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Research Article

Evaluation of Acute and Sub-Acute Toxicity of Alkaloids from *Datura stramonium* Sp. in Mice

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ABSTRACT

Datura stramonium L. or jimson weed is a wild shrub which belongs to Solanaceae family. It contains a number of toxic alkaloids such as atropine, hyoscamine and scopolamine. The objective of this study was to evaluate the acute and sub-acute toxicity of alkaloids extracted from the seed of *Datura stramonium* sp. In mice. Biochemical, hemodynamic, histological parameters were investigated. Experiments on acute toxicity on alkaloids, injected intraperitoneally, showed that these substances are moderately toxic (lethal dose 50 %: 303.4 mg/kg). The resulting intoxication triggered tachycardia, difficulty in breathing, convulsions and a decrease in locomotor activity in mice. The acute treatment for 24 hours and 5 days at a dose of 1/5 LD_{50}, *i.e*., 60 mg/kg of alkaloids, did not show any difference in body weight. There was an increase in heart beat and respiratory cycle on day 1 in injected mice compared to control animals. These parameters became normal after 5 days of treatment. The hematological observations show that alkaloids at a dose of 60mg/kg induced a significant decline in the number of red blood cells (RBC), hemoglobin and hematocrit. The plasma AST, ALT, LDH, K^+, urea and creatinine levels were significantly affected after alkaloid administration. The sub-acute dose (16 mg/kg) after 28 days resulted in a significant increase in AST, ALT, GGT, PLA, Na^+ and K^+ concentrations accompanied by a significant decrease in RBC, hemoglobin, hematocrit and platelets levels. Heart beat and respiratory cycle also increased in treated-animals compared to the controls. Histopathological examination revealed high degree of vacuolization and inflammation in liver and the same up to lesser extent in kidney. In conclusion, the *Datura stramonium. sp* alkaloids modulate biochemical, hematological and histological parameters in mice.

Keywords: Toxicity, *Datura stramonium*, tropane alkaloids, Histopathological, hematological parameters, atropine.

INTRODUCTION

Datura stramonium L. is a hallucinogenic plant[1,2] widely found in urban and rural areas[3]. It is characterized by tubular white or lavender flowers and fruits consisting of spiny egg-shaped capsules[4]. *Datura stramonium* (DS) is also known as Thorn Apple or Jimson Weed. It is an alkaloid-containing plant that is entirely toxic[5,6]. The toxic compounds found in all parts of the plant, especially in the seeds, are tropane alkaloids which possess strong anticholinergic properties[7,8]. These alkaloids include, hyoscyamine (stems, leaves, roots, seeds), hyoscine (roots); atropine (*d, l*-hyoscyamine) and scopolamine (*l*-hyoscine)[9]. Atropine and scopolamine are muscarinic antagonists that cause anticholinergic syndrome, which is characterized by ocular effects (mydriasis and paralysis of accommodation), dryness of the mucosal lining (which affects the mouth, sinuses, etc.), mental disorders (excitation, agitation, confusion, delirium, hallucinations, etc.), neurological effects (colonic spasms and seizures), hyperventilation, hyperthermia, sinus tachycardia, and disordered heart rhythm[10,11]. 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. The toxicity of *D. stramonium* in grazing animals have been suspected by livestock owners and field veterinarians especially at time of drought or after ingesting freshly harvested maize that will be used for ensiling and heavily contaminated with young *D. stramonium*[12]. *Datura stramonium* is a natural source of antioxidants and phytochemical having antimicrobial activities. Its extracts show significant antimicrobial activity against *Staphylococcus aureus*, *Proteusvulgaris*, *Pseudomonasaeruginosa*, *Escherichia coli*, *Aspergillus niger* and *Fusariumspecies*[13]. The objective of this study is to evaluate the acute and sub-acute toxicity of the seeds extract (total alkaloids) of *Datura stramonium*on liver and kidney in mice.

