

STUDIES ON THE PHYTOCHEMICAL SCREENING AND DELETERIOUS EFFECTS OF AQUEOUS EXTRACT OF *DATURA* *STRAMONIUM* ON ALBINO RATS

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ABSTRACT

Datura stramonium is a commonly known natural plant throughout the world for its hallucinogenic effects. It is also known to produce other effects such as euphoria. The phytochemical constituents of *Datura stramonium* was determined quantitatively, and its effects on albino rats was determine using some biochemical parameters such as AST, ALT, ACP, and total protein as well as histological examinations of the liver, kidney and intestine of the rats. Twelve adult male albino rats constituted groups of four (4). Three (3) albino rats each was treated with 2.0g/kg, 1.5g/kg, and 0.75g/kg per body weight of the aqueous

extract of *Datura stramonium* respectively and the equivalent volume of distilled water was given to the remaining three animal that served as control, daily for twenty one (21) days. The levels of AST, ALT, ACP, and Total Protein in the serum were higher in the animals treated with *Datura stramonium* relative to those of animals in the control group. There was a statistically significant difference ($p>0.05$) in the mean value of AST and ACP of animals treated with *Datura stramonium* relative to the control group but a non statistically significant difference ($p<0.05$) in the mean value of ALT, and total proteins were observed. Histological findings showed no apparent sign of injury or lesions on the liver, kidneys and intestine of the animals administered with 2.0g/kg, 1.5g/kg, and 0.75g/kg per body weight per day of the plant extract for the 21 days. The findings in this study suggested that *Datura stramonium* altered protein metabolism and normal liver function processes by elevating the levels of AST, ALT, ACP and total proteins in the blood serum, as well altered the cellular and functional integrity of the rats. The plant has some benefits when taken in correct doses because of the presence of some relevant phytochemicals which are known for their medicinal properties.

KEY WORDS: *Datura stramonium*, ACP, AST, ALT.

INTRODUCTION

Datura stramonium, known by the common names Jimson weed, Devil's snare, is a plant of the solanaceae (nightshade) family, believed to have originated from the Americans, but now found everywhere around the world. Other common names for *D. stramonium* include thorn-apple and moon-flower, and it has a Spanish name Toloache (University of Texas, 2013). It is popularly called "gegemu" or "ewe ikan" by the Yoruba speaking part of Nigeria. Its use has a very wide array because of its hallucinogenic property and it differs from one continent to another. It is also known to have a wide array of uses especially in medicine. The plant was used all over the world from historical times. Folk uses include cure for cancer, local analgesic for burns, sedatives in epilepsy, influenza, cough remedy, treatment of Asthma, healing of wounds and treatment of acne. It is used as a ritualistic herb and for inebriation purposes because of its hallucinogenic effects.

The main toxic alkaloids in *D. stramonium* are the Tropane alkaloids of which atropine (dl-hyoscyamine) and scopolamine (l-hyoscyne) (Friedman, 1989). Atropine and scopolamine are competitive antagonists of muscarinic cholinergic receptors and are central nervous system depressants (Halpern, 2004). All parts of the plants are toxic, but the highest amount of the alkaloids is contained in ripe seeds. Many cases of accidental poisoning by *D. stramonium* have been reported when these plants were eaten accidentally, or decoction prepared from herbal prescription (Al-shaikh and Sablay, 2005). Intentional poisoning has also been reported in several cases, namely a fatal poisoning with *D. stramonium* for its mind altering properties and the eating and chewing of *Datura* in a suicides attempts. *Datura* belongs to the classic "witches' weeds", along with deadly nightshade, henbane, and mandrake. Most parts of the plants are toxic, and *datura* has a long history of use for causing delirious states and death. It was well known as an essential ingredient of potions and witches' brews (Preissel and Preissel, 2002).

The word *datura* comes from Sanskrit dhatūr ("thorn apple"); In Sanskrit it is referred to as "Kanak" and "Unmatt" in the ancient scriptures of Ayurveda by sage Sushrut and Charaka. Records of this name in English dates back to 1662. In India it has been referred and attributed to as "Poisonous" and aphrodisiac. In little measure, it was used in Ayurveda as a medicine from the ancient times. It is used in rituals and prayers to Lord Shiva.

Description

Datura species are herbaceous, leafy annuals and short-lived perennials which can reach up to 2m in height. The leaves are alternate, 10-20 cm long and 5-18 cm broad, with a lobed or toothed margin. The flowers are erect or spreading (not pendulous like those of *Brugmansia*), trumpet-shaped, 5-20 cm long and 4-12 cm broad at the mouth; colours vary from whit to yellow, pink and pale purple. The fruit is spiny capsule 4-10 cm long and 2-6 cm broad, splitting open when ripe to release the numerous seeds.

Cultivation

Datura species are usually planted annually from the seed produced in the spiny pods, but with care, plants can be overwintered. Most species are suited to being planted outside or in containers. As a rule, they need warm, sunny places and soil that will keep their roots dry. When grown outdoors in good locations, the plants tend to reseed themselves and may become invasive. In containers, they should have porous, aerated potting soil with adequate drainage. The plants are susceptible to fungi in the root area, so organic enrichment such as compost and manure should be avoided (Preissel and Preissel, 2002).

Toxicity

All *Datura* plants contain tropane alkaloids such as scopolamine, hyoscyamine, and atropine, primarily in their seeds and flowers. Because of the presence of these substances, *Datura* has been used for centuries in some cultures as a poison (Adams and Garcia, 2005). There can be a 5:1 toxin variation between plants, and a given plant's toxicity depends on its age, where it is growing, and the local weather conditions. This variation makes *Datura* exceptionally hazardous as a drug (Boumba *et al.*, 2004).

There are several reports in medical literature of deaths from *D. stramonium* and *D. ferox* intoxication (Micalodimitrakis and Koutselinis 1984). In some parts of Europe and India, *Datura* has been a popular poison for suicide and murder. From 1950 to 1965, the State Chemical Laboratories in Agra, India, investigated 2,778 deaths caused by ingesting *Datura* (Andrews, 2013).

