

Full Length Research Paper

Toxicity studies of alkaloids of seeds of *Datura stramonium* and synthesis alkaloids in male rats

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The effects of acute, subacute and chronic administration of alkaloids atropine and scopolamine, the main constituents of the active principle of *Datura stramonium*, with toxic properties, were studied in male Albino-Wistar rats. After acute i.p administration of dose 100 mg/kg (1/4 DL₅₀) of total alkaloids to the seeds of *D. stramonium*, there were no remarkable changes in general appearance and no deaths occurred in any experimental group. 24 h after total alkaloids of seeds, a significant reduction in tissues (liver, spleen and brain) was observed. The red blood cells (RBC), Hematocrit (HCT), Hemoglobin (HGB) and white blood cells (WBC) were significantly higher in the treated groups than the control group. There were no statistical differences in Glutamic-oxaloacetic transaminase (GOT), Glutamic-Pyruvic Transaminase (GPT) and alkaline phosphatase (ALP) observed between groups. Histological examination of liver showed no histopathological changes. Subacute study for four weeks showed no resulting mortality or signs of toxicity. The relative weight of kidneys showed a significant decrease, however, these doses of synthetic alkaloids (5.2 mg/kg of atropine and 2.6 mg/kg of scopolamine) produced significant increase of lungs in comparison with the control group. RBC, HGB, HCT and PLT values of control group were significantly higher than those of the treated group. The enzyme activities of GOT, GPT and ALP were significantly increased. The microscopic examination of liver showed normal conservative lobular architecture and necrotic areas. In chronic study, the synthetic alkaloids administered i.p at daily doses 4.2 mg/kg of atropine and 1.6 mg/kg of scopolamine, did not produce death, However the diarrhoea and hypoactivity were observed. The relative weight of liver was significantly less than that of the control group. The haematological analysis revealed a significant decrease in RBC, HCT, HGB and WBC and we observed manifold centrolobular necrotic areas, and blood congestion and dilated central veins in treated groups.

Key words: Toxicity, *Datura stramonium*, alkaloids, atropine, scopolamine, rat.

INTRODUCTION

The solanaceae plant, *Datura stramonium*, is locally known as Sikrane and is prevalent in north Algeria. It is also called Devil's trumpet, Devil's apple, Jamestown weed (SteenKamp et al., 2004). The main toxic alkaloids in *D. stramonium* are the Tropane alkaloids of which atropine (dl-hyoscyamine) and scopolamine (l-hyoscyne) (Friedman, 2004; Steenkamp et al., 2004). Atropine and scopolamine are competitive antagonists of muscarinic cholinergic receptors and are central nervous system depressants (Halpern, 2004). All parts of the plant are

toxic, but the highest amount of the alkaloids is contained in ripe seeds (Miraldi et al., 2001; Friedman and Levin, 1989). Many cases of accidental poisoning by *D. stramonium* species have been reported when these plants were eaten accidentally, or decoction prepared from herbal prescription (Al-Shaikh and Sablay, 2005; Hirschmann et al., 1990). Intentional poisoning with *D. stramonium* 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 attempt (Klein-Schwartz and Odera, 1984; Kurzbaum et al., 2001; SteemKamp et al., 2004; Forrester, 2006; Monteriol et al., 2007). The toxicity of *D. stramonium* in grazing animals have been suspected by livestock owners and field veterinarians especially at time

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Figure 1. Plant of *Datura stramonium*.

of drought or after ingesting freshly harvested maize that will be used for ensiling and heavily contaminated with young *D. stramonium* (Nelson et al., 1982). The aims of this study were to evaluate the acute toxicity of the total alkaloids of the ripe seeds, the subchronic and chronic toxicity of the synthetic alkaloids, atropine and scopolamine, administered in 2:1 ratio as *D. stramonium* seeds, where the biochemical, hematological and histopathological parameters was investigated.

MATERIALS AND METHODS

Plant material

Seeds of *D. stramonium* were collected in South Setif (East Algeria) between August and September. The seeds were stored at room in dry place. After drying, the seeds were kept in tightly – closed containers prior to use (Figures 1 and 2).

Extraction of total alkaloids

100 g of air dried powdered of seeds was defatted with petroleum ether under reflux, and then the seeds were witted with 150 ml of

NH_4OH (25%, m/m) for 4 h, and were extracted to exhaustion with CHCl_3 using a soxhlet apparatus for 6 h. The organic extract (containing free alkaloids + lipophilic impurities) is then shaken three times with 150 ml aqueous sulphuric acid (2%, m/m). The acid extracts (alkaloids salts) are treated three times with 50 ml NH_4OH (25%, m/m) to pH 10 to liberate the free alkaloids which are separated by extraction with 150 ml CH_2Cl_2 , and then dried with Na_2SO_4 and concentrated to dryness under reduced pressure to obtain crude alkaloids (Bruneton, 1999). The yield of this extract was approximately $0.089 \pm 0.02\%$ (w/w).