MATERIALS AND METHODS

Plant material

Datura stramonium seeds were collected during September and October 2014, from Belimour, district Bordj Bouarreridj province in the northeast of Algeria. The climate in the region is characterized as semi-arid. The mean annual temperature is 14.3 °C, and the average

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annual rainfall is 351mm. The plant has been identified based on morphological aspects. Seeds were separated manually from the fruits and then minced with electrical grinder (Muleinex) into a powder and finally stored in airtight containers prior to use. (See Fig. 1, Fig. 2).

Extract preparation

The air-dried powder of seeds (100g) was defatted with petroleum ether under reflux conditions. Defatted dry powder was alkalized with 40 ml for ammoniac (0.5 N) for 8hours at room temperature, and was extracted to exhaustion with dichloromethane (250 ml) using a soxhlet apparatus for 6 h. The organic extract is then shaken three times with 150 ml aqueous sulphuric acid (0.5 N), and then the aqueous layer was adjusted to pH10 with concentrated ammonium cloride. The aqueous basic solution was extracted three times with chloroform. The combined chloroform extract was dried over sodium sulphate and evaporated to give the crude alkaloid mixture.

Experimental animals

Experiments were performed on adult male Swiss albino mice **(**8 weeks of age**)**, weighing 25 to 30 g. The animals obtained from Institut Pasteur d'Algérie, Algiers. Mice were placed in metallic cages (53 × 32× 19 cm) in the animal house of Faculty of Sciences University Ferhat Abbas, Setif, Algeria and acclimatized for 2 weeks prior to conduct the experiments. They were provided with standard pellet diet and water *ad libitum.* All animals were kept in standard environmental conditions.

Toxicological studies in mice

Determination of LD_{50}

The total alkaloids, extracted from the seeds of *Datura stramonium*, were dissolved in 600 µl of ethanol and diluted by normal saline solution (v/v 0.9%) before administration. Animals were deprived of food for 24 hours prior to administration of alkaloids and randomly divided into five groups plant was studied by preparing 5 different concentrations of alkaloids 297.5, 300, 302.5, 305, and 307.5 mg/kg and administered in traperitoneally (single administration) to five groups of animals. The mice were individually observed for the first 30 min after treatment, periodically observed for the next 24 h (with special attention given during the first 4 h), and observed also daily for a period of 14 days[14]. The number of deaths within this period was recorded. The LD_{50} was determined according to probit method (method of least squares) using the [Software Stat Plus] (Ver. 2015 Build 5.9.8.5 ©2015).

Acute toxicity

The mice were divided at random, into 3 groups, each of them consisted of 10 mice. The first group was administered with normal saline and considered as a control. The second and third groups were administered a single intraperitoneal dose of 60 mg/kg (≈ 1/5 LD_{50}) of alkaloids. The mice were killed after 24 hours of administrated on 5th day of the treatment. An acute poisoning in humans by *D. stramonium* needs hospitalization and the recovery takes between 1-5 days[15]. At the end of all experimental periods, all treated animals were fasted overnight, but allowed access to water and sacrificed by decapitation under anesthesia. Blood samples were collected in two tubes: tube 1 containing EDTA was processed immediately for hematological parameters analysis; tube 2 containing heparin was centrifuged at 4000 r/min at 4 °C to obtain serum (stored at -20°C until analysis). Various tissues (kidneys, liver, heart and spleen) were dissected and weighed, and wet sections from the liver and kidneys were examined histopathologically. Paraffin sections of liver and kidney were made and stained with hematoxylin/eosin then microscopic evaluation was carried out in the laboratory of Anatomo-pathology of Bordj Bouarreridj.

Subacute toxicity

Two groups of 10 mice were used. The first group was given normal saline and taken as a control. The second group was given a single intraperitoneal dose of 16 mg/kg (≈ 1/20LD_{50}) of alkaloids extracted from the seeds of *Datura stramonium* once daily for 28 days. During the period of administration, the animals were weighed and observed daily to detect any signs of toxicity. After 28 days, all surviving animals were investigated in the same way that was used in the acute toxicity study.

Statistical analysis

All data were expressed as mean ± standard error of mean (SEM), comparison was performed by the student's *T*-test using Graphpad Prism version5.00. Values of $p < 0.05$ were considered statistically significant. LD_{50} is determined using stat PLUS 5.9.8.5 (2015).