Due to the potent combination of anticholinergic substances it contains, *Datura* intoxication typically produces effect similar to that of an anticholinergic delirium; hyperthermia; tachycardia; bizarre and possible violent behavior; and severe mydriasis (dilated pupils) with resultant painful photophobia that can last several days (Freye, 2010).

Classification

Kingdom: Plantae

(Unranked): Angiosperms

(Unranked): Eudicots

(Unranked): Asterids

Order: Solanales

Genus: *Datura*

Species: *D. stramonium*

Binomial name: *Datura stramonium* L.

Datura stramonium, known by the common names Jimson weed, Devil's snare in the United States, or datura, is a plant in the solanaceae (nightshade) family. It is believed to have originated in the Americas, but is now found around the world. Other common names for *D. stramonium* include thornapple and moonflower, and it has a Spanish name Toloache (University of Texas, 2013).

For centuries, datura has been used as herbal medicine to relieve asthma symptoms and analgesic during surgery or bonesetting. It is also a powerful hallucinogen and deliriant, which is used spiritually for the intense visions it produces. However, the tropane alkaloids which are responsible for both the medicinal and hallucinogenic properties are fatally toxic in only slightly higher amounts than the medicinal dosage, and careless use often results in hospitalizations and deaths.

Description

D. stramonium is a foul-smelling, erect annual, freely branching herb that forms a bush up to 5 feet (60-150cm) tall (Stace, 1997). The roots are long, thick, fibrous and white. The stem is stout, erect, leafy, smooth, and pale yellow-green. The stem forks off repeatedly into branches and at each fork forms a leaf and a single, erect flower.

The leaves are approximately 3 to 8 inches (8-20cm) long, smooth, toothed, soft, irregularly undulate. 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 the extracts of the herb, and remains even after the leaves have been dried.

Datura stramonium generally flowers throughout the summer. The fragrant flowers are trumpet-shaped, white to creamy or violet, and 2 ½ to 3 ½ inches (6-9cm) long, and grow on short stems from either the axils of the leaves or the places where the branches fork. The calyx is long and tubular, swollen at the bottom, and sharply angled, surmounted by five sharp teeth. The corolla, which is folded and only partially open, is white, funnel-shaped, and has prominent ribs. The flowers open at night, emitting a pleasant fragrance and are fed upon by nocturnal moths (Grieve, 1971).

The egg-shaped seed capsule is 1 to 3 inches (3-8cm) in diameter and either covered with spines or bald. At maturity it splits into four chambers, each with dozens of small black seeds.

Range and Habitats

Datura stramonium is native to North America, but was spread to the Old World early. It was scientifically described and named by Swedish botanist Carl Linnaeus in 1753, although it had been described a century earlier by herbalists, such as Nicholas Culpeper. Today it grows wild in all the world's warm and moderate regions, where it is found along roadsides and at dung-rich livestock enclosures. In Europe, it is found as a weed on wastelands and in garbage dumps (Preissel, 2002).

Datura prefers rich, calcareous soil. Adding nitrogen fertilizer to the soil will increase the concentration of alkaloids present in the plant. *Datura* can be grown from seed, which is sown with several feet between each plant. *Datura* is sensitive to frost, and so should be sheltered during cold weather.

Medicinal use

Datura has long been used as an extremely effective treatment for asthma symptoms. The active anti-asthmatic agent is atropine, which causes paralysis of the pulmonary branches of the lungs, eliminating the spasms that cause asthma attacks (Pennachio, 2010). The Zuni used to use *datura* as an analgesic, to render patients unconscious while broken bones are set (Turner, 2009). The Chinese also used it in this manner, as a form of anaesthesia during surgery (Nellis, 1997).

Atropine and scopolamine (both of which are found in very high concentrations in *datura*) are muscarinic antagonists which can be used to treat Parkinson's disease and motion sickness,

and to inhibit parasympathetic stimulation of the urinary tract, respiratory tract, GI tract, heart and eye (Biaggioni, 2011).

Toxicity

All parts of *Datura* plants contain dangerous levels of tropane alkaloids atropine, hyoscyamine and scopolamine which are classified as delirants, or anticholinergics. There is a high risk of fatal overdose for uninformed users, and many hospitalizations occur amongst recreational users who ingest the plant for its psychoactive effects (Preissel *et al.*, 2002).

Datura intoxication typically produces delirium, hypothermia, tachycardia, bizarre behavior, and severe mydriasis with resultant painful photophobia that can last several days. Pronounced amnesia is another commonly reported effect (Freye, 2009).

Phytochemical studies

The presence of diverse phytochemicals in *Datura stramonium* is highly responsible for its hallucinatory, medicinal and toxic properties. Some of this phytochemicals include: Alkaloids, Tannins, Flavonoids, Phenols, Saponins, Glycosides, Terpenoids, Oxalates, etc.

Flavonoids

Flavonoids are also known as vitamin and citrin. They are a class of plant secondary metabolites (IUPAC compendium). According to IUPAC nomenclature, they can be classified into three. The three flavonoids are ketone-containing compounds and as such are flavanoids and flavonols (Spencer *et al.*, 2008). Flavonoids are the most common group of polyphenolic compounds in the human diet. Flavonoids have evidence of health-modulating effects in animal which eat them.

Flavonoids have potential to be biological response modifier such as anti-allergic, anti-inflammatory, and anti-cancer activity shown from in vitro studies.

Alkaloids

Alkaloids are commonly defined as plant substances that contain at least one nitrogen atom in a heterocyclic system per molecule, and which are basic in character. They are cyclic organic compounds containing nitrogen in a negative oxidation state, which is of limited distribution among living organism. This definition has been further qualified by the statement that almost all alkaloids are toxic, and a large majority possesses some pharmacological activity (Popl *et al.*, 1990).

Saponins

Saponins are amphipathic glycosides grouped phenomenologically by the soap-like foaming they produce when shaken in aqueous solutions, and structurally by having one or more hydrophilic glycoside moieties combined with a lipophilic tri-terpene derivative. Saponins are being promoted commercially as dietary supplements and nutraceuticals. Oral administrations might be expected to lead to hydrolysis of glycoside from terpenoid.

Tannins

Tannins are high molecular weight, water soluble polyphenols that form reversible complexes with proteins through pH-dependent hydrogen binding and hydrophobic interactions.