Thin layer chromatography assay

Thin layer chromatography (TLC) was carried out using silica gel (gel plates Alugram sel G/UV254 20x20) procured from Macherey – Nagel, Germany. The total alkaloids were dissolved in alcohol and 10 μl was applied to plate. Methanol, chloroform, ammonia (78.5:20:1.5) solvent system was used for TLC. The TLC spots containing alkaloids were visualized by spraying with Dragen-dorff reagent according to Kurt's method with modifications (Kurt, 1971).

HPLC analysis

A Pye unicam HPLC was used to quantify atropine and scopolamine in total alkaloids of *D. stramonium* seeds. The HPLC system consisted of a isocratic pump, a rheodyne injector equipped with a 20 μl sampling loop and photodiode array detector. Separation was achieved by a Varian/Chrompack column C_{18} (125 x 4 mm i.d, particle size 5 μm) preceded by a guard column, at temperature 35°C. The mobile phase (delivered at a flow rate of 1 ml/min) consisting of acetonitrile – phosphate buffer (pH 3.8)(15/85, v/v), was filtered through a 0,45 μm membrane and degassed before use, according to Kirchhoff et al. (2004) method with some modifications.

Experimental animals

Male Albino-Wistar rats weighing between 200 to 250 g were obtained from animal center of Pasteur's Institute (Algiers – Algeria).

Rats were housed in hanging transparent plastic cages (55 x 33 x 19 cm) in the animal room of Faculty of Sciences University Ferhat Abbas Setif Algeria and acclimated for 3 weeks prior to experiment. The litter was renewed every 3 days. They were fed with a standard pellet and tap water *ad libitum*. All animals were kept in standard environmental conditions (temperature 20 to 25°C and 12 light/12 h dark cycle). Each rat was identified by body marks using 1% picric acid solution. All experimental procedures were conducted in accordance with the guide for care and use of laboratory animals and in accordance with the scientific council of the Faculty of Natural Sciences and Life of the University Ferhat Abbas, Setif – Algeria.

Preparation of drugs

The drugs were dissolved in 100 μl of ethanol and diluted with normal sterile saline water.

Acute toxicity

Two groups of 10 Wistar albino rats were given single dose of 100 mg/kg (1/4 DL_{50}) body weight of alkaloids by intraperitoneal route (but not lethal dose to try to investigate the target organs) (Antov et



Figure 2. Fruit and seeds of *Datura stramonium*.

al., 1991). The control group (10 rats) received saline water with few drops of ethanol at the same volume. Animals were observed and recorded systematically 1, 2, 3, 4, 5, and 6 h and daily after test substance administration. The visual observations included changes in skin and fur (hair), eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous system. The first group was sacrificed after 24 h of treatment; and the second group after 5 days. The maximum acute liver toxicity is expressed at day 5 (Szymanowicz and Danel, 2005). In humans after acute poisoning by *D. stramonium*, hospitalization and recovery takes between 1 to 5 days (Bouzidi et al., 2000).

Subacute toxicity

A separate was conducted to evaluate the subacute toxicity of sulfate atropine and bromide scopolamine (were obtained from Fluka - USA) on one group of 10 rats. The alkaloids (sulfate atropine et bromide scopolamine) were dissolved in 100 μ l alcohol and then diluted with normal sterile saline water and administered daily by intraperitoneal route at dose 4.5 mg/kg sulfate atropine and 2.25 mg/kg bromide scopolamine for four weeks, while control group (10 rats) was given saline water with alcohol at the same volume. The animals were observed daily for abnormalities and the body weights were recorded at weekly intervals. At the end of experiment, the animals were examined and sacrificed under the same conditions as described previously, the acute toxicity.

Chronic toxicity

A 120 day subchronic toxicity study of alkaloids (sulfate atropine and bromide scopolamine) was carried out with dose 4.5 mg/kg

sulfate atropine and 2.25 mg/kg of bromide scopolamine. The alkaloids were administered daily by intraperitoneal injection. The animals were observed daily for any changes in behavior and manifestations of toxic symptoms. The body weights were recorded every week. At the end of all experimental periods, animals were anaesthetized with urethane at the dose 760 mg/kg. Two kinds of blood were obtained from the retro-orbital vein, a sample for hematology containing ethylenediaminetetraacetic acid with apparatus MEDONIC (Beckman Coulter – USA) and sample for serum and used for measurement of activities Glutamic-oxaloacetic transaminase (GOT), Glutamic- Pyruvic Transaminase (GPT) (using commercial Kits –SGM Rome-Italy) and alkaline phosphatase (ALP) (using commercial Kits – CYPRESS DIAGNOSTIC Langdrop –Belgium) with apparatus TECHNICON RA-1000-USA. After blood collection, the animals were sacrificed by cervical dislocation. After autopsy, all tissues were examined grossly and major's organs (liver, brain, heart, kidneys, spleen, testicles, and lung) were weighted. The relative organ weight (weight of organ as a proportion of the total weight of each rat) was calculated and compared with the value of the control. Tissues from liver of all animals were fixed in 10% buffered formalin solutions then embedded in paraffin and cut with a microtome set at 5 μ m, stained with hematoxylin and eosin and examined by light microscopy.

Statistical analysis

The statistical significance of the differences between means was calculated using one-way ANOVA followed by Tukey's test for multiple comparisons with control group, * $P \leq 0,05$.