RESULTS

Median lethal dose

The administration of alkaloids, extracted from the seeds of *Datura stramonium*, triggered severe clinical symptoms, including tachycardia, convulsions and difficulty in breathing. Besides, we also observed a decrease in locomotor activity of mice. Though there were deaths 15th minute to 24 hours; however, the animals which survived exhibited a normal behavior, similar to the animals of the control group. The toxicity was observed to be a dose-dependent phenomenon. The LD_{50} value of the extracts was calculated to be 303.4 mg/kg (See Fig. 3).

Acute toxicity

No deaths were attributable to treatment. The changes in the physiological parameters like body temperature, heart and respiratory rates are presented on Table 1. In general, no significant differences were observed in body weight between treated and control. Moreover, no morphological changes were observed in the organs of treated animals. The effects of alkaloids of *D. stramonium* seeds on the weight of different organs (liver, kidney, heart and spleen) are presented in Table 2. There were statistically significant decreases in the weight of kidney and spleen of the treated mice of the first group (sacrificed after 24 h). However, the group sacrificed after 5 days of treatment exhibited a significant reduction in the weights of kidney, liver, heart and spleen compared to control group. The hematological parameters of the treated and control groups are presented in Table 3. There were no remarkable differences in hematological parameters after 24h, nonetheless, after 5 days of the treatment with alkaloids, the RBC, HCT, Hb markedly decreased. The values for the biochemical parameters in treated and control mice are

Figure 1: Plant of *Datura stramonium.*

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

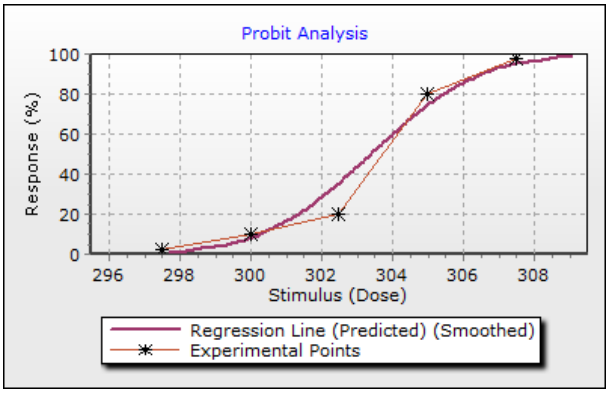

Figure 3: Median lethal dose (LD50) of total alkaloids of *Datura stramonium* seeds in mice (probit method: Least squares [Normal Distribution]), LD_{50} = 303.40 mg/kg

presented in Table Table 4. We notice an increase in urea, AST, ALP, and LDH and a significant decrease in K level, after 24h and 5 days of treatment, compared to control. Datura alkaloids also increased the concentrations of creatinine after 24 h of treatment.

Sub-acute toxicity

No deaths were observed after 28 days of treatment with Datura alkaloids. The changes in the physiological parameters like body temperature, heart and respiratory rates are presented on Table 5. The changes in organ weights in 28 days-treated mice are shown in Table 6. There was no significant ($p>0.05$) differences in the weight of heart, spleen and kidney of treated and control rats, although, the liver weight of the extract-treated mice was lower than the control. The hematological parameters of the treated and control groups are shown in Table 7. The RBC, HCT, HGB and platelets were lower in the alkaloid-treated group than control group. The values for the biochemical parameters in treated and control mice are presented in Table 8. Significant increase was noticed in the levels of AST, ALT, GGT, ALP, Na, and K in treated animals, as compared to the respective controls.

Histopathological examination

The examination of kidneys revealed the presence of a vascular congestion to proximity of vessels in acute and subacute intraperitoneal administration of alkaloids. Histological examination of the liver of treated animals in acute toxicity (group sacrifed after 24h) conditions showed moderate congestion, the severity of the lesions progressed with the progression of the study period, section in the liver in treated mice (after 5 and 28 days) showed severe congestion and hepatocyte degeneration (See Fig. 4 and 5).

