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RESEARCH ARTICLE

Biochemical and Histopathological study of aqueous and methanolic extracts of *Datura innoxia* on Wistar rats.

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Abstract

Different parts of *Datura innoxia* have been identified traditionally to possess various medicinal uses in Sudan and Africa such as skin treatment eruptions, colds, nervous disorders, narcotic for surgical procedures, anti-spasmodic, anti-asthmatic, narcotic, anti-microbial agent and neuro-sedative. This study was done for evaluate the toxicity of methanolic and aqueous extraction of seeds and leaves of the Datura innoxia on Wistar rats by using two different doses in three weeks orally consumption. Rats were allotted at random to nine groups, each group contains 6 rats; group one served as control, group two and three treated with 100 and 300 mg kg^{-1} of methanol extraction of seeds, group four and five treated with 100 and 300 mg kg^{-1} of methanol extracted of leaves, group six and seven treated with 100 and 300 mg kg^{-1} of water extracted of seeds, group eight and nine treated with 100 and 300 mg kg^{-1} water extract of leaves. The results revealed significant decrease ($p \leq 0.05$) in body weight gain for rats in groups 3, 7 and 8 after the three weeks, while in the other groups there is no significant change in the body weight gain comparing to the control group and also it is clearly observed that an alteration in enzyme of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) activities. Changes in concentration of cholesterol, urea, serobiochemical parameters and pathological changes in vital organs were also observed. **The results** concluded that *Datura innoxia* is toxic causing due to alteration in various serobiochemical and hematological parameters, this toxicity correlated with dysfunction of vital organs (kidney, liver and brain) due to present of toxic material in plant studied.

1.0 INTRODUCTION

The uses of traditional medicines and medicinal plants in most developing countries for the treatment of various diseases have been widely observed [(1] The medicinal properties of plants could be based on the antioxidant, antimicrobial antipyretic effects of the phytochemical in them [2 and 3]. According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs [4]. Those plants are widely used by all sections of community, whether directly as folk remedies or the medicaments of the different indigenous systems as well as in modern medicine system[5, 6 and 7]. Datura innoxia (Family: Solanaceae, locally known as Elsakran) is used for many medicinal purposes. Datura precise and natural distribution is uncertain, owing to its extensive cultivation and naturalization throughout the temperate and tropical regions of the globe. In Sudan D. innoxia is widely spread in AL Jazeera State and other States. It is native to Central and South America, and introduced in Africa, Asia, Australia and Europe [8]. It contains atropane alkaloids such as scopolamine, hyoscyamine, hyoscine,

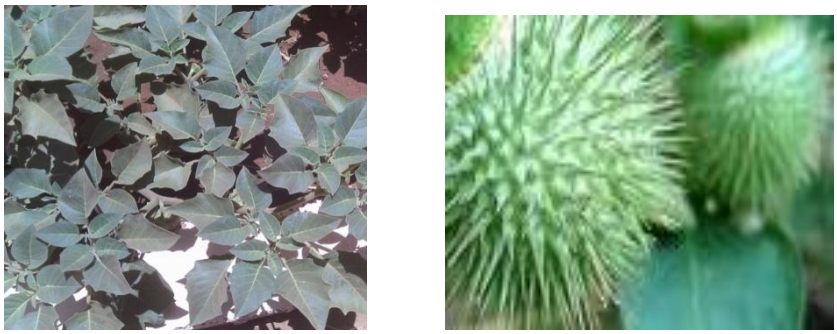
norscopolamine, meteloidine [9 and 10], flavonoids, cardiacs glycosides, Assential oils, saponins and phenols [11 and 12]. Traditional medicine uses flowers, leaves and seed of *D. innoxia* medically treat for skin eruptions, colds, and nervous disorders [13]. It has been used in the past as a antispasmodic, hallucinogenic, hypnotic and narcotic and also in the treatment of insanity, impotence, asthma, diarrhea, as an analgesic, to control fever, kill parasites, and skin diseases [14].