RESULTS

Thin layer and HPLC chromatography assays

The evaluation of TLC of alkaloids of seeds of *D. stramonium* revealed two distinct spots. The comparison with witnesses' samples (sulphate atropine and bromide scopolamine) showed that the first spot is atropine and the second is scopolamine which are the majors' alkaloids in *D. stramonium* (Figure 3). Representative chromatograms for tropane alkaloids are shown in (Figure 4) under chromatographic conditions used. The retention times for scopolamine and atropine were 9.05 and 16.11 min, respectively. The concentrations of atropine and scopolamine were 4 and 2 mg/100 g of *D. stramonium* seeds respectively.

Acute toxicity study of total alkaloids

The results of the study of animals administered with single dose of 100 mg/kg body weight of total alkaloids did not show any toxic symptoms such as paralytic, ataxic, lacrimation, laboured breathing or death, immediately after injection or at the end of 5 days. There were no statistically significant differences in average body weight of the control group and total alkaloids – treated groups during the acute toxicity (Table 1). The effects of total alkaloids of *D. stramonium* seeds on



Figure 3. Thin layer chromatography of alkaloids seeds of *Datura stramonium*. Mobile phase: methanol /chloroform /ammonia / 78.5/20/1.5 (v/ v/ v). Detection of alkaloids was performed by use of Dragendorff's reagent.

relative organ (liver, lungs, kidney, heart, brain, testis and spleen) weights are presented in Table 2. There were statistically significant decreases in the relative organ weight of liver, spleen and brain of the treated rats of the first group (sacrificed after 24 h). The haematological parameters of the rats treated with total alkaloids are presented in Table 3. The RBC (red blood cells), HCT (Hematocrit), HGB (Hemoglobin) and WBC (white blood cells) were significantly higher in the treated groups than the control group.

The results of the indices of liver function GOT (glutamic-oxaloacetic transaminase), GPT (glutamic-pyruvic transaminase) and ALP (alkaline phosphatase) are given in Figure 5. It was observed that the values of GOT, GPT and ALP on day 1 and 5 were comparable with the values of the control and treated groups. There were no statistical differences in GOT, GPT and ALP observed between groups. The histological examination of liver from males' rats was performed in both control and treated groups. All the sampling tissue sections showed no histopathological changes. They were within normal limits. Neither degenerative nor infiltrative lesions were observed.

Subacute toxicity study of synthetic alkaloids

A separate experiment was carried out to evaluate the subacute toxicity of synthetic alkaloids (5.2 mg/kg sulphate atropine and 2.6 mg/kg bromide scopolamine) in the Wistar rats. The administration of saline water or synthetic alkaloids to four weeks did not induce any marked changes in the general behaviour or physiological activities of the rats, however, the diarrhoea was observed.

The treatment of rats with saline or dose synthetic alkaloids (sulphate atropine and bromide scopolamine), did not induce mortality during the whole study period. The administration of dose synthetic alkaloids resulted in a non significant reduction in the final body weight of the animals when compared with the control (Figure 6).

The saline and synthetic alkaloids did not cause any gross morphological abnormality in various organs of the animals. The relative weight of kidneys showed a significant decrease, however, these doses of synthetic alkaloids produced significant increase of lungs in comparison with the control group (Table 4).

The haematological values of treated rats were significantly different from those of control group (Table 5). RBC (Red blood cells), HGB (Haemoglobin), HCT (Hematocrit) and PLT (Platelets) values of the control group were significantly higher than those of the treated group.

Biochemical and haematological observation

The estimation of various enzymes of subacute toxicity revealed significant differences. The GOT, GPT and ALP exhibited a drug dose dependent elevation, when compared with saline control (Figure 7). Under the microscopic examination, the liver of treated-group of subacute toxicity study showed normal conservative cellular and lobular architecture and necrotic areas.

Chronic toxicity study of alkaloids

In the chronic toxicity study, the alkaloids sulphate atropine and bromide scopolamine at doses 4.2 mg/kg and 1.6 mg/kg respectively, given intraperitoneally for 120 days, did not produce death of rats during the experimental period, however, the diarrhoea and hypoactivity were observed. Significant differences were observed in weight of rats treated with total alkaloids of seeds of *D. stramonium*, but the changes in animal weight control and treated group follows a similar appearance (Figure 8). No significant differences were detected in the weight gain of male (control 41.00 ± 8.16 g; experimental 43.81 ± 20.71 g) rats treated with the synthetic alkaloids, as compared to control group. The relative organ weights of the synthetic rats were differed from those of the control group. The relatives' weights of