Glycosides

Glycosides are secondary metabolites created by plants and animals. They are usually toxic but may have drug-like therapeutic effects when used appropriately. In humans, small amounts of cardiac glycosides slow down and strengthen the beat of the heart; they do this by blocking the sodium/potassium pump of heart cells which leads to a delay in the electrical signal between the atrium and ventricle.

Terpenoids

Terpenes are about the largest group of natural substances produced by plants, and are all based on varieties of structural modifications of the simple C₅ isoprenoid unit (Nwankwo, 2011).

Phenolic acid

They are class of chemical compounds consisting of a hydroxyl group bonded directly to an aromatic hydrocarbon group. Phenols have high acidity due to the aromatic ring tight coupling with oxygen and hydrogen.

Oxalates

Oxalate occurs in many plants, where it is synthesized via the incomplete oxidation of carbohydrates.

Phytates

A saturated cyclic acid is the principal storage form of phosphorus in many plant tissues. Although indigestible for many animals, phytate and its metabolites as they occur in seeds

and grains have several important roles for the seedling plant. Most notably, phytate functions as a phosphorous store, as an energy store, as a source of cations.

Enzymes as biological markers

A biological marker, or biomarker, generally refers to a measured characteristic which may be used as an indicator of some biological state or condition. Biomarkers are often measured and evaluated to examine normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention (Aronson, 2005).

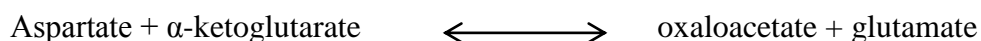
Some enzymes are also used as biological markers. They include but not limited to: aspartate transaminase, alanine aminotransferase, acid phosphatase, alkaline phosphatase, etc.

The enzymes used for the toxicity studies on *D. stramonium* include the following:

Aspartate Transaminase (AST)

Aspartate transaminase (AST), also called aspartate aminotransferase is commonly known as (AspAT / ASAT/ AAT) or serum glutamic oxaloacetic transaminase (SGOT), is a pyridoxal phosphate (PLP) - dependent transaminase enzyme (EC 2.6.1.1). AST catalyzes the reversible transfer of α -amino group between aspartate and glutamate and, as such, is an important enzyme in amino acid metabolism. AST is found in the liver, heart, skeletal muscle, kidneys, brain, and red blood cells, and it is commonly measured clinically as a marker for liver health.

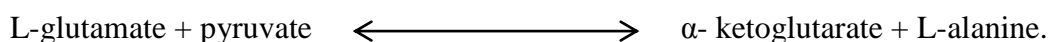
Aspartate transaminase catalyzes the interconversion of aspartate and α -ketoglutarate to oxaloacetate and glutamate.



AST (SGOT) is commonly measured clinically as part of diagnostic liver function tests, to determine liver health (Nalpas *et al.*, 1986).

Alanine aminotransferase (ALT)

Alanine aminotransferase (ALT) was formerly called serum glutamic pyruvic transaminase (SGPT). It catalyzes the transfer of an amino group from L-alanine to α -ketoglutarate, the products of this reversible transamination being pyruvate and L-glutamate.



ALT is measured to see if the liver is damaged or diseased especially by cirrhosis and hepatitis caused by alcohol, drugs or viruses. When the liver is damaged or diseased, it

releases ALT into the bloodstream, which makes ALT levels go up; it is measured in international units/ liter (U/ L) (Wang *et al.*, 2012).

Total protein

This is the amount of protein in the blood. Measurements obtained are used in the diagnosis and treatment of a variety of diseases involving the liver, kidney and bone marrow as well as other metabolic or nutritional disorders.

Elevated levels of total protein can indicate dehydration, infection, cancer and Waldenstrom's disease. Low levels are associated with liver disease, glomerulonephritis, malnutrition and nephrotic syndrome.

Acid phosphatase (ACP)

Acid phosphatase (ACP) is an enzyme present in almost all weaves of the organism, being particularly high in prostate, stomach, liver, muscle, spleen, erythrocytes and platelets. High levels of ACP are found in prostatic pathologies as hypertrophy, prostatitis or carcinoma. In haematological disorders, bones or liver diseases as well as in Paget's or Gaucher's diseases.

Aim of study

The aims of this study is to evaluate the presence of phytochemical constituents, (alkaloids, flavonoids, tannins, saponins, terpenoids, and others) present in *D. stramonium* and as well the toxic effects of the leaves extract on the enzymes and histopathological activity of albino rats.

MATERIALS AND METHODS

Experimental Animals

Eighteen (18) male albino rats weighing between 120-150 grams were obtained from Abdul rat ventures in Kogi State University Staff Quarters, Anyigba and served as test animals.

Datura stramonium

The leaves of *Datura stramonium* were collected from the Staff Quarters of Kogi State University and were identified by Professor S.S Usman of biological department (botany option), Kogi state University, Anyigba, Nigeria.

Other Reagents

Reagents used includes but not restricted to the following: Ferric Chloride, Bromine water, Methanol, Distilled water, Hydrochloric acid, ammonia solution, Mayers reagent, Dragendorff's reagent, 80% alcohol, Lead acetate, 20% NaOH, Benedict's solution, chloroform, concentrated sulfuric acid, acetic anhydride, normal saline.

Apparatus and Equipments

The apparatus and equipment used in the course of this research work include: test-tubes, refrigerator, weighing balance, glass rod, test-tube rack, water bath, spectrophotometer, volumetric flasks, measuring cylinder, filter paper, rotary evaporator, mixer, and oven.

All these equipment and apparatuses were obtained at the Biochemistry Laboratory, Kogi State University Anyigba.

METHODS

Collection and preparation of leaves

The fresh leaves were collected from the garden at the Staff Quarters in Kogi State University, Anyigba, Kogi State. The leaves were air dried and pounded in the laboratory to a uniform texture using standard laboratory mortar and pestle, and used for further analysis.

Extraction Procedures

The pounded leaves was then weighed using a standard laboratory weighing balance and crucible – the weight of the pounded fresh leaves was varied according to Kg/bodyweight of the experimental animal (Albino rats); it was then transferred into a conical flask for extraction process.