The objectives present study were investigated the toxic effect of seeds and leaves of *D. innoxia* by using two extraction (methanol and water) on body weight gain, hematology, enzyme activities(AST, ALT and ALP) in Wister rats.

2.0 MATERIALS AND METHODS

2.1 Materials

D. innoxia (Fig. 1) was collected from AL Jazeera State, Sudan in February, 2012. The plant tissues were cleaned, shade-dried and ground by a mechanical grinder and the methanol and aqueous extracts were prepared and used in this study.



(a) (b)

Fig. 1. *Datura innoxia* leaves (a) and fruits (b)

Fifty four both sexes Wister rats, 3 month old and of (100-110g) average body weight ranged, were used in the present study. The rats were clinically healthy and housed within the premises of the Faculty of Science and Technology, Al-Neelain University, Khartoum.

Animal house under standard husbandry conditions (30°C ± 2°, 60–70% relative humidity and 12h: 12h day-night cycle) and fed on the rat diet (flour 55.6%, meat 35%, edible oil 7.5%, sodium chloride 1.2% and vitamins and minerals 0.7) and water provided ad. Libitum. Animal experiments were designed and conducted in accordance with the guidelines of institutional animal ethical committee.

2.2 Experimental Design

Animals were divided into nine groups, each group containing six animals. Group1 served as control, received distilled water for 21 days. Groups 2 and 3 received methanol extracted seeds dose of 100 and 300 mg/kg/day respectively and Groups 4 and 5 received methanol leaves extracted dose of 100 and 300 mg/kg/day respectively. Groups 6 and 7 received dose of 100 and 300 mg/kg/day of aqueous seeds extract and Groups 8 and 9 received dose of 100 and 300 mg/kg/day of aqueous leaves extract respectively, for 21 days orally through cathedral tube. Clinical signs, average body weight and body weight gain were reported for each group. On the 22nd day, animals were anesthetized, blood for hematological and serobiochemical parameters were immediately collected. At necropsy, all rats were examined to identify gross lesion. The specimen of the liver, kidney and brain were quickly removed after autopsy and fixed in 10% formalin for Histopathological study.

2.2 Methods

2.2.1 Preparation of methanol and water extract of seeds and leaves of *D. innoxia*

The method was performed by using Soxhlet apparatus. 500g of the coarsely powdered seeds and leaves were weighed precisely and subjected to extraction with 250 ml petroleum ether (60-80C) for 2 hrs then, the extract was separated from solvent using rotary evaporator, air dried the plant residues were further dried, weighted and extracted with 250 ml methanol (99.8%) for 2 hrs, then, the extract was separated from solvent using rotary evaporator and the air dried powder were used for the treatment (methanol extract). The residues of plant seeds were further dried, weighed and extracted with

500ml of distilled water overnight at room temperature, and filtered through What man paper (NO.1) and dried further by freeze drier (aqueous extract) [15]

2.2.2 Hematological analysis

Blood samples were collected in EDTA container for determination of hemoglobin concentration (Hb), packed cell volume (PCV), red blood cells (RBCs), total white blood cells (WBCs) and differential WBC count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). This analysis has been conducted by Hematology analyzer (Sysmex KX-21, Japan, 1999).

2.2.3 Biochemical analysis

Blood samples were collected at slaughter in plane tubes, and centrifuged immediately at 3500 rpm for 5min and analyzed for the activities of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP), concentration of total protein, albumin, globulin, bilirubin, cholesterol and urea by Roche diagnostic Hitachi 902 analyzer, Germany, 1996).

2.2.4 **Histological study**

Necropsy was conducted to identify gross lesion and specimens of the liver, kidneys, brain, spleen and intestines were collected after slaughtering of rats and immediately fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned at 5 m and stained routinely with haematoxylin and eosin (H & E) using Harris's heamalun.**[**16].

2.3 Statistical analysis

The results were analyzed for statistical significance by one way ANOVA using the statistical package of social sciences (SPSS) version 16. All data were expressed as means (mean ± Standard error (M± S.E). P. Values ($P<0.05$) were considered statistically significant. [17].