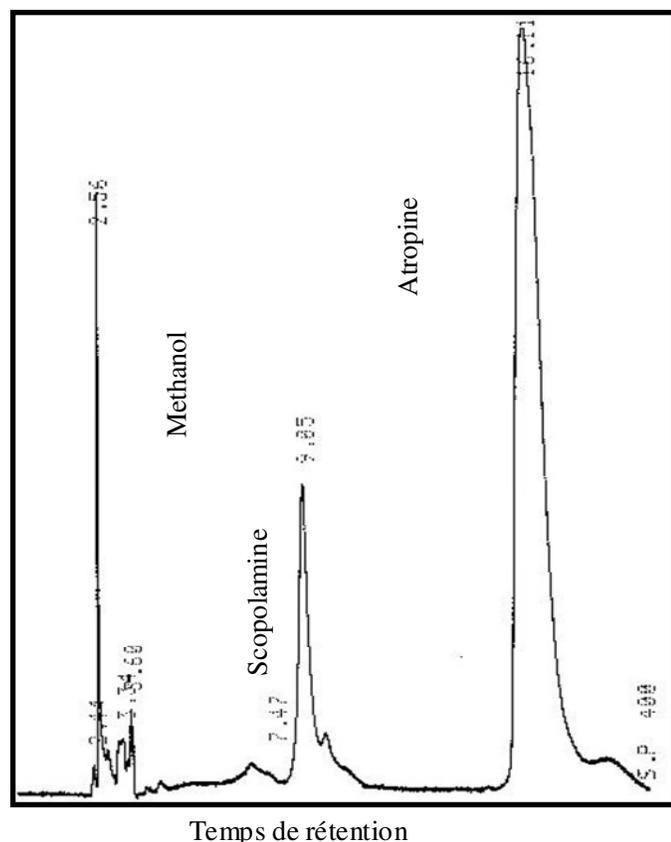


Figure 4. HPLC chromatogram of the total alkaloids from the seeds of *Datura stramonium*. Varian/ Chrompack column C₁₈, the mobile phase, 85% phosphate buffer, 15% acetonitrile, flow rate 1 ml/ min, pressure 140 bar, 35°C temperature, wavelength 210 nm.

liver of the treated group were significantly less than that of the control group (Table 7). The analysis of haematological profile revealed a significant decrease in the RBC, HCT, HBG and WBC. Blood chemistry values of rats are shown in Figure 9. GOT, GPT were significantly increased when compared with those of the control group. In the liver of the alkaloids exposed rats, we observed manifold centrolobular necrotic areas, hyperplasia of hepatocytes, as well as cytoplasmic dystrophy characterized by vacuoles tightly packed in the cytoplasm. Additionally blood congestion and sharply dilated central veins were noted.

DISCUSSION

The D.S has been used in folklore and traditional systems of medicine, as a cure for the asthma (Pretorius and Marx, 2006), stimulation of central nervous system, treatment of dental and skin infections (Gidado et al., 2007). D.S (called Jimson weed) has been reported as a drug of abuse (Aroukou et al., 2003; Chan, 2002; Birmes

et al., 2002). D.S is known to contain highly toxic tropane alkaloids, including the pharmacologically active compounds atropine and scopolamine (Dugan et al., 1989; Desachy et al., 1997). Tropane alkaloids are easily separated and detected TLC (Steenkamp et al., 2004; Bruneton, 1999; Papadoyannis, 1995). HPLC still remains as one of the most commonly used techniques to analyze for alkaloids (Steenkamp et al., 2004).

The use of TLC allowed separating and identifying the two majors' alkaloids atropine and scopolamine in total alkaloids. HPLC enabled to evaluate the content of the two mains alkaloids. The atropine concentration is higher than that of scopolamine. The separation and values of scopolamine and atropine were in agreement with the ranges previously reported in available literature (Friedman, 2004; Miraldi et al., 2001). The toxic effects of a test substance can be basically terminated by physical examination, daily observation, visual examination, measure of food and water consumption, body and organ weight, haematology, urinalysis, biochemical organ function tests and pathology studies (Maruo et al., 2003). The liver, know to be key organ in the metabolism and detoxification of xenobiotics, is vulnerable to damage induced by a huge variety of chemicals (Udem et al., 2009).

An obvious sign of hepatic injury is leakage of cellular enzyme into plasma. When the liver cell membrane is damaged, a variety of enzymes normally located in the cytosol are released into blood stream. The estimation of the GPT (glutamic-pyruvic transaminase) and GOT (glutamic-oxaloacetic transaminase) in the serum is a useful quantitative marker for the extent and type of hepatocellular damage (Udem et al., 2009; Kumar et al., 2004). An increase in the level ALP (alkaline phosphatase) is an indication of biliary obstruction (Udem et al., 2009; Kaneko et al., 1997). The group treated with 100 mg/kg total alkaloids did not show a change in the levels of these enzymes. Microscopic examinations of treated-groups show no histopathological changes. It is likely that rat quickly eliminates the alkaloids of *D. stramonium*.

In the subchronic toxicity study in males rats given the alkaloids intraperitoneally at doses. Since, the changes in body weight seen was used as an indicator of adverse effects of drugs and chemicals (El Hilaly et al., 2004) there was no change weight gains were not significantly different in the treated rats as compared to the controls. This observation of body weight is in agreement to the earlier reports of Gidado et al. (2007) and Crawford and Friedman (1990) who have noted no change in body weight of seeds *D. stramonium* treated rat. With the exception of an increase in the relative lung weight, there is no significant change in other relative organ weight.

Alterations in serum constituents were clearly evident in the treated animals; they included increased serum ALP, GOT and GPT. GOT and GPT are elevated following tissue damage in which cellular enzymes are released

Table 1. Effect of Acute administration of total alkaloids of seeds of *Datura stramonium* (100 mg/kg) on body of male rats. Values are mean \pm S.D.