50 ml of distilled water was added into the conical flask containing the pounded fresh datura leaves and allowed to soak for two (2) hours. The mixture was then filtered to obtain the pure filtrate which was believed to contain most of the actives ingredients present in the leaves.

Phytochemical screening

Flavonoids

1 gram of freshly blended *D. stramonium* was extracted with 25 ml of 95% ethanol under 2000 rpm and shaken for 24 hours. After filtration, 0.5 ml of the filtrate was taken into a test-tube and 0.5 ml of 50% ethanol was put into as separate test-tube as blank. The reading was taken with a spectrophotometer at an absorbance of 490 nm.

Total Flavonoid in mg/g = $\frac{\text{absorbance} \times \text{gradient factor} \times \text{dilution factor}}{\text{Weight of sample}}$

Weight of sample

Alkaloids

To 5 gram of sample, 50 ml of 10% acetic acid in ethanol was added and shake for 2000 rpm for 24 hours. After filtration, ammonia was added to the filtrate in a drop-wise manner in order to precipitate the alkaloids. With a pre-heated and weighed filter paper, the solution was filtered and the precipitate on the filter paper was dried in the oven for 30 minutes at 60°C.

$$\% \text{ Alkaloids} = \frac{W2 - W1}{W} \times 100$$

Where: W = weight of sample

W1 = weight of empty filter paper

W2 = weight of paper plus precipitate

Tannins

To 2 gram of the sample, 10 ml of distilled water was added, shaken and left for 24 hours. After filtration, 2.5 ml of filtrate was put into a 50 ml volumetric flask. 1 ml of Folin-Denis reagent was added to both blank and sample and also 2.5 ml of saturated Na₂CO₃. The solution was made up to 50 ml with distilled water and allowed to stand for 30 minutes. Using Agilent Spectrophotometer that is capable of reading visible and invisible rays, readings were taken with the reagent blank at zero. The tannin content was given as follows:

$$\% \text{ Tannin} = \frac{A_n}{A_s} \times C \times 100/w \times V_f / V_a$$

Where: A_n = absorbance of test sample

A_s = absorbance of standard solution

C = concentration of standard solution

W = weight of sample used

V_f = total volume of extract

V_a = volume of extract analyzed

Steroids

Crude extract was mixed with 2 ml of chloroform and concentrated H₂SO₄ was added sidewise. A red colour produced in the lower chloroform indicated the presence of steroids.

Terpenoids

Crude extract was dissolved in 2mL of chloroform and evaporated to dryness. To this, 2 ml of concentrated H_2SO_4 was added and heated for about 2 minutes. A grayish colour indicated the presence of terpenoids.

Glycosides

5 grams of sample was soaked for 24 hours in 50 ml of distilled water. 1 ml of filtrate was put into a test-tube and 4 ml of picric acid was added to both the sample and blank. Both test-tubes were put in a water bath for 5 minutes at 36°C . After colour development, reddish brown colour, the absorbance of the test-tubes, i.e. the blank and sample, were read at 490 nm.

Saponins

1 gram of sample was soaked in 50 ml of butanol and kept for 24 hours. 10 ml of 40% saturated MgCO_3 was added and thoroughly shook. After filtration, 1ml of filtrate and 1ml of methanol which served as blank was put into a 50 ml volumetric flask. 2 ml of 10% FeCl_3 was added to the sample and blank and was made up to 50 ml with distilled water. Using the Agilent spectrophotometer at 380 nm, the absorbance was read.

$$\% \text{ Saponin} = \frac{\text{Absorbance of sample} \times \text{Gradient factor} \times \text{Dilution factor}}{\text{Wt of sample} \times 10,000}$$

Oxalate

Oxalate determination was carried out as follows; 2 grams of sample was boiled in 40 ml of distilled water for 30 minutes in a reflux condenser. 10 ml of 20% Na_2CO_3 was added and boiled for another 30 minutes. The liquid was extracted and the residue was washed with hot water until the wash water stopped showing any alkaline reaction. The combination of wash water and filtrate was concentrated to a small volume and cooled. With constant stirring, HCl was added (1:1) drop wise until the final acid concentration after neutralization was about 1% at which stage, a heavy precipitate appeared (which was allowed to flocculate). The extract was carefully filtered into a 250 ml flask and made up to mark, it was kept overnight, and then the supernatant liquid was filtered through a dry filter paper in a dry beaker.

An aliquot of this filtrate was taken into a 400 ml beaker, diluted with water with 200mL and made just ammoniacal and reacted with acetic acid. In the cold medium, 10 ml of a 10% calcium chloride solution was added and stirred well to induce calcium oxalate precipitate to appear and it was allowed to settle overnight. The clear supernatant liquid was carefully

decanted off through whattman No 20 filter paper without disturbing the precipitate. The precipitate was dissolved in HCl (1:1). Oxalic acid was re-precipitated by adjusting the pH with ammonium hydroxide solution. Contents were boiled and allowed to settle overnight. Oxalate was determined by titrating against 0.05N KMnO₄ solution.

1 ml of 0.05N KMnO₄ = 0.00225 anhydrous oxalic acid

$$\% \text{ Oxalate} = \frac{\text{Titre value} \times 0.00225}{2}$$

Phytate

The phytic acid was determined by weighing of 2g of sample into 250 ml conical flask. 100 ml of 2% concentrated hydrochloric acid was used to soak sample in the conical flask for 3 hours. This was filtered through a double layer of hardened filter papers. 50 ml of filtrate was placed in 250 ml beaker and 107 ml of distilled water was added to give proper acidity. 10 ml of 0.03% Ammonium thiocyanate solution was added into each solution as indicator. This was titrated with standard iron chloride solution, which contained 0.00195g iron pertul.

$$\% \text{ Phytate} = \gamma \times 1.19 \times 100$$

Where X = titre value x 0.00195g

Phenolic acid

To a test tube add 0.03 gram of sample. Add 10 ml of 50% aqueous ethanol and extract for 2 hours shaking every 15 minutes (vortex). Using a micropipette, take 200µl of filtrate (50% ethanol as blank) into a 50 ml volumetric flask and add 2.5 ml Folin-Denis reagent to both sample and blank. Add 5 ml saturated Na₂CO₃ and add distilled water to make it up to the 50 ml mark. Leave for 20 minutes. Read at 760 nm.