3.0 RESULTS

3.1 Growth changes

Table 1 indicated the body weight and body weight gain of rats given daily oral doses of *D. innoxia* seeds and leaves extracts at 100mg/kg for groups 2, 4, 6 and 8 and 300 mg/kg for groups 3, 5, 7 and 9 within 3 weeks. The seeds methanol extract of *D. innoxia* for group 3 that received dose of 300 mg/kg had lower ($p\leq0.05$) body weight gains than control after 3 weeks, but seeds aqueous extract of *D. innoxia* for groups 7 that received 100 mg/kg and group 8 that received 300 mg/kg of leaves extract were lower ($p\leq0.05$) body weight gains than control. While other groups show no significant changes in body weight gain compared to control.

3.2 **Hematological changes**

Table 2 summarized the hematological changes for given daily oral of *D. innoxia* seeds and leaves of methanol and water extracts at 100mg /kg for groups 2, 4, 6 and 8 while dose of 300mg/kg for groups 3, 5, 7 and 9 within 3. Three weeks after treatment, the values of WBC were lower ($p\leq0.05$) in group 4 that treated with 100 mg/kg for leave methanol extracted, but it is higher ($p\leq0.05$) than groups 2, 3, 5, 6 and 8 compared with control plus other groups, Lymphocytes were lower ($p\leq0.05$) in group 6 and 7 while other groups show no significant changes compared to control. Neutrophils were higher ($p\leq0.05$) in all groups comparing with control, The PCV values were lower ($p\leq0.05$) in group 5 compared with control, but other groups show no significant changes compared to control, MCH values were lower ($p\leq0.05$) in group 5 and7 compared with control and higher ($p\leq0.05$) in group 6 than control group, MCHC, Hb and RBC show no significant changes compared to control group.

3.3 Serobiochemical changes

Table 3 illustrated the serobiochemical changes for rats given daily oral doses of *D. innoxia* seeds and leaves methanol and water extracts at 100mg /kg groups 2, 4, 6 and 8 and 300mg/kg groups 3, 5, 7 and 9 for 3 weeks.. After three weeks of treatment the activities of ALP, ALT and AST were lower ($p\leq0.05$) in group 4 and 5 comparing with control group. While the concentrations of urea were higher ($p\leq0.05$) in groups 3 and 6 than control group, concentration of cholesterol were lower ($p\leq0.05$) in groups 3, 4, 5, 7, 8, and 9 compared with control group, while group two show no significant changes compared to control group.

3.4 Histological changes:

Figure 2 shows the affects of methanolic extracts of leaves which were more obvious observed in water leaves extract. The effects were observed mainly in three organs (kidney, liver and brain), in kidneys of rats that received 100/mg/kg/day; glomerular alteration reflected as fatty cytoplasmic change, also segmentation and packing in the cortex were noted. While Figure 3 shows dilatations of renal tubules of the cortex of kidneys. Whereas, in the brain, cerebral neuronal vacuolation and lymphocyte infiltration were noticed Figure 4. Liver shows necrosis and lymphocytic infilteration in the central labulor zone figure 5.

Table 1. Body weight and body weight gains in rats orally given *D. innoxia* seeds and leaves methanol and aqueous extracts.

Treatment groups	Body weight (g) 0 week	Body weight gain (g) Three weeks
1.Control (normal diet)	106±7.0	40±10.5
2.Seed methanol extract (100/mg/kg/day)	104±10.4	38±5.4^{NS}
3.Seed methanol extracts (300/mg/kg/day)	110±21.6	22±2.3*
4. Leave methanol extracts (100/mg/kg/day)	106±18.1	43±4.4^{NS}
5.Leave methanol extracts (300/mg/kg/day)	105±6.3	45±4.8^{NS}
6. Seed water extracts (100/mg/kg/day)	106±3.4	49±5.3^{NS}
7. Seed water extracts (300/mg/kg/day)	108±6.4	22±10.5*
8. Leave water extracts (100/mg/kg/day)	104±17.6	22±5.6*
9. Leave water extracts (300/mg/kg/day)	106±4.1	42±5^{NS}