Group	1st day	5th day	Difference
Group 1st day	208.1 \pm 37		
Group 5th day	210.1 \pm 10.81	235.2 \pm 8.43	25.1 \pm 2.83*
Control	239.8 \pm 22.58	254.5 \pm 15.39	25.1 \pm 2.83*

Table 2. Relative organ weights of male rats treated with 100 mg/kg of seed's total alkaloids of *Datura stramonium*. Values are mean \pm S.D.

Group	Liver	Brain	Kidney	Lungs	Heart	Spleen	Testes
Control	0.0399 \pm 0.0024	0.0089 \pm 0.0011	0.007 \pm 0.0007	0.0081 \pm 0.0022	0.0038 \pm 0.0004	0.0045 \pm 0.0007	0.0087 \pm 0.0021
Group 1st day	0.0358 \pm 0.00421	0.0076 \pm 0.0006*	0.0069 \pm 0.0075	0.0093 \pm 0.0041	0.0038 \pm 0.0003	0.0038 \pm 0.0003*	0.0073 \pm 0.0022
Group 5th day	0.0398 \pm 0.00371	0.0089 \pm 0.001	0.0075 \pm 0.0025	0.008 \pm 0.0013	0.0039 \pm 0.0005	0.0056 \pm 0.0016*	0.0106 \pm 0.0020

Table 3. Effect of Acute administration of total alkaloids of seeds of *Datura stramonium* (100 mg/kg) on some haematological parameters in male rats. Values are mean \pm S.D.

Group	RBC 10 ⁶ /mm ³	MCV	RDW	HCT%	PLT 10 ⁶ /mm ³	MPV	WBC 10 ⁶ /mm ³	HGB g/l
Control	7.83 \pm 0.56	52.75 \pm 3.23	16.11 \pm 1.86	41.26 \pm 2.30	431 \pm 73.92	6.6 \pm 0.31	8.56 \pm 2.69	13 \pm 0.8
Group 1st day	8.46 \pm 0.56*	52.09 \pm 0.98	14.76 \pm 1.14	44.67 \pm 2.29*	487.88 \pm 85.59	7.22 \pm 0.14*	10.51 \pm 1.42	14.14 \pm 0.63*
Group 5th day	8.47 \pm 0.66*	53.77 \pm 2.69	16.13 \pm 0.95	46 \pm 2.51*	433 \pm 73.29	6.81 \pm 0.23	10 \pm 2.40	14.1 \pm 0.44*

from cells into the bloodstream. GOT is found in high constitutive levels in the heart and liver, whereas GPT is most active in the liver. Serum ALP is found in most tissues, including bone, liver and kidney. Elevations are seen following eating and osteoblastic activity, impairment of liver function, and obstruction of bile flow, depressions are seen in malnutrition (Udem et al., 2009; Maruo et al., 2003). The results of the present study show alterations in haematological parameters associated with the subchronic and intra peritoneal exposure to alkaloids (atropine and scopolamine) in rats. Although the exact moment

in which these alterations took place was not established; it was observed that a decreased number of red blood cells (RBC), haemoglobin concentrations and hematocrit value after 4 weeks of alkaloids exposure. These haematological alterations suggest possible dehydration (Dugan et al., 1989). This is consistent with the known pharmacological action of atropine in producing thirst and dryness of the mouth (Dugan et al., 1989). Also, there may be a relationship between RBC, HCT HBC and increased activity transaminases especially the ALT. In the 120 day chronic experiment the rats treated with synthetic

alkaloids, did not show reduction in body – weight gain but showed a decrease in the relative weight of liver. This observation on growth body and relative weight organ is not consistent with studies by Dugan et al. (1989) in which the male rats fed Jimson weed at 0.5% in the diet decreased body weight and increased the relative weight of liver. The toxicity of seeds of *D. stramonium* may be due to alkaloids but also to other components present in the seeds of plant. This chronic experiment produced high levels of transaminases. This is an indication of organ damage (El Hilaly et al., 2004). The mechanism of