Procedure for Toxicity Studies

The male albino rats were procured from Abdul ventures and acclimatized in the clean and very hygienic Laboratory animal house for 7 days after which they were placed into different groups in a metabolic cage and their initial weight taken. The animals were placed on standard feed (animal growers) for the whole time. The rats were divided into three broad groups corresponding to the 3 weeks (21 days) in which the experiment was to be carried out – i.e. one group for each week respectively.

Each group, containing ten (10) animals, was further sub-divided based on their individual dose requirements. Three (3) animals each was given a particular dosage (i.e. extract concentration) per body weight and were labeled correspondingly A, B, and C.

For the first group, which was labeled as week 1, 2ml of 2.0g/ml, 1.0g/ml, and 0.75g/ml of the aqueous extract was administered to each animal labeled as Tr1, Tr2, and Tr3 respectively (with Tr1, Tr2 and Tr3 further sub-classified into ABC groups each) for 7 days at about 10:00 am each day; while a fourth animal was given the placebo (i.e. distilled water without extract) which served as the control animal. The administration was done orally using a standard laboratory syringe.

The same dosage of the aqueous extracts was given to the second and third group of the male albino rats corresponding to the week two (2) and week three (3) of the experimental duration respectively; and all other factors including environmental factors were seen to be constant throughout the period of the experiment. Also, the physiological changes and reactions in the animals were noted throughout the whole experiment.

Table 1: Distribution of experimental animals into groups.

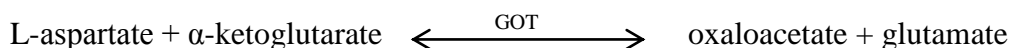
	Week 1	Week 2	Week 3
Tr 1	A	A	A
(2.0g / kg)	B	B	B
	C	C	C
Tr 2	A	A	A
(1.5g / kg)	B	B	B
	C	C	C
Tr3	A	A	A
(0.75g / kg)	B	B	B
	C	C	C

At the end of each week, the animals corresponding to that particular group was sacrificed using jugular puncture while their blood was collected into an EDTA-free Laboratory bottle; these bottles were tightly capped and was centrifuged at 10000 RPM for 30 minutes in order to obtain the upper clear layer called the blood serum which was to be used for the toxicity studies. The liver, kidneys, and a portion of the intestine were also harvested for the purpose of histopathology assessment of damage.

The blood serums collected were then used for some specific enzyme assay to check for physiological damages within the animals while the serum of the control animal served as a standard. Specific enzymes that were assayed include: aspartate transaminase (AST), alanine aminotransferase (ALT), acid phosphatase (ACP) and Total protein.

Procedure for Enzyme Assay**AST determination**

Principle: According to Reitmann (1957) AST is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine.



Wavelength:

Hg 546 nm

Cuvette:

1cm light path

Incubation Temperature:

37°C

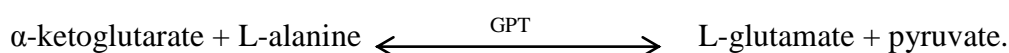
Table 2: Procedure for AST determination.

Pipette into test tubes	Reagent blank	Sample
Sample	----	0.1 ml
Reagent 1	0.5ml	0.5ml
Distilled Water	0.1ml	----
Mix, incubate for exactly 30 mins. At 37°C		
Reagent 2	0.5ml	0.5ml
Mix, allow standing for exactly 20 mins. At 20 to 25°C		
Sodium hydroxide	5.0ml	5.0ml

Mix, read the absorbance of sample against reagent blank after 5 minutes.

Procedure for ALT determination

Principle: ALT is measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine.



Wavelength:

Hg 546 nm

Cuvette:

1cm light path

Incubation Temperature:

37°C

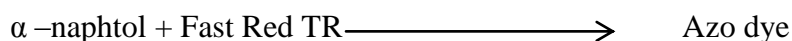
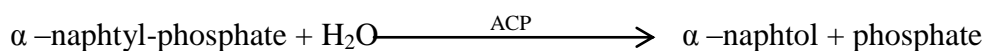
Table 3: Procedure for ALT determination.

Pipette into test tubes:	Reagent Blank	Sample
Sample	----	0.1ml
Solution R1	0.5ml	0.5ml
Distilled Water	0.1ml	----
Mix, incubate for exactly 30 mins. At 37°C		
Solution R2	0.5ml	0.5ml
Mix, allow standing for exactly 20 mins. At 20 to 25°C		
Sodium hydroxide	5.0ml	5.0ml

Mix, read the absorbance of sample against reagent blank after 5 minutes

Procedure for ACP determination

Principle: Acid phosphatase activity present in the sample is determined according to the equation.



α -naphthol reacts with a diazoted compound forming a colour with a maximum of absorbance at 405nm. Tartarate is used as specific of the prostatic function.

Wavelength:

405 nm

Cuvette:

1cm light path

Incubation Temperature:

30, 37°C

- Adjust the instrument to zero with distilled water

Table 4: Procedure for ACP determination

Pipette into cuvette	
	ACP Total (T)
WR (mL)	1.0
Sample (μ L)	100

- Mix, incubate for 5 minutes.
- Read initial absorbance (A) of the sample, start the stopwatch and read absorbance at 1 minute intervals thereafter for 3 minutes.
- Calculate the difference between absorbance and the average absorbance differences per minutes ($\Delta A/\text{min}$).