Values are expressed as means ± SE; NS = not significant;* Significant

Table2.Hematologicalchangein the rats given *D. innoxia* methanolic and aqueous extracts orally for 3weeks:

		Methanol extracts groups				**Water extracts groups**			
Parameters	**1. Control (Normal diet)**	**2. Seeds** (100 mg /kg/d**ay)**	**3 Seeds (300 mg /kg/day)**	**4. Leave**s (100 mg /kg/day)	**5. Leave**s (300 mg /kg/day)	**6. Seeds** (100 mg /kg/day)	**7. Seeds** (300 mg /kg/day)	**8. Leave**s (100 mg /kg/day)	**9. Leave**s (300 mg /kg/day)
Hb(g/dl)	13.6±0.9	12.4±0.4^{NS}	13.9±0.6^{NS}	12.3±0.2^{NS}	11.3±0.7^{NS}	13.0±0.2^{NS}	12.2±0.7^{NS}	14.2±0.3^{NS}	13.1±0.4^{NS}
RBC(x10^6 mm^3)	7.1±0.7	7.4±0.4^{NS}	7.5±0.2^{NS}	6.7±0.1^{NS}	6.8±0.8^{NS}	07.1±0.7^{NS}	7.4±0.8^{NS}	7.3±0.1^{NS}	7.2±0.1^{NS}
PCV (%)	42.0±3.5	39.2±0.8^{NS}	42.8±2.0^{NS}	37.6±0.3^{NS}	36.0±1.8*	39.8±0.3^{NS}	38.9±2.7^{NS}	42.9±0.4^{NS}	40.3±1.8^{NS}
MCV (m^3)	58.9±0.81	53±2.3^{NS}	56.7±1.5^{NS}	55.3±0.8^{NS}	53±3.5^{NS}	43.3±2.8*	52.6±2.3^{NS}	57.7±1.0^{NS}	55.8±0.8^{NS}
MCH(Pg)	19.2±0.6	16.9±1.3^{NS}	18.4±0.5^{NS}	18.1±0.4^{NS}	16.7±1.8*	23.0±4.7*	16.5±1.3*	19.6±0.3^{NS}	18.2±0.3^{NS}
MCHC (%)	32.6±0.5	31.8±1.2^{NS}	32.5±0.5^{NS}	32.8±0.4^{NS}	31.4±1.6^{NS}	37.6±4.9^{NS}	31.5±1.2^{NS}	33.2±1.0^{NS}	32.5±0.1^{NS}
WBC(x10^6 mm^3)	7.7±0.2	9.5±0.9*	12.3±3.5*	06±0.6*	11.7±9.1*	13.2±0.5*	15.9±1.2*	7.20±0.1^{NS}	8.3±0.4*
Lymphocytes (%)	78.4±7.8	67.4±7.6^{NS}	69.7±7.4^{NS}	78±8.8^{NS}	70.4±6.6*	54.2±9.0*	65.7±10.2*	67.1±2.9^{NS}	74.3±5.9^{NS}
Neutrophils (%)	21.6±7.8	32.6±7.7*	30.3±7.0^4*	22±8.8*	29.6±8.6*	45.8±9.0*	34.3±8.2*	32.9±2.9*	29.1±7.6*

Values are expressed as means ± SE; NS = not significant;* Significant

Table 3.Serobiochemical analysis of rats given D. innoxa seeds and leaves methanol and aqueous extracts orally for 3 weeks:

parameters		Methanol extracts groups				Water extracts groups			
	1. Control (Normal diet)	**2. Seeds** (100 mg/kg/day) (methanolic extract	**3. Seeds** (300 mg/kg/day)	**4. Leaves** (100 mg/kg/day)	**5. Leaves** (300 mg/kg/day)	**6. Seeds** (100 mg/kg/day)	**7. Seeds** (300 mg/kg/day)	**8. Leaves** (100 mg/kg/day)	**9. Leaves** (300 mg/kg/day)
ALP (iu)	228.1±5.1	189.2±6.8*	138.8±2.9*	99.4±.8*	190.0±0.1*	183.6±2.6	187.2±6.7*	156.6±8.1*	186.2±1.4*
ALT (iu)	34.50±1.5	29.2±5.0*	29.8±0.1*	9.7±0.5*	11.6±0.2*	21.9±0.7*	18.0±0.1*	28.1±0.8*	15.4±0.2*
AST(iu)	196.7±4.2	190±3.1^{NS}	183.5±4.5^{NS}	104.5±3.8*	149.1±1.7*	179.1±2.2^{NS}	197.9±1.2^{NS}	188.0±4.4 ^{NS}	202.2±1.1^{NS}
Total protein (g/dl)	7.4±0.1	6.9±0.2^{NS}	7.1±0.1^{NS}	7.1±0.3^{NS}	6.9±0.1^{NS}	7.0±0.3^{NS}	7.0±0.3^{NS}	6.8±0.5^{NS}	7.0±0.1^{NS}
Albumin (g/dl)	3.7±0.4	4.0±0.3^{NS}	2.9±1.3*	4.2±0.2^{NS}	3.9±0.2^{NS}	4.1±.2^{NS}	3.7±0.2^{NS}	3.6±.5^{NS}	4.1±0.1^{NS}
Globulin (g/dl)	3.7±0.5	2.9±0.1^{NS}	4.2±0.1^{NS}	2.8±0.2^{NS}	3.0±0.1^{NS}	2.9±0.1^{NS}	3.3±0.1^{NS}	3.2±0.3^{NS}	2.8±0.2^{NS}
Bilirubin(mg/dl)	0.7±0.1	0.70±0.2^{NS}	0.56±0.1^{NS}	0.1±0.2^{NS}	0.5±0.1^{NS}	0.5±0.2^{NS}	0.6±0.1^{NS}	0.5±0.1^{NS}	0.7±0.1^{NS}
Urea (mg/dl)	50.6±0.4	39.4±0.3*	55.1±0.4*	45±0.3^{NS}	42.5±0.9*	67.6±3.2*	47.7±0.4^{NS}	45.6±0.5^{NS}	44.7±0.5^{NS}
Cholesterol (mg/dl)	87.0±0.4	86.6±0.4^{NS}	74.6±0.6*	75.0±0.9*	100±0.6*	85±0.6^{NS}	75.0±0.6*	74.0±0.2*	98.5±0.3*

Values are expressed as means ± SE; NS = not significant;* Significant

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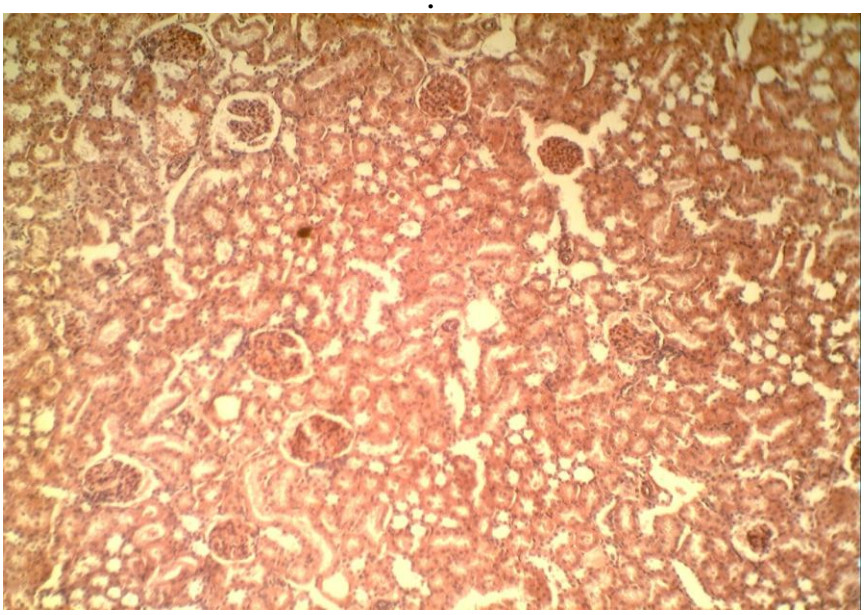

Fig. 2 Seeds water extract (100/mg/kg/day) kidneys showing glomerular alteration (fatty cytoplasmic change), segmentation and packing in the cortex. H&E. X10.