DISCUSSION

All parts of *Datura stramonium* are very noxious to health. Its intake in excess may be fatal if ingested by humans or animals. The toxins in jimsonweed are tropane belladonna alkaloids which possess strong anticholinergic properties[16]. The intraperitoneal LD_{50} value of total alkaloids was 303.4 mg/kg. In view of the results of the LD50 ($50<LD_{50}<500$ mg/kg), according to Hodge and and Sterner (1949)[17], total alkaloids of *Datura stramonium* can be classified in the category of moderately toxic products. The clinical picture of male mice treated with alkaloids was characterized by relatively rapid appearance of symptoms, including tachycardia, they increase heart rate through their effect on the parasympathetic nervous system by blocking vagal stimulation[9], severe agitation and convulsions by reaching the central nervous system. The acute treatment for 1and 5 days at a dose of 60 mg/kg of total alkaloids did not show any difference in body weight. Organ weights have been used as sensitive indicator to evaluate the toxic effects of drugs in toxicological studies[18]. Relative kidney and spleen weight (After 1 and 5 days) and relative liver and heart (after 5 days) height were significantly decreased in response to alkaloids of jimson weed seeds. Heart and respiratory rates were accelerated on day 1compared to the control values, the increased heart rate is due to its effects on the parasympathetic component of vagal block[19]. The hematopoietic system is one of the most sensitive target for toxic compounds, and an important index of physiological and pathological status in human and animals[20]. In this study, the results on the hematological parameters showed a significant decrease in RBC, hemoglobin and hematocrit in the treated groups (scarified after 5 days) as compared to the control group, this result can probably be explained by the effect of total alkaloids on the erythropoiesis and the destruction of cells. The evaluation of serum biochemical parameters has significant importance on toxicological changes[21]. Liver is the major site for metabolism including drugs[22]. When the liver cell membrane is damaged, a variety of enzymes normally located in the cytosol are released into blood stream. The transaminases (AST and

Table 1: Change in clinical indices in mice intraperitoneally treated with 60 mg/kg (≈ 1/5 DL_{50}) of total alkaloids of *Datura stramonium* seeds.

Indices	Days after treatment		
	Group Control	Group 1st day	Group 5th day
Body temperature (C°)	37.09 ± 0.30	37.26± 0.39	37.45± 0.12
Heart rate (min-1)	388.9± 14.42	479.85 ± 28.85 *	388.8 ± 12.73
Respiratory rate (min-1)	172.1 ± 2.45	190.6 ± 2.23*	172.2± 1.49

Values are Mean±SEM, * Significantly different at P< 0.05.

Table 2: Relative weight of different organs of mice treated with 60 mg/kg (≈ 1/5 DL_{50}) of total alkaloids of *Datura stramonium* seeds.

Organ	Control	Group 1st day	Group 5th day
Liver	0.0423 ± 0.0011	0.0423 ± 0.022	0.0360 ± 0.0020 *
Kidney	0.0130 ± 0.00053	0.0108 ± 0.000340*	0.00918 ± 0.00164 *
Heart	0.0063 ± 0.00026	0.00457 ± 0.000202	0.00475 ± 0.000313*
Spleen	0.00258 ± 0.00020	0.00151 ± 0.289*	0.00181 ± 0.000213 *

Values are Mean±SEM, * Significantly different at P< 0.05.

Table 3: Hematological parameters in mice treated with 60mg/kg(≈ 1/5LD_{50}) of total alkaloids of *Datura stramonium* seeds.

Hematological Parametres	Days after treatment		
	Control	Group 1st day	group 5 thday
RBC (10^6/ul)	8.71 ± 0.55	8.84 ± 0.29	7.75 ± 0.38*
WBC (10^3/ul)	4.42 ± 0.55	3.85 ± 0.48	3.01 ± 0.45
Hb (gr/ L)	11.48 ± 0.38	11.06 ± 0.28	9.60 ± 0.55*
HCT (gr/ L)	32.38 ± 0.91	33.06 ± 0.83	28.13 ± 1.68*
VGM (UI/L)	37.25 ± 0.76	37.13 ± 0.74	35.36 ± 0.83
TCMH (UI/L)	13.20 ± 0.46	12.49 ± 0.26	12.08 ± 0.28
Platelets (10^3/ul)	911.1 ± 89.24	821.6 ± 101.9	830.8 ± 89.71
Lymphocytes (%)	92.83 ± 1.25	93.96 ± 1.57	84.39 ± 2.88

Values are Mean±SEM, * Significantly different at P< 0.05.

