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Evaluation of Toxicological Activity (Acute and Sub-Chronic Toxicities) of the Aqueous Extract of *Lawsonia inermis* Seeds on Wistar Rats

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Abstract: This study was carried out on the effect of aqueous extract of *Lawsonia inermis* seeds on rats. The 78.57 mg kg^{-1} of the extract administered for 4 weeks, caused body weight gain to rats and a significant decrease on hematological parameters and potassium concentration. Also there was a significant increase in the AST, ALP, total protein, albumin and urea concentrations with no obvious histopathological changes. 78.57, 392 and 785.7 mg/kg/day administered orally to rats for 1 week, caused an increase in AST, ALP and total protein concentrations. 785.7 mg kg^{-1} of the extract caused an increase in the ALT activity and a decrease in the potassium concentration. 78.57 and 785.7 mg kg^{-1} of the extract caused an increase in urea and cholesterol concentrations, while 392 and 785.7 mg kg^{-1} of the extract had caused hepatocytic necrosis, dilatation of the renal tubules and desquamation of the intestinal epithelium.

Key words: *Lawsonia inermis*, toxicity, Wistar rat and aqueous extract

INTRODUCTION

Medicinal plants occupied an important position in the socio-cultural, spiritual and medicinal arena of rural people in many parts of the world. The World Health Organisation (WHO, 1991) estimated that 80% of the population of developing countries rely on traditional medicine mostly plant drugs, for their primary health care. Demand for medicinal plants is increasing in both developing and developed countries but 90% of these materials harvested from wild sources without applying scientific management. Hence, many species are under threat to become extinct, their sustainable management and harvesting can conserve biodiversity, sustain human and environmental health, generate employment and enhance export earnings.

Lawsonia inermis is a flowering plant, the sole species in the genus *Lawsonia* in the family Lythraceae. It is native to tropical and subtropical regions of Africa, Southern Asia and Northern Australia in semi-arid zones (Chopra *et al*., 1992). It is also found in Northern and Central Africa, Asia and in expatriate communities from these areas of the world (Habbal *et al*., 2005). Analysis of henna seeds gave the following values: moisture, 10.6%,

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protein, 5.0%, fatty oil, 10-11%, carbohydrate, 33.62%, fiber, 33.52% and ash 4.75%. Extraction of seeds with petroleum ether yields non-drying viscous oil, with the following characteristics: saponification value 1.49, iodine value, 59.98; acid value, 18.2; acetate value, 9.2 and unsaponification matter, 10.5% (Manjunath, 1962). Phytochemical investigations of the aerial parts of *L. inermis* have shown the presence of alkaloids, flavonoids, tannins and glucosinolate (Al-Yahya *et al*., 1990), In addition to lepidimoids (Hasegawa *et al*., 1992) and an enzyme designated rubisco ribulose 1.5-biophosphate carboxylase oxygenase (Mummenhoff and Hurka, 1979). Black Henna is the name given to an artificial product by the addition of Paraphenylene-Diamine (PPD) to natural Henna. PPD is traditionally used in black hair dye but is sensitizer and may be life threatening. Therefore, immediate medical attention is deemed necessary (Kok *et al*., 2005). *Lawsonia inermis* seeds oil is devoid of behavioral and CNS effects and failed to produce any effect on isolated tissue though it possesses significant analgesic activity (Bagi *et al*., 1988). Crude and ethonolic extract of *Lawsonia inermis* leaves showed dose dependent analgesic, antipyretic and anti-inflammatory effect in rats (Ali *et al*., 1995). The 70% ethanol extract of *L. inermis* leaves extract showed that the feeding of 0.8 g kg^{-1}, BW, decreased the blood glucose concentration from 194 mg dL^{-1} to normal condition after 14th day in diabetic mice. Similar results occurred in total cholesterol concentration, which decreased from 148.9 to 55.3 mg dL^{-1} and triglyceride concentration decreased from 225.7 to 76.0 mg dL^{-1} (Symsudin and Winarno, 2008). Henna's anticarcinogenic property was reported by Endrini *et al*. (2002) using a chloroform extract of *Lawsonia inermis* by the culture tetrazollium salt (MTT) assay on the human breast, colon and liver carcinogenic cell lines and normal human liver cell lines. *Lawsonia inermis* was reported to have an affect against CCl_4-induced liver toxicity in mice (Anand *et al*., 1992). The naphthoquinones in *L. inermis* inhibited bacteria *Escherichia coli*, *Klebsiella pneumonia*, *Campylobacter jejuni*, *Staphylococcus* sp., *Bacillus* sp., *Mycobacterium* sp., *Coynebacterium diphtherium* (Dama *et al*., 1998). The antidermatophytic activity of ethanol, ethyl acetate and hexane extract of *L. inermis* were tested on 5 strains each of *Tinea rubrum* and *T. mentagrophytes*. All these extract showed significant antidermatophytic properties *in vitro* (Natarajan *et al*., 2003).

The objectives of the study was evaluation of toxicological activity (acute and sub-chronic toxicities) of the aqueous extract of *Lawsonia inermis* seeds on Wistar rats.