Procedure for Total Protein determination

Table 5: Procedure for total protein determination

Pipette into test tubes:		
	Reagent Blank	Sample
Sample	----	0.1ml
Solution R1	0.5ml	0.5ml
Distilled Water	0.1ml	----
Mix, incubate for exactly 30 mins. At 37°C		
Solution R2	0.5ml	0.5ml
Mix, allow standing for exactly 20 mins. At 20 to 25°C		
Sodium hydroxide	5.0ml	5.0ml

Histopathological Screening

Tissue Processing

1. Fixation: The tissue was fixed in a Fixative (10% Phosphate buffered Formalin).

2. Dehydration: The tissue was dehydrated in ascending grades of alcohol to remove water.
3. Clearing: The tissue was put in Xylene to remove (clear) the alcohol.
4. Infiltration: The tissue was infiltrated with Paraffin Wax.
5. Embedding: The tissue was embedded in Paraffin Wax using an embedding mould and cassette.
6. Microtomy: The tissue was sectioned using a microtome to form ribbons (3-5 microns thick).
7. Floating: These ribbons were floated in a warm water bath onto labelled slides.
8. Hot plate: These slides were placed on a warm plate to dry and allow adherence.

Staining (Haematoxylin and Eosin)

1. Tissue section was dewaxed using Xylene.
2. Tissue section was hydrated using descending grade of alcohol: 100%, 70% and 50%.
3. Tissue was rinsed in distilled water.
4. Tissue section was stained using Haematoxylin (Harris) for 5 minutes.
5. Tissue section was rinsed in distilled water.
6. Tissue section was differentiated using 1% acid alcohol briefly.
7. Tissue section was rinsed in water.
8. Tissue section was blued with tap water for 10 minutes.
9. Tissue section was counterstained using aqueous Eosin for 1 minute.
10. Tissue section was rinsed in distilled water
11. Tissue section was dehydrated using ascending grades of alcohol (50%, 70% and 100%).
12. Tissue section was cleared using Xylene at 3 changes.
13. Tissue section was viewed under the microscope using x10 then x40 objective.
14. The nucleus appeared blue and the cytoplasm appeared red.

RESULTS

Phytochemical screening result

Table 6: Results for Phytochemical Screening

Phytochemicals	Concentration (mg/g)	(%)	State (Present/ Absent)
Alkaloids		0.60 %	Present
Flavonoids	1.15		Present
Tannins	3.16		Present
Saponins	0.64		Present
Glycoside compounds	0.054		Present

Phenolic compounds	20.04		Present
Phytate		0.84%	Present
Oxalate	0.016		Present
Terpenoids	-		Present (++)
Steroids	-		Present (++)

++ indicate more presence

Toxicity test result

Table 7: Results of aspartate transaminase (AST)

Dose concentration	Treatment	Week 1	Week 2	Week 3
Tr1	A	17.32	13.38	35.01
2.0g / kg	B	17.87	14.01	34.55
	C	17.31	13.56	34.39
Tr2	A	23.93	24.05	25.35
1.5g / kg	B	24.20	23.69	25.60
	C	24.32	23.66	25.70
Tr3	A	24.35	31.72	28.88
0.75g / kg	B	24.62	31.38	29.15
	C	24.53	31.38	29.02
	Control	12.95	25.55	26.60

Σ Control = 21.70

Table 8: Statistical analysis of AST

	2.0g / kg	1.5g /kg	0.75g / kg
Control	21.70 \pm 0.00 ^a	21.70 \pm 0.00 ^a	21.70 \pm 0.00 ^b
A	21.90 \pm 6.65 ^a	24.44 \pm 0.45 ^a	28.32 \pm 2.15 ^a
B	22.14 \pm 6.30 ^a	24.50 \pm 0.57 ^a	28.38 \pm 1.99 ^a
C	14.72 \pm 1.30 ^a	27.46 \pm 3.47 ^a	27.20 \pm 2.12 ^{ab}

a, b: Mean with same superscript is not significantly different.

Mean with different superscript are significantly different at 0.05% probability or ($p < 0.05$).

Table 9: Results of Alanine Aminotransferase (ALT)

Dose concentration	Treatment	Week 1	Week 2	Week 3
	A	24.15	26.15	17.05
2.0g / kg	B	24.17	26.45	17.22
	C	24.16	26.12	17.09
	A	31.70	26.19	19.44
1.5g / kg	B	31.66	26.13	19.23
	C	31.68	26.40	19.41
	A	13.55	22.88	18.65
0.75g / kg	B	13.79	23.25	18.80
	C	13.94	22.99	18.71
	Control	18.88	31.36	19.52

Σ Control = 23.25

Table 10: Statistical analysis of ALT

	2.0g / kg	1.5g /kg	0.75g / kg
Control	23.25 ± 0.00 ^a	23.25 ± 0.00 ^a	23.25 ± 0.00 ^a
A	22.45 ± 2.76 ^a	25.78 ± 3.55 ^a	18.36 ± 2.70 ^a
B	22.61 ± 2.78 ^a	25.67 ± 3.60 ^a	18.61 ± 2.73 ^a
C	24.16 ± 1.13 ^a	25.06 ± 4.26 ^a	18.78 ± 2.63 ^a

a, b: Mean with same superscript is not significantly different.

Mean with different superscript are significantly different at 0.05% probability or ($p < 0.05$).

Table 11: Results of acid phosphatase (ACP)

Dose concentration	Treatment	Week 1	Week 2	Week 3
	A	23.85	21.55	25.65
2.0g / kg	B	24.15	22.02	25.60
	C	24.00	21.68	25.25
	A	26.15	25.30	26.20
1.5g / kg	B	26.33	25.55	26.33
	C	26.27	25.65	26.22
	A	32.35	34.00	35.67
0.75g / kg	B	32.15	33.78	36.21
	C	32.25	33.47	36.12
	Control	39.75	23.25	33.00

Σ Control = 32.00

Table 12: Statistical analysis of ACP

	2.0g / kg	1.5g /kg	0.75g / kg
Control	32.00 ± 0.00 ^a	32.00 ± 0.00 ^a	32.00 ± 0.00 ^a
A	23.68 ± 1.19 ^b	25.88 ± 0.29 ^b	34.00 ± 0.96 ^a
B	23.92 ± 1.04 ^b	26.07 ± 0.26 ^b	34.05 ± 1.18 ^a
C	22.84 ± 1.16 ^b	25.72 ± 0.30 ^b	30.65 ± 2.24 ^a

a, b: Mean with same superscript is not significantly different.

Mean with different superscript are significantly different at 0.05% probability or ($p < 0.05$).