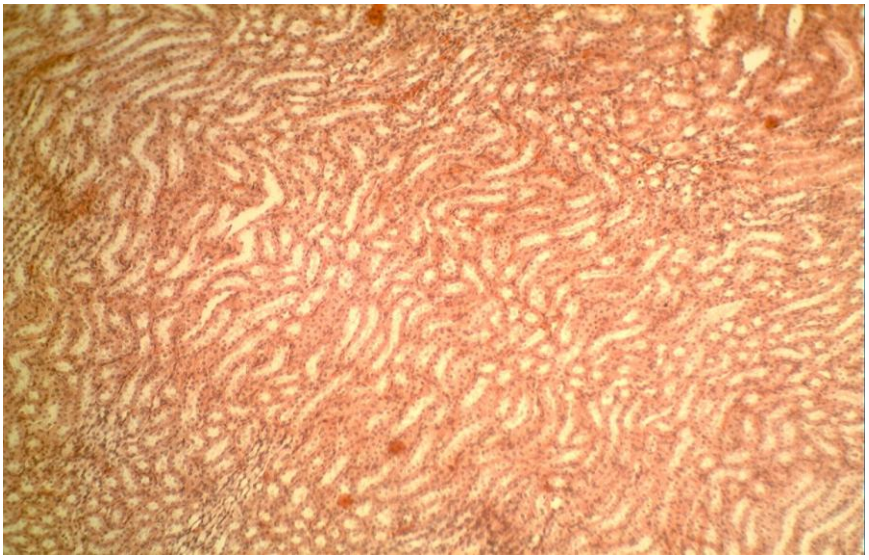

Fig. 3 Leaves water extract (100/mg/kg/day) kidneys showing dilatation of the renal tubules in the cortex. H&E. X10.

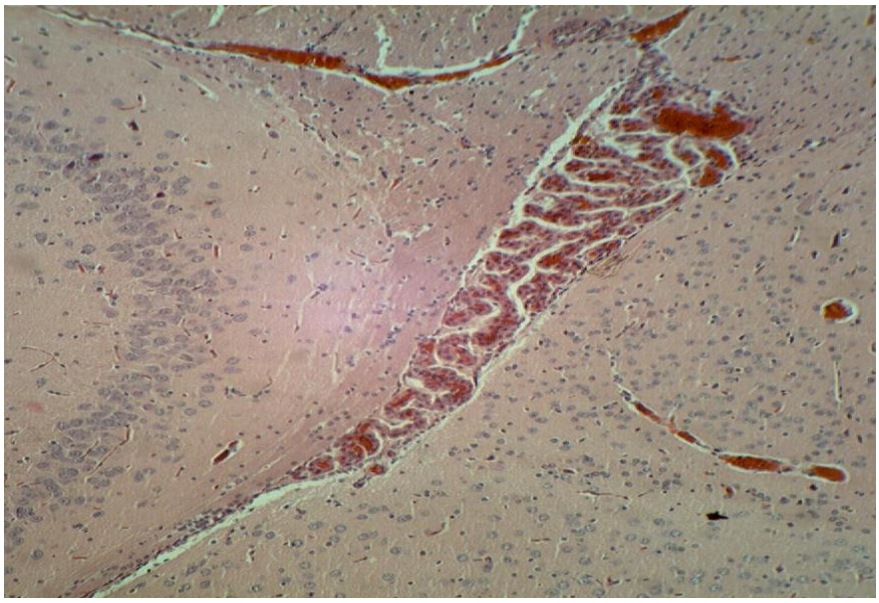

Fig.4 Seeds water extract (300/mg/kg/day) brain Showing cerebral neural vacuolation and lymphocytic infiltrations H&E X 10.