MATERIALS AND METHODS

Plant Material

Lawsonia inermi seeds were purchased from a local market in Khartoum (April, 2009), Sudan. The plant seeds were cleaned and shade-dried.

Preparation of Plant Extract

Extraction of the *L. inermis* seeds was done by means of decoction (boiling of 5.5 g of the plant seeds in 375 mL distilled water mimicking its use in the traditional medicine.

Experimental Design

Twenty four 2-week-old Wistar rats were housed within the premises of the Medicinal and Aromatic Plants Research Institute, National Centre for Research, Khartoum, with feed and water provided *ad libitum*. The rats were allotted into four groups each of 6 rats. Group 1 fed the normal diet and served as control group. Group 2-4 were given the aqueous extract of the *L. inermis* seeds at doses of 78.57, 392 and 785.7 mg/kg/day via the oral route, respectively for seven days duration period.

In the second type of experiments, twelve 3-week-old Wistar rats were allotted into two groups, each of 6 rats. Group 1 continued to be fed with the normal diet and served as control group. Group 2 was given the aqueous extract of the *L. inermis* seeds at a dose of 78.57 mg/kg/day via the oral route for 4 weeks duration time. At the end of each experiment, Average body weight and body weight gain were measured for each group, rats from each group were killed under diethyl ether anesthesia to identify gross lesions and specimens of the liver, intestines, kidneys, spleen and heart were immediately fixed in 10% neutral buffered formalin and processed for histopathology. Blood samples were collected from the cervical blood vessels of each rat for serum analysis and hematology.

Blood Analysis

Serum samples were analyzed for the activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) and for concentrations of total protein, albumin, globulin, bilirubin, cholesterol, urea, sodium and potassium.

Hemoglobin (Hb) concentration, Red Blood Cell (RBC) counts, Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and White Blood Cell (WBC) counts were determined by standard methods (Schalm *et al*., 1975).

Statistical Analysis

The significance of differences between means was compared at each time point using Duncan's multiple range test after ANOVA for one-way classified data (Snedecor and Cochan, 1989).

RESULTS

The effect on the body weight and body weight gain of rats given daily oral doses of *L. inermis* seeds aqueous extract at 78.57 mg kg^{-1} (group 2), 392 mg kg^{-1} (group 3) and 785.7 mg kg^{-1} (group 4) for 1 week is presented in Table 1, the control rats (group 1) had the lowest ($p<0.05$) body weight gains than group 2 at week one and the other groups shows no significant changes.

Haematological changes for rats given daily oral doses of *L. inermis* seeds aqueous extract at 78.57 mg kg^{-1} (group 2), 392 mg kg^{-1} (group 3) and 785.7 mg kg^{-1} (group 4) for 1 week are presented in Table 2, after 1 week of treatment, the value of MCV in (group 2) and granuloytes in (group 3) were lower ($p<0.05$) than in the control group (group1).

Serobiochemical changes of rats given daily oral doses of *L.inermis* seeds aqueous extract at 78.57 mg kg^{-1} (group 2), 392 mg kg^{-1} (group 3) and 785.7 mg kg^{-1} (group 4) for 1 week are presented in Table 3, 1 week after treatment, the activity of AST activity was higher ($p<0.05$) in group 2, 3 and 4, ALT was higher ($p<0.05$) in group 4 and the activity of ALP was higher ($p<0.05$) in group 2, 3 and 4 than control (group 1). The concentration of total protein was lower ($p<0.05$) in group 2, 3 and 4 than the control (group 1). Globulin concentration was higher ($p<0.05$) in group 2 and 3, while the cholesterol and urea concentration was higher ($p<0.05$) in group 2 and 4 than the control (group 1).

Table 1: Body weight and body weight gain in rats orally given *L. inermis* extracts for 1 weeks

Treatment groups	Body weight (g) 0 week	Body weight gain (g) 1 week
Control (normal diet)	85.0±3.16	8.7±4.59
L. inermis (78.57 mg/kg/day)	83.3±2.79	10.0±2.76*
L. inermis (392 mg/kg/day)	83.3±2.79	9.4 ± 2.46^{NS}
L. inermis (785.7 mg/kg/day)	83.3±2.79	8.5 ± 3.01^{NS}

Values are expressed as Mean±SE; NS: Not significant; *Significant at $p<0.05$

Table 2: Haematological analysis of rats given *L. inermis* aqueous extract orally for 1 week