Table 13: Results of Total Protein

Dose concentration	Treatment	Week 1	Week 2	Week 3
	A	7.35	9.28	7.52
2.0g / kg	B	7.53	9.39	7.41
	C	7.40	9.38	7.35
	A	7.38	13.76	8.30
1.5g / kg	B	7.50	13.73	8.28
	C	7.40	13.76	8.17
	A	9.89	11.38	9.00
0.75g / kg	B	10.15	11.57	9.15

	C	10.50	11.70	9.08
	Control	10.45	11.55	10.175

Σ Control = 10.73

Table 14: Statistical analysis of total protein

	2.0g / kg	1.5g /kg	0.75g / kg
Control	10.73 \pm 0.00 ^a	10.73 \pm 0.00 ^a	10.73 \pm 0.00 ^a
A	8.05 \pm 0.62 ^a	9.81 \pm 1.99 ^a	10.09 \pm 0.69 ^a
B	8.11 \pm 0.64 ^a	9.84 \pm 1.96 ^a	10.29 \pm 0.70 ^a
C	17.83 \pm 9.51 ^a	9.50 \pm 2.13 ^a	10.12 \pm 1.04 ^a

a, b: Mean with same superscript is not significantly different.

Mean with different superscript are significantly different at 0.05% probability or ($p < 0.05$).

Table 15: showing weight difference in the experimental animals treated with the plant extract

	Week 1	Week 2	Week 3
A	17.50	21.35	16.00
B	17.22	21.22	16.11
C	18.02	21.30	16.25
A	27.25	24.55	25.50
B	28.02	24.18	25.58
C	28.52	24.14	25.75
A	27.15	39.00	17.02
B	26.95	38.75	16.85
C	27.17	39.19	17.04
Control	29.22	29.24	30.48

Histopathological screening result

Table 16: Tabular presentation of histopathological screening result.

	INTESTINE	LIVER	KIDNEY
CONTROL	Normal	Normal	Normal
WEEK 3	Normal	Normal	Normal
WEEK 2	Normal	Normal	Normal
WEEK 1	Normal	Normal	Normal

Magnifications: X 40
X 100
X 400

Stains: H&E (Haematoxylin and Eosin)

Kidney

NORMAL – Normocellular Glomerular tufts displayed on a background containing tubules.

No necrosis. (See micrograph 1 below).

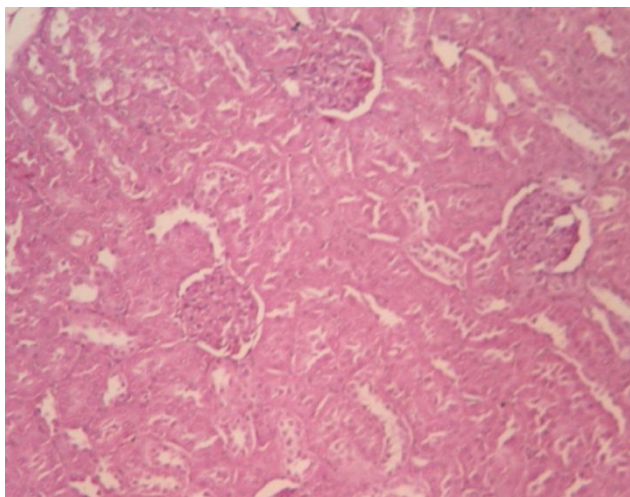


Figure 1: Normal kidney.

LIVER

NORMAL - Histologic section of liver shows preservation of hepatic architecture. The hepatocytes are arranged as parallel plates, and extend from the portal tracts to the central veins. The sinusoids are not congested. No areas of necrosis, haemorrhage or inflammation is seen. No intracellular inclusions are present (see Micrograph 2 below)

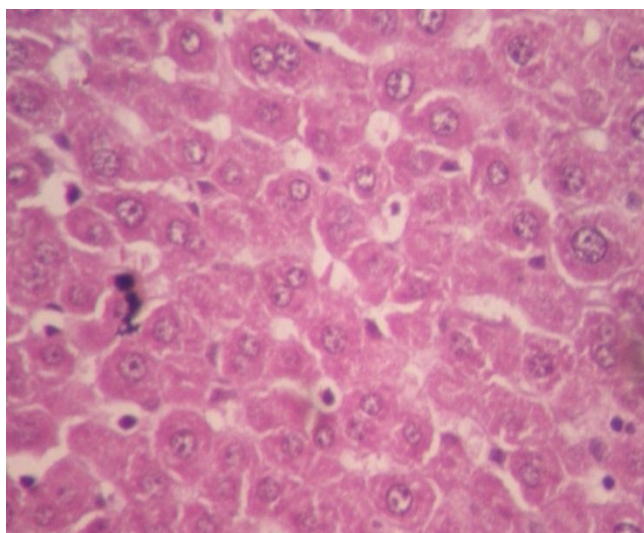


Figure 2: Normal liver.

INTESTINE

NORMAL – Histologic section of intestinal tissue show lining cells composed of tall columnar cells with interspersed mucous containing goblet cells (see Micrograph 3 below).

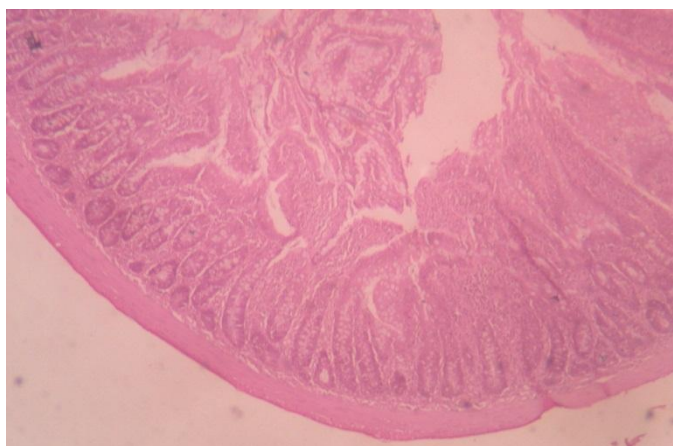


Figure 3: Normal intestine.