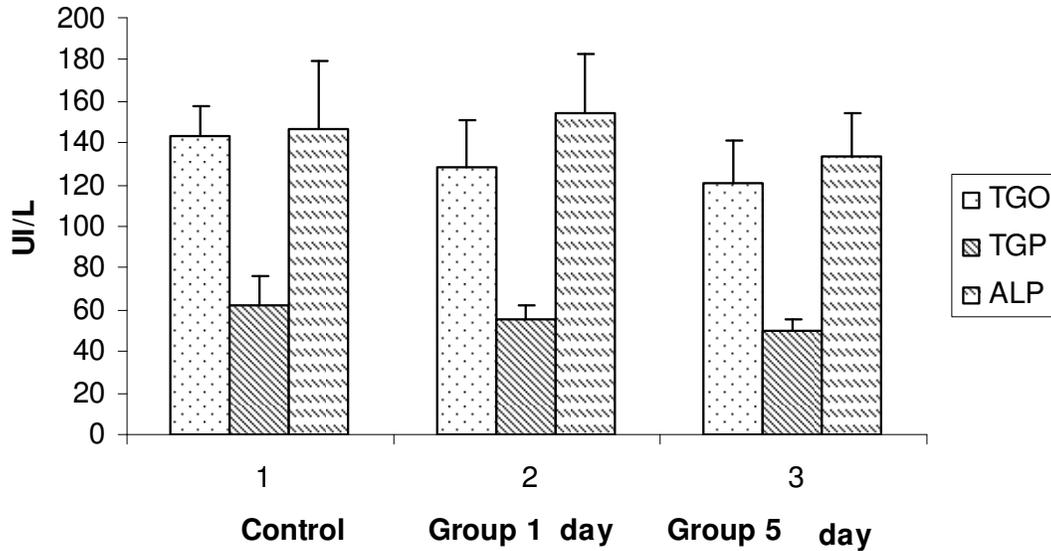


Figure 5. Effect of Acute administration of total alkaloids of seeds of *Datura stramonium* (100 mg/kg) on some biochemical parameters in male rats. Values are mean \pm S.D.

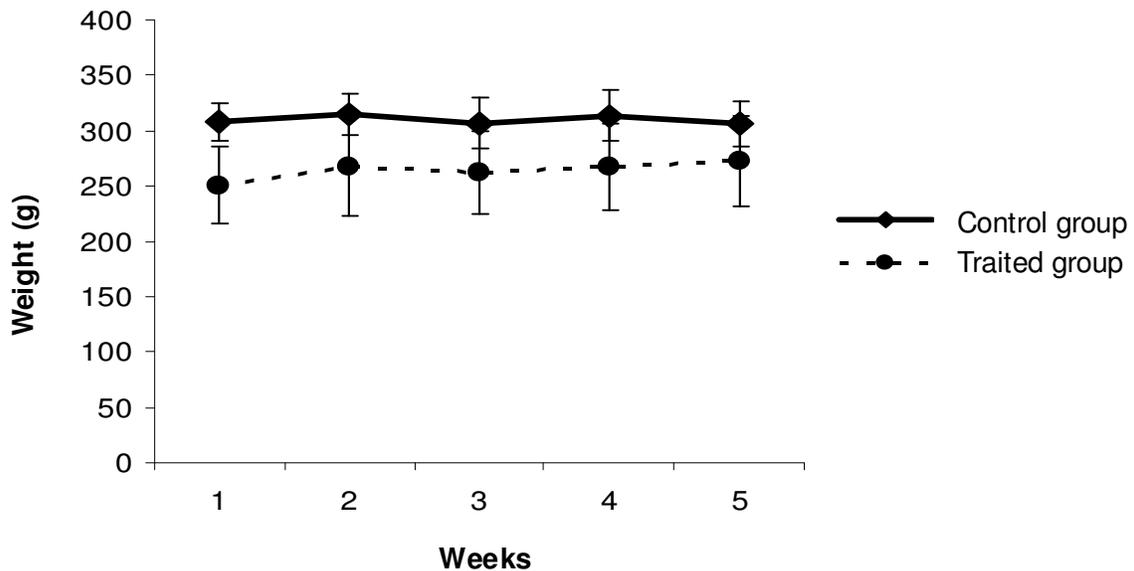


Figure 6. Effect of subchronic administration of synthetic tropan alkaloids (atropine and scopolamine for 4 weeks) on body weight of male rats. Values are mean \pm S.D.

this damage was not investigated. There may be a relationship between RBC, HCT HBC and increased activity transaminases especially the GPT. The lower of RBC may be attributed to the increase in GPT. Increased GPT is indicative of liver damage. If that is the case, serum protein synthesis in the liver will be suppressed and the bone marrow may not have enough proteins to synthesize RBC. Also, these haematological alterations suggest possible dehydration (Dugan et al., 1989). Ours

resultants show that the alkaloids (atropine and scopolamine) are able to produce alterations on erythrocyte parameters after a long period of exposure.

The increase or decrease in relative organ weights results when organ weights change without change in body weights and are often indicative of underlying disease or damage of such organs (Udem et al., 2009). The significant change in the relative organ weight in association with the increased activities of the

Table 4. Effect of subchronic administration of synthetic tropan alkaloids (atropine and scopolamine) on some Haematological parameters in male rats. Values are mean ± S.D.

Group	RBC 10 ⁶ /mm ³	MCV	RDW	HCT%	PLT 10 ⁶ /mm ³	MPV	WBC 10 ⁶ /m ³	HGB g/l	MCH	MCHC
Control	8.96±0.52	52.58±1.95	16.01±1.15	47.17±2.17	460.75±129.81	7.05±0.37	12.57±2.74	15.77±0.62	17.4±0.59	33.67±1.97
Group treated for 4 weeks	8.15±0.38*	53.73±2.8	14.76±1.14	42.34±2.90*	448.4±102.29*	7.02±0.30	11.92±2.48	14.72±0.40*	17.4±0.65	33.36±0.65

Table 5. Effect of subchronic administration of synthetic alkaloids (atropine and scopolamine) on relative organ weights of male rats. Values are mean ± S.D.

