, 2017). The elevation is a consequence of hepatic injury, resulting in the leakage of enzymes that are normally localized within the hepatocytes. Similarly, kidneys as the principal organ for the excretion of xenobiotics and their metabolites are particularly prone to their toxic effects. Estimation of serum levels of these enzymes are considered as important indicators of the functional integrity of hepatocellular membranes, Pari and Amali, (2005). According to Adeneye *et al.* (2006) elevation in these enzymes is related to hepatic and heart disorders. Mild reduction in levels of both the liver and the kidney enzymes in treated groups could indicate that *D. stramonium* leaves possesses hepato-protective, nephron-protective and cardio-protective properties. Ogunmoyole *et al.* (2019) reported that *D. stramonium* extract at 200mg/kg dose, generally caused a significant decrease in ALT, AST, ALP and MDA in selected tissue homogenates while these parameters increased significantly in the serum relative to the control group. Abbas (2013) and Başaran *et al.* (2018), in their separate studies reported low level of serum AST and ALP relative to the control animals and concluded that extract of *D. stramonium* are hepatoprotective at 200mg/kg. The decrease in ALP with the reference range showed that the *D. stramonium* extract does not cause harmful effect, Clementine and Tar, (2010). However, the result of this study did not agree with the work done by Jaroslaw *et al.* (2009) and Udem *et al.* (2009), who in separate studies reported significant increase in the serum ALP by *D. stramonium* extract leading to biliary obstruction and heart failure.

Cholesterol is synthesized in the liver, and it is important for cell wall structure, cell immunity, steroid hormones production by the adrenal glands and gonads, membrane strength, nerve protection and formation of bile acids, which accounts for about 80 %. Cholesterol is also necessary for formation of antibodies and enzymes and is also used to evaluate risk for atherosclerosis (Soetan *et al.,* 2013). Cholesterol is among the prominent lipids

responsible for membrane integrity (Bouzidi *et al*., 2011). Total cholesterol in this study was decreased in the serum of experimental animals treated with the 200 mg/k dose of the extract relative to the control. The lowering of cholesterol level in the sera of the experimental rats administered with the 200 mg/kg dose might suggest that the ingredients contained in the leaf extract at the medium dose was capable of inhibiting the activities of hepatic lipogenic and cholesterogenic enzymes, such as malic enzyme, fatty acid synthase, glucose 6-phosphate dehydrogenase and HMG-CoA reductase (Vega *et al.,* 2003) which are all required for cholesterol synthesis. This cholesterol lowering action of the crude extract might be attributed to the presence of a bioactive phyto-constituents, i.e. β-sitosterol (Berkov *et al*., 2006; Swathi *et al*., 2012), and N,N'-Bis(salicylidene)-3,3'-bis(aminopropyl)aminocobalt(II) contained in the leaves as identified by Otuokere *et al.*, (2020), which is an Antitopoisomerase-II, Casein-Kinase-II-Inhibitor, Topoisomerase-II-Inhibitor known to decrease cholesterol synthesis in the liver. This finding partially agreed with the studies of Tariq *et al*. (1989) and Gharaibeh *et al*. (1988), but completely agreed with recent studies of Rasekh *et al*. (2001); Couladis *et al*. (2003) and Ogunmoyole *et al.* (2019), who in their separate studies reported that the crude extract of *D. stramonium* leaves at moderate doses has a significant cholesterol lowering action.

Triglycerides are form of fat and a major source of energy for the body and are made up of three fatty acids attached to a glycerol molecule, hence the term triglycerides (Purves *et al.,* 2003). Triglyceride is synthesized in the liver, and the result obtained in this study demonstrated the ability of *D. stramonium* to influence liver metabolism towards decreasing the synthesis of lipids (hypolipidemic potential) in the 100 and 200 mg/kg doses. The low levels of serum triglyceride may be due to a number of factors such as the decreased availability of fatty acids for esterification (Bopanna *et al.*, 2017), increased catabolism of LDL, enhancing of tissues lipases, activation of acetyl-CoA carboxylase (McCarty, 2021) and production of triglycerides precursors such acetyl-CoA and glycerol phosphate (Campillo *et al.,* 2014). The result obtained in this study shows *D. stramonium* to have hypolipidemic potential. HDL-cholesterol is otherwise called 'good cholesterol' which returns cholesterol to the liver where it is converted into bile and subsequently removed from the body, Nwanjo, (2005). It is important to note that the serum level of HDL-cholesterol and LDL were within the normal range at both lowest and highest doses used in this study, whereas, a noticeable increase and decrease in HDL-cholesterol and LDL, respectively, were observed in the sera of the rats treated with the 200 mg/kg dose suggesting hepato- and cardio-protection of *D. stramonium* crude extract at moderate dose. This observation could explain why *D. stramonium* leaf extract had no alteration on the lipid profile, hence, do not compromise the membrane integrity (its stability and fluidity) and their functions. The Very Low Density Lipoprotein Cholesterol (VLDLc) serum levels at 100 and 200 mg/kg doses were significantly higher than the normal value compared with the serum level obtained in the highest dose. This also gave support to the hepato- and cardio-protective ability of the crude extract. The outcome of this result strongly agreed with that of Ghasi *et al.* (2000); Boumba *et al.* (2005); Nandave *et al.* (2009) and Başaran *et al.* (2018).

CONCLUSION

D.stramonium had no significant effect on the evaluated blood parameters (Hb, PCV and RBC) of male Wistar rats used, but however, improved the leucocytes counts and most differentials (lymphocytes, neutrophils and monocytes) at lower doses much better than the high dose, implying an improved index of immune function. The decreases in the kidney and liver enzymes suggested hepato-protective, nephron-protective and cardio-protective properties of the leaf extract. The study also revealed a lowering effect of bad cholesterol (TC), while increasing the good cholesterol (HDL-c) at lower doses of the extract, implying that the extract even at the low dose of 100 mg/kg, may have inhibited the activities of hepatic lipogenic and cholesterogenic enzymes, required for bad cholesterol synthesis. Therefore, apart from the excitatory effect which occurred in a dose dependent manner, it can be concluded that the extract of *Datura stramonium* exhibited desired pharmacological and biochemical activities at lower doses, than at higher doses.

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