Discussion

Phytochemical Analysis

Phytochemical analysis conducted on the leaves of *D. stramonium* revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities. Analysis of the leaves revealed the presence of phytochemicals such as alkaloids, flavonoids, phenolic acids, tannins, saponins, glycosides, steroids, terpenoids, and oxalate and phytate. Phenolic compound possess biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflammation, as well as inhibition of angiogenesis and cell proliferation activities (Han *et al.*, 2007). Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds. Natural antioxidants mainly come from plants in the form of phenolic compounds. Tannins bind to proline-rich proteins and interfere with protein synthesis. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infections and they have been found to be antimicrobial substances against wide array of microorganisms in vitro. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall (Marjorie, 1966). They also have antioxidant and show strong anticancer activities.

The plant extract were also revealed to contain saponins which are known to produce inhibitory effect on inflammation, as well as the property of precipitating and coagulating red blood cells (Okwu, 2004). Steroids have been reported to have antibacterial properties and they are very important compounds especially due to their relationship with compounds such as sex hormones (Okwu, 2001). Glycosides are known to lower blood pressure according to many reports. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity (Harborne, 1973).

Toxicity studies

The plant extract appeared to have an initial psycho-stimulating effect on the animal which makes them to be restless and more aggressive few hours after administration. Several hours after administration of the plant extract, the animals were docile and inactive. It is suggested that the sedative effects of the extract began after the psycho-stimulating effects and was more pronounced. The plant extract did not appear to have any weight losing effect, this could be as a result of its other medicinal properties and perhaps the dosage was not high enough to induce weight loss.

The increased level of AST suggests the generation of hepatic (liver) stress and injury following administration of the plant extract. AST catalyzes the reversible transfer of α -amino group between aspartate and glutamate and, as such, is an important enzyme in amino acid metabolism. AST is found in the liver, heart, skeletal muscle, kidneys, brain, and red blood cells, and it is commonly measured clinically as a marker for liver health (Kirsch *et al.*, 1984).

AST is mostly found in the liver and plays a biological role especially as a biomarker to determine liver's state of health. Though higher, there is no statistically significant difference in the mean value of AST in the animals in the 2.0g/kg and 1.5g/kg extract treated group; however there is a statistical significant difference in the animals in the 0.75g/kg extract treated group when compared to the control.

ALT catalyzes the transfer of an amino group from L-alanine to α -ketoglutarate, the products of this reversible transamination being pyruvate and L-glutamate. It is measured to see if the liver is damaged or diseased especially by cirrhosis and hepatitis caused by alcohol, drugs or viruses. When the liver is damaged or diseased, it releases ALT into the bloodstream, which makes ALT levels goes up and it is measured in international units / liter (U/ L) (Wang *et al.*, 2012).

Though higher at 2.0g/kg and 1.5g/kg and lower at 0.75g/kg than the control, there is no statistically significant difference in the value of ALT in the animals in the extract treated group.

Acid phosphatase (ACP) is an enzyme present in almost all weaves of the organism, being particularly high in prostate, stomach, liver, muscle, spleen, erythrocytes and platelets.

Because of its wide distribution throughout the body and especially in the liver, cellular damage causes an elevation in the total serum of ACP, such that when there is an injury to the tissue, the cells increase in ACP and thus releasing it into the blood stream, where it is identified in higher than normal values. The mean ACP level of *Datura* treated group of 2g/ml and 1.5g/ml is less than that of the control and shows a significant difference ($P < 0.05$). The extract treated group is higher than the control at 0.75g/kg treatment and shows no significant difference ($P < 0.05$). This could be explained that at that quantity (2.0g/kg and 1.5g/kg), it may not be deleterious but could have other physiological implications.

High levels of ACP are found in prostatic pathologies as hypertrophy, prostatitis or carcinoma. In haematological disorders, bones or liver diseases as well as in Paget's or Gaucher's diseases.

Total protein measures the amount of protein in the blood. Measurements obtained are used in the diagnosis and treatment of a variety of diseases involving the liver, kidney and bone marrow as well as other metabolic or nutritional disorders.

Elevated levels of total protein can indicate dehydration, infection, cancer and Waldenstrom's disease. Low levels are associated with liver disease, glomerulonephritis, malnutrition and nephrotic syndrome. This could explain the reason why there is low levels of protein in the serum of the extract treated animals (2.0g/kg, 1.5g/kg and 0.75g/kg). Although there is no statistically significant difference in the values of total protein ($p < 0.05$) the low values of protein in treated animals compared to the control, could be an indication of liver damage. Although there was an apparent increase in the overall weight of animals, this may be due to the other medicinal properties of *Datura* or some other physiological effects on the overall metabolism of the animal.

Also, there appeared to be no physiological injuries in the liver, kidney and intestine of extract treated animals when compared to the control. Perhaps the concentration of the extract was not high enough to induce deleterious effects, or that the duration as well as the other medicinal constituents of the plant prevented any observable injuries.

CONCLUSION

From the study, it has been demonstrated that aqueous leaf extract of *Datura stramonium* has deleterious effects on certain organs in the body such as the liver. Histochemically, the leaf

extract alters protein metabolism, caused cellular damage through mediation of metabolic stress. The plant extract as well altered the activities of aspartate transaminase, alanine aminotransferase, acid phosphatase, and total protein content in the hepatic and other organs of the treated animals. Histologically, sections of the liver, kidney and intestines showed no evidence of physical damage. The results revealed the presence of medicinally important constituents in the plants studied. Many evidences gathered in earlier studies which confirmed the identified phytochemicals to be bioactive. Several studies confirmed that the presence of these phytochemicals constitute medicinal as well as physiological properties to the plant studied in the treatment of different ailments. Therefore, extracts from *D. stramonium* could be seen as a good source for useful drugs. Hence, the use of this plant for its hallucinogenic, medicinal effects and for other application by man should be done with great cautions, as there is a high vulnerability of toxicity.

Recommendation

The traditional medical practitioners making use of *D. stramonium* should do some scientific findings, especially LD₅₀, before administration. However it is suggested that further work should be carried out to isolate, purify, and characterize the active constituents responsible for the activity of *D. stramonium*. Additional work is encouraged to elucidate the possible mechanism of action of the active components and quantify relative safe dosage of the plant to the users.

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