Group	Liver	Brain	Kidney	Lungs	Heart	Spleen	Testes
Control	0.033±0.0041	0.035±0.0042	0.0067±0.0009	0.0077±0.0012	0.003±0.0003	0.0026±0.0003	0.01±0.001
Group treated for 4 weeks	0.035±0.0042	0.012±0.0018	0.0065±0.0006	0.01±0.002	0.003±0.0003	0.026±0.0003	0.011±0.001

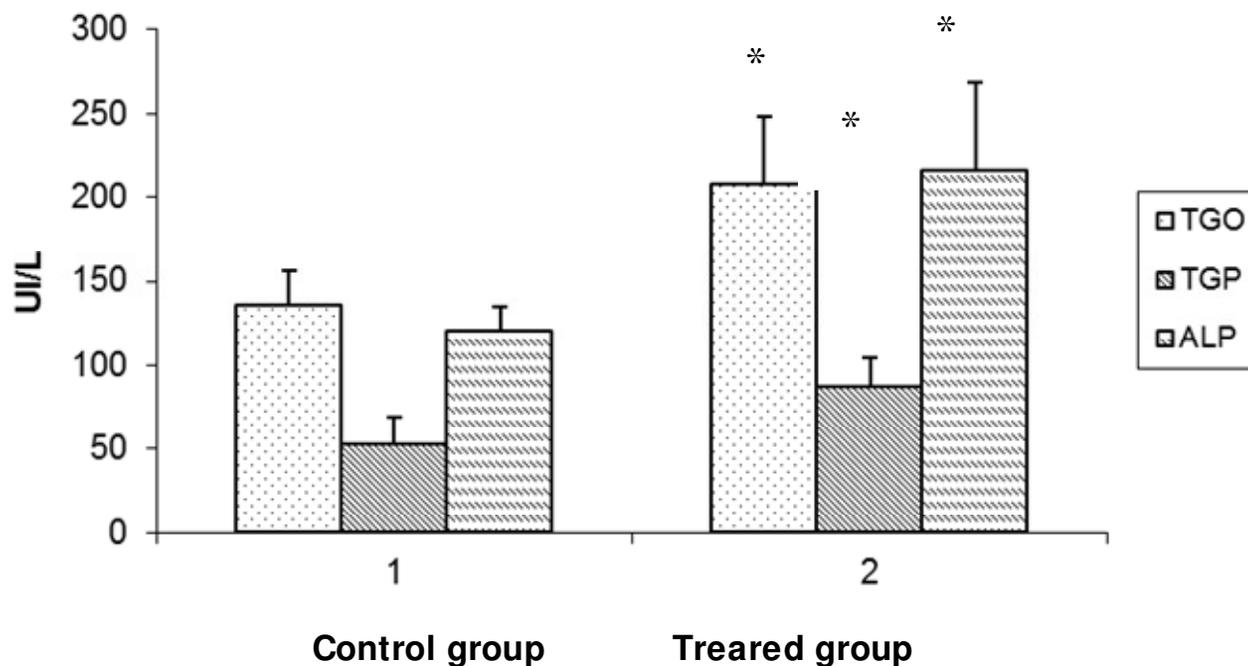


Figure 7. Effect of subchronic administration of synthetic alkaloids (mg/kg atropine and mg/kg scopolamine) on some biochemical parameters in male rats. Values are mean ± S.D.

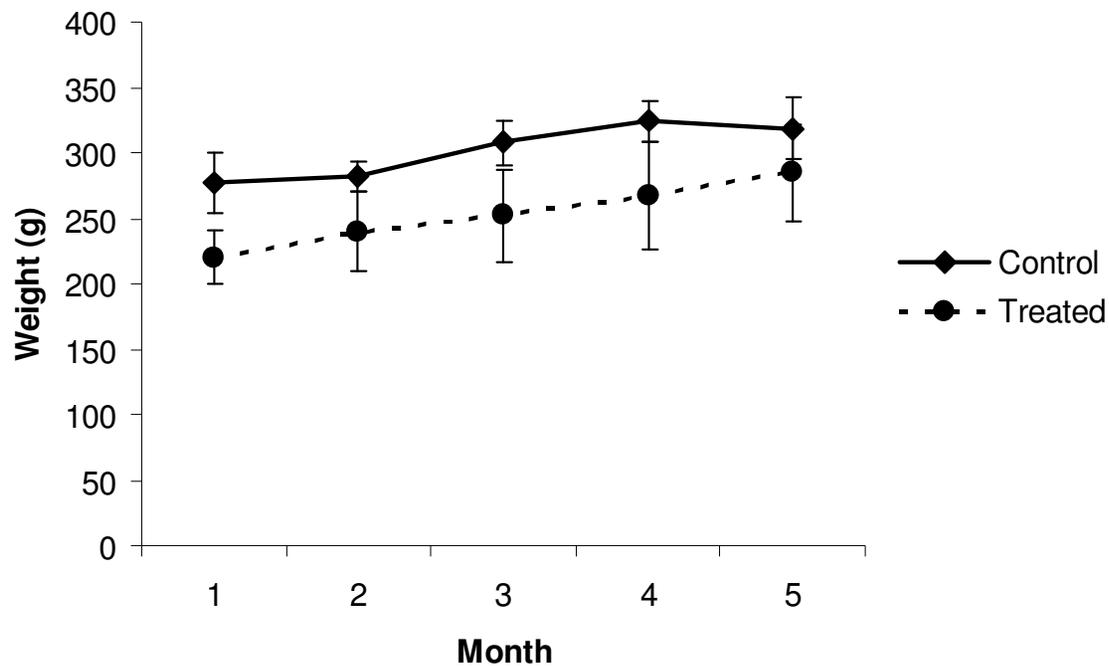


Figure 8. Effect of chronic administration of synthetic tropan alkaloids (atropine and scopolamine for 4 months) on body weight of male rats. Values are mean \pm S.D.