Table 4: Serum biochemical parameters in male mice intraperitoneally treated with 60 mg/kg (≈ 1/5 DL50) of total alkaloids of *Datura stramonium* seeds.

Biochemical parametres	Days after treatment		
	Control	Group 1st day	Group 5th day
Urea (gr/ L)	0.64 ± 0.03	1.44 ± 0.12*	0.97 ± 0.12 *
ALP (gr/ L)	55.43 ± 4.33	68.40 ± 2.46*	59.00 ± 1.65*
AST (UI/L)	14.60 ± 1.63	22.46 ± 2.90*	24.06 ± 2.35*
ALT (UI/L)	49.63 ± 5.39*	50.00 ± 3.54	43.13± 2.02
Creat (mg/ L)	37.79 ± 1.50	43.21 ± 0.95*	38.50 ± 1.60
Na (mmol/l)	134.1 ± 1.95	135.8 ± 1.54	133.6± 1.11
K (mmol/l)	4.47 ± 0.34	3.53± 0.08*	3.38 ± 0.17*
Cl (mmol/l)	95.63 ± 5.14	99.63 ± 2.14	102.9 ± 1.80
GGT (UI/L)	32.43 ± 2.47	28.50 ± 1.56	35.83 ± 3.02
LDH (UI/L)	274.2 ± 17.05	356.9 ± 17.21*	357.6 ± 15.96*

Values are Mean±SEM, * Significantly different at P< 0.05.

Table 5: Change in clinical indices in mice intraperitoneally treated with 16 mg/kg (≈ 1/20 DL50) of total alkaloids of *Datura stramonium* seeds for28 days.

Indices	Group control	Group treated for 4 weeks
Body temperature (c)	36.73 ± 0.23	37.06± 0.17
Heart rate (min-1)	366.4± 46.67	475.3 ± .97*
Respiratory rate (min-1)	177.8 ± 3.35	201.6± 2.65 *

Values are Mean±SEM, * Significantly different at P< 0.05.

ALT) and PLA are well-known enzymes used as good indicators of liver function[23] and as biomarkers predicting possible toxicity[24,25]. Since AST found in the serum is of both mitochondrial and cytoplasmic origin and any rise can be considered as a first sign of cell damage that leads to the