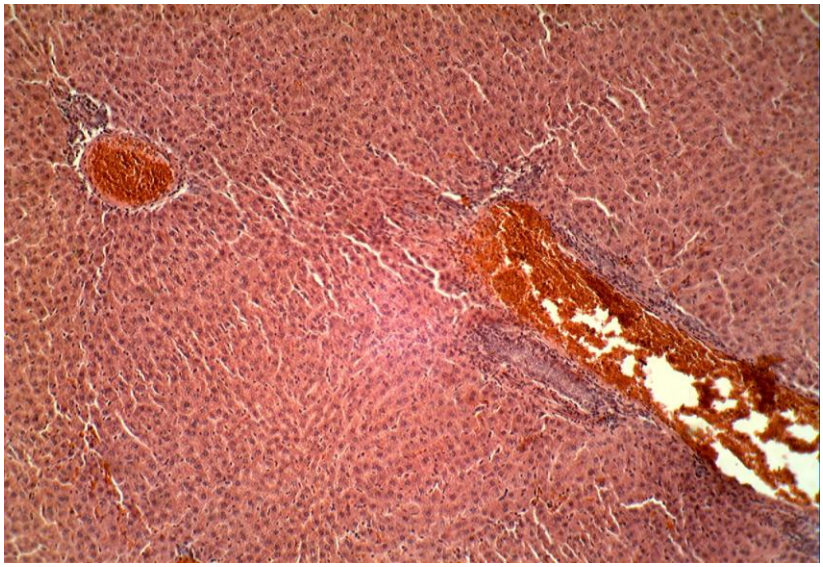

Fig. 5 Leave methanol extracts (100/mg/kg/day) liver showing Necrosis and lymphocytic infilteration in the central labulor zone. H&E X 10.

4.0 DISCUSSION

Medicinal plants have been used for centuries as remedies for human diseases because they contain chemical components of therapeutic value [18]. The most important of these bioactive constituents of plants are alkaloids, tannin, flavonoid and phenolic compounds.[19]. Within the recent years, infections have increased to some great extent and antibiotics

resistance effects become an ever-increasing therapeutic problem [20]. Although the *D. innoxia* seeds are used in traditional medicine in Sudan and Africa for the treatment of different types of diseases, The leaves and seeds are widely used in herbal medicine as anesthetic, antispasmodic, bronchodilator and as hallucinogenic. [21 and 22]. No research has been done to investigate the safety of *D. innoxia* seeds and leaves for human been or livestock, bird and rodent. Our experiments investigate the toxicity of seeds and leaves extracts given orally at 100 and 300 mg/kg to Wistar rats. The results of the present investigation indicated that *D. innoxia* seeds and leaves extracts were toxic, but not fatal to rats given orally doses of 100 and 300 mg/kg for 21 days. The pathological data were indicated that the plant constituent (s) affecting mainly in liver, kidneys and brain. Causing damage to this organ was described and included hepatocellular fatty vacuolation, fatty change and dilatation and alteration of glomeruli, cerebral neuronal vacuolation and lymphocytic infiltration. Similar results were obtained when the effects of Datura on fishes were examined [23]. The mechanism whereby the plant constituent(s) injured body tissue cannot be stated from the present study. This toxicity of *D. innoxia* seeds and leaves might be related to the compounds in *D. innoxia* seeds and leaves. Wistar rats suffered from damage in the liver and kidneys, which may contribute to the raised of serum AST activity and urea concentration. These findings are agreed with [24].

Conclusion

The toxicity from oral administration of 100 and 300mg/kg of *D. innoxia* seeds and leaves extracts for 3 weeks serves as evidenced by consistent extensive damage to liver, kidneys and brain. The damage to these organs caused by daily oral of D. innoxa extracts at 100 and 300mg/kg/day for 3 weeks was less marked.

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