Table 6. Effect of chronic administration of synthetic tropane alkaloids (atropine and scopolamine) on relative organ weights of male rats. Values are mean \pm S.D.

Group	Liver	Brain	Kidney	Lungs	Heart	Spleen	Testes
Control	0.0347 \pm 0.0031	0.0061 \pm 0.0007	0.0072 \pm 0.0005	0.0064 \pm 0.0003	0.0035 \pm 0.0008	0.0030 \pm 0.0005	0.009 \pm 0.0013
Group treated for 4 weeks	0.0315 \pm 0.0037*	0.0067 \pm 0.0009	0.0062 \pm 0.0011	0.0074 \pm 0.0017	0.0034 \pm 0.0006	0.0034 \pm 0.0006	0.00913 \pm 0.001

Table 7. Effect of chronic administration of synthetic tropan alkaloids (atropine and scopolamine) on some biochemical parameters in male rats. Values are mean \pm S.D.

Group	RBC 10 ⁶ /mm ³	MCV	RDW	HCT%	PLT 10 ⁶ /mm ³	MPV	WBC 10 ⁶ /mm ³	HGB g/l	MCH	MCHC
Control	9.42 \pm 0.46	49.54 \pm 1.75	15.13 \pm 0.45	46.7 \pm 2.42	676.77 \pm 89.62	6.86 \pm 0.16	10.12 \pm 1.87	15.82 \pm 0.58	16.66 \pm 0.55	33.65 \pm 0.49
Group treated for 4 weeks	7.89 \pm 0.52*	50.24 \pm 1.65	14.22 \pm 0.89	38.63 \pm 3.27*	593.18 \pm 144.56	6.76 \pm 0.262	6.6 \pm 2.92*	13.62 \pm 0.38*	17.03 \pm 0.76*	34.3 \pm 0.68

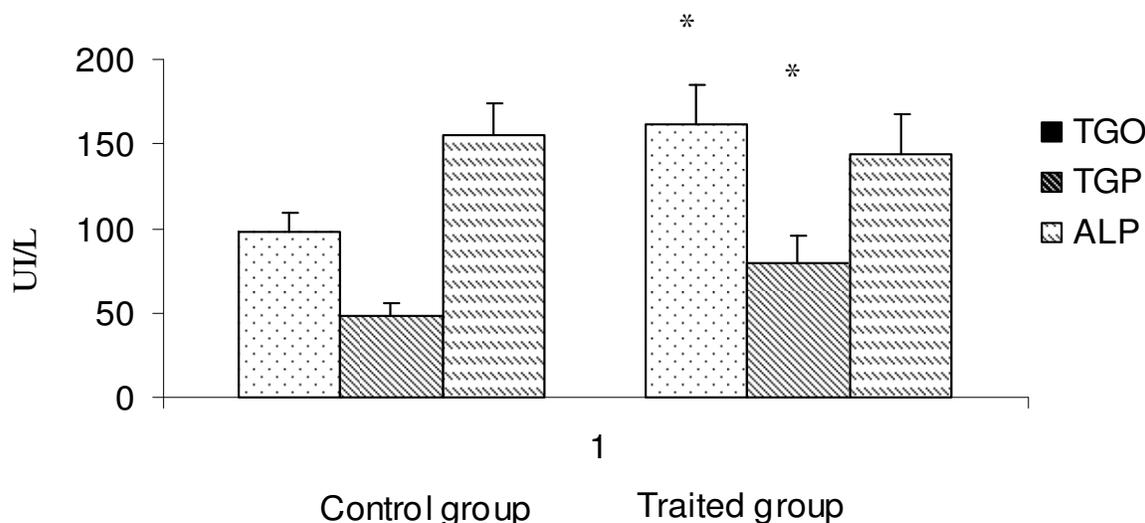


Figure 9. Effect of chronic administration of synthetic alkaloids (atropine and scopolamine) on some biochemical parameters in male rats. Values are mean \pm S.D.

transaminases indicate organ damage, especially that of liver. This is not a good sign changes in the relative organ weight of the liver may affect the metabolic processes such as detoxification, biotransformation, and synthesis of serum proteins that take place in the liver.

The histopathological changes observed in the liver were characteristic of circulatory disturbances and necrotic changes. This is in line with the observations made in the blood biochemistry. The severity of the changes increased with increased of duration of the treatment, suggesting a cumulative effect of the alkaloids. The liver showed necrosis in the hepatocytes. For a fair assessment of the toxicity of alkaloids from *D. stramonium*, it is useful to study the effect of these alkaloids on the kidney and especially the brain in rats of both sexes.

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