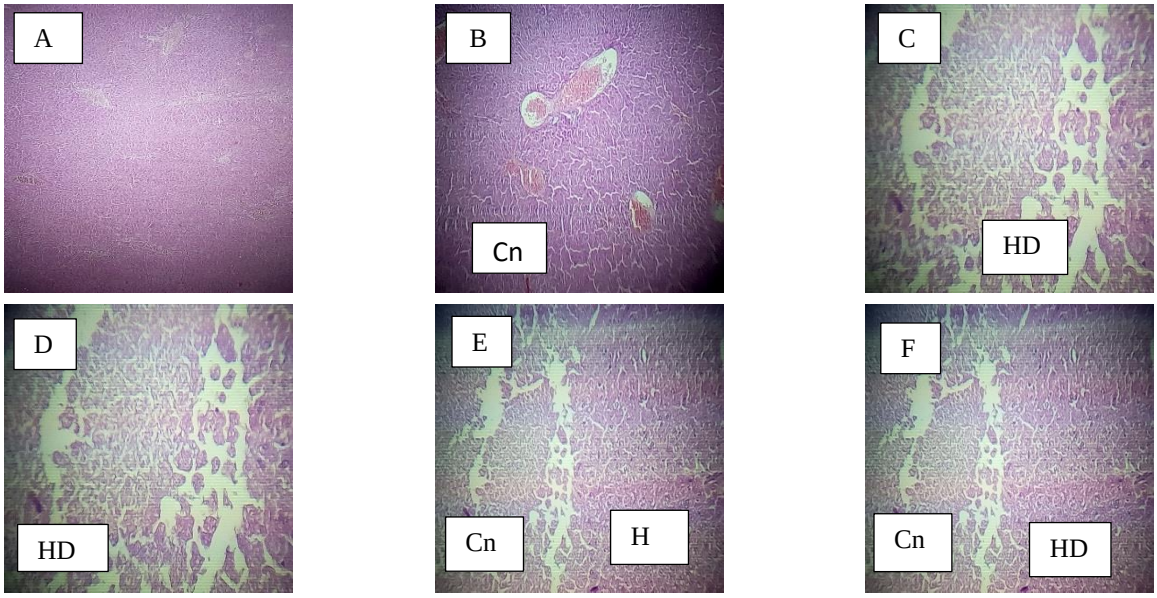

Figure 4: Hepatic histological sections of control animals (A), and mice treated with a dose of 60 mg/kg (≈ 1/5DL50) (B: After 24h, C and D After 5 days) and 16 mg/kg (≈ 1/20 DL50) (E, F: After 28 days) of total alkaloids of *Datura stramonium* (Hematoxylin/eosin stain; X 40). Cn: congestion, HD: Degeneration of hepatocytes.

Table 6: Relative organ weight of male mice treated with 16 mg/kg (≈ 1/20 DL50) of total alkaloids of *Datura stramonium* seeds for 28 days.

Organ	Control	Group treated for 4 weeks
Liver	0.0451± 0.0021	0.037 ± 0.0018 *
Kidney	0.011± 0.00063	0.0011± 0.00067
Heart	0.005± 0.00027	0.005± 0.00046
Spleen	0.002± 0.00019	0.002± 0.0001

Values are Mean±SEM, * Significantly different at P< 0.05.

Table 7: Hematological parameters for male mice intraperitoneally treated with 16mg/kg (≈ 1/20 DL50) of total alkaloids of *Datura stramonium* seeds for 28 days.

	Days after treatment	
Hematological parametres	Control	Group treated for 4 weeks
RBC (10^6/ul)	8.52 ± 0.27	6.44 ± 0.55 *
WBC (10^3/ul)	2.27± 0.30	2.48 ± 0.32
Hb (gr/ L)	10.20 ± 0.37	7.80 ± 0.63*
HCT (gr/ L)	30.15 ± 0.28	23.18 ± 1.85*
VGM (UI/L)	35.79 ± 0.29	32.36 ± 0.46
TCMH (UI/L)	12.39 ± 0.17	12.74 ± 0.45
Platelets (10^3/ul)	1012 ± 94.00	618.7 ± 55.54 *
Lymphocytes (%)	86.84 ± 1.36	85.63 ± 2.28

Values are Mean±SEM, * Significantly different at P< 0.05.

outflow of the enzymes into the serum[26]. LDH can be used to look at liver problems and also might indicate the presence of red cell hemolysis[23]. ALP is often employed to assess the integrity of the plasma membrane of the liver[27,28]. Results from these studies (acute toxicity) indicated a significant increase in AST, PLA and LDH in 60 mg/kg extract-treated animals (after 1 and 5 days). The increase in serum LDH and AST activity may indicate hepatic damage probably by the altered cell membrane permeability leading to the leakage of the enzymes from the tissues to the serum[29]. The elevation observed in liver enzymes (AST, PLA) in response to total alkaloids is in agreement with is in the work reported by Bouzidi *et al.* (2011)[30]. Histopathological result of liver sections confirmed these effects indicating severe congestion and hepatocyte degeneration. These results can be supported by the study of Raimundo (2006)[31]. The kidneys excrete metabolic waste products and regulate the serum concentration of a variety of substances. The urea and creatinine are important biomarkers of renal toxicity[32,33], and any rise in the levels of these parameters indicates a marked renal damage[34,35]. The results showed a decrease in serum levels of potassium (24h and 5 days). but an increase in the serum levels urea (24h and 5 days), and creatinine (group sacrifed after 24h) in the test group. These results suggest possible renal damage. Kidneys of treated mice in our study were clearly damaged and their

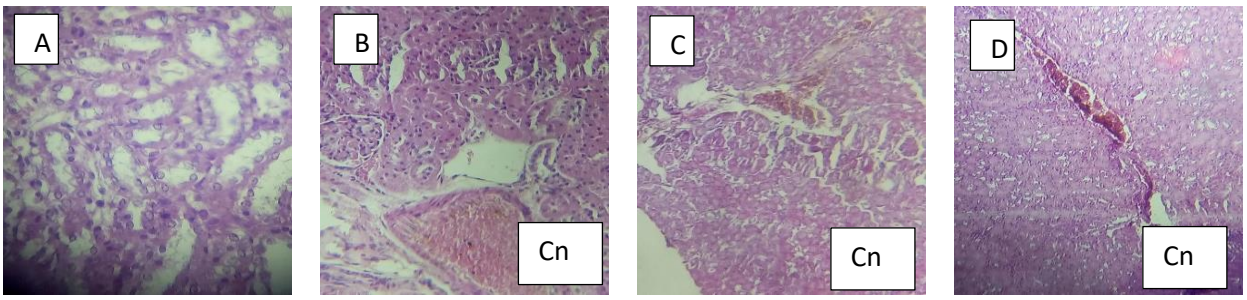

Figure 5: Renal Histological sections of control group (A) and treated-mice with a single dose of 60 mg/kg (≈ 1/5 DL50) (B: After 24h, C: After 5 days) and 16 mg (≈ 1/20 DL50) mg/kg (D: After 28 days) mg/kg of total alkaloids of *Datura stramonium* (Hematoxylin/eosin stain, X 10). Cn: Congestion.

Table 8: Serum biochemical parameters of mice intraperitoneally treated with 16mg/kg (≈ 1/20 DL50) of total alkaloids of *Datura stramonium* seeds For 28 days

Biochemical parametres	Days after treatment	
	Control	Group treated for 4 weeks
Urea (gr/ L)	1.08 ± 0.06	0.88 ± 0.05
ALP (gr/ L)	52.75 ± .49	64.40 ± 2.52*
AST (UI/L)	21.64 ± 2.11	29.12 ± 1.30*
ALT (UI/L)	36.83 ± 2.45	47.00 ± 2.89*
Creat (mg/ L)	35.75 ± 1.87	40.87 ± 2.45
Na (mmol/l)	136.4 ± 1.28	143.7 ± 1.49*
K (mmol/l)	4.01 ± 0.13	5.10 ± 0.36 *
Cl (mmol/l)	102.3 ± 1.78	101.5 ± 2.71
GGT (UI/L)	30.60 ± 2.22	38.00 ± 1.82*
LDH (UI/L)	275.3 ± 27.48	262.7 ± 24.15

Values are Mean±SEM, * Significantly different at P< 0.05.

histological aspects indicated a severe congestion.-In the sub-acute toxicity study, no mortality was recorded during 4 weeks of compounds administration in doses approximately 1/20 LD_{50}, i.e. hematological data obtained in our study indicated a significant decrease in RBC, hemoglobin; hematocrit and platelets in the treated groups, this is probably due to cell lyses and might explain the effect of total alkaloids on erythropoiesis.–Biochemical studies of plasma parameters of mice treated with total alkaloids caused an increase in plasma transaminases (AST, ALT), PLA and GGT after 28 days, and further explain a dysfunction of the liver. Histopathological observations of liver sections confirmed these effects, indicating important congestion and hepatocyte degeneration. In the present study, there was not any significant difference in urea and creatinine following treatment with total alkaloids but an increase was significantly observed in serum sodium ions with a concomitant significant increase in potassium ion levels, and this phenomenon may be due to renal dysfunction resulting from the inability of the kidney to regulate the electrolyte balance. Besides, histopathological examination also revealed a moderate congestion in kidneys.

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