Parameters	Groups: Control (normal diet)	*L. inermis* (78.57 mg/kg/day)	*L. inermis* (392 mg/kg/day)	*L. inermis* (785.7 mg/kg/day)
One week				
Hb (g dL^{-1})	15.7±2.19	15.2±0.21^{NS}	16.2±0.59^{NS}	15.6±0.36^{NS}
RBC ($\times 10^6$ mm^3)	10.2±1.52	10.2±0.11^{NS}	10.2±0.37^{NS}	9.6±0.67^{NS}
PCV (%)	67.4±9.70	65.0±1.04^{NS}	69.9±3.24^{NS}	66.0±1.57^{NS}
MCV (m^3)	66.5±1.06	63.3±0.84*	68.5±1.20^{NS}	68.8±0.75^{NS}
MCH (pg)	15.6±0.32	14.8±0.19^{NS}	15.9±0.17^{NS}	16.3±0.26^{NS}
MCHC (%)	23.4±0.22	23.4±0.10^{NS}	23.2±0.34^{NS}	23.7±0.25^{NS}
WBC ($\times 10^3$ mm^3)	6.0±1.50	6.3±0.70^{NS}	6.0±0.99^{NS}	5.9±0.62^{NS}
Lymphocytes (%)	58.3±2.10	60.5±4.31^{NS}	63.4±2.77^{NS}	60.9±2.90^{NS}
Granulocytes (%)	41.7±2.10	39.6±4.31^{NS}	36.6±2.77*	39.1±2.90^{NS}

Values are expressed as Mean±SE; NS: Not significant; *Significant at $p<0.05$

Table 3: Serobiochemical analysis of rats given *L. inermis* aqueous extract orally for 1 week

Parameters	Groups: Control (normal diet)	*L. inermis* (78.57 mg/kg/day)	*L. inermis* (392 mg/kg/day)	*L. inermis* (785.7 mg/kg/day)
One week				
AST (iu)	22.3±0.60	25.8±0.29*	26.6±0.58*	26.3±0.73*
ALT (iu)	45.0±0.67	46.2±0.53^{NS}	46.2±0.53^{NS}	50.0±0.56*
ALP (iu)	232.2±0.55	243.3±0.88*	280.0±1.53*	284.0±0.97*
Total protein (g dL^{-1})	9.6±0.38	7.7±0.33*	7.3±0.42*	6.5±0.19*
Albumin (g dL^{-1})	5.2±0.18	4.9±0.18^{NS}	5.2±0.16^{NS}	5.0±0.20^{NS}
Globulin (g dL^{-1})	1.7±0.38	2.9±0.39*	2.2±0.32*	1.5±0.30^{NS}
Bilirubin (mg dL^{-1})	1.5±0.08	1.2±0.16^{NS}	1.4±0.14^{NS}	1.5±0.14^{NS}
Urea (mg dL^{-1})	31.5±5.28	38.8±4.25*	30.7±3.04^{NS}	37.5±4.1*
Cholesterol (mg dL^{-1})	101.2±0.21	118.2±0.60*	101.2±1.02^{NS}	110.0±1.65*
Sodium (mmol L^{-1})	140.0±1.44	139.3±0.99^{NS}	143.0±0.58^{NS}	142.0±0.37^{NS}
Potassium (mmol L^{-1})	5.0±0.12	5.1±0.05^{NS}	5.1±0.09^{NS}	4.3±0.19*

Values are expressed as Mean±SE; NS: Not significant; *Significant at $p<0.05$

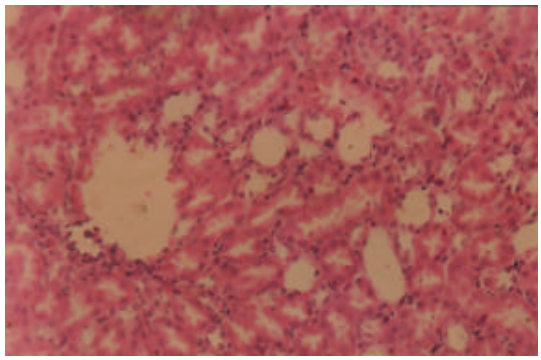

Fig. 1: Liver of rats receiving daily aqueous extracts of *Lawsonia inermis* seeds at 392 mg kg^{-1} for 1 week showing fatty cytoplasmic vaculation of the centerlobular and isolated cell necrosis, H and E X100

Potassium concentration was lower ($p<0.05$) in group 4 than control group (group 1) and the other groups.

After 1 week of treatment of the daily oral doses of *L. inermis* seeds extract rats in group 3 and 4 there were fatty cytoplasmic vaculation of the centirlobular hepatocytes and isolated cell necrosis (Fig. 1). Segmentation and packing of the glomerular tubules and

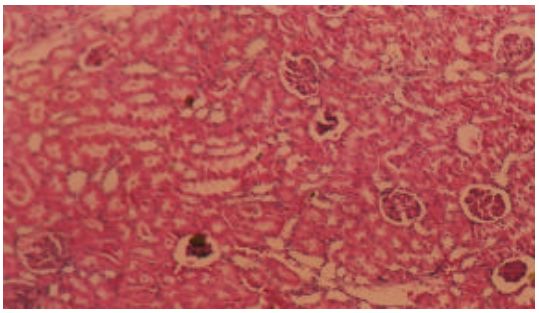

Fig. 2: Kidney of rats receiving daily aqueous extracts of *Lawsonia inermis* seeds at 78.57 mg kg^{-1} for 4 weeks showing segmentation, packing and necrosis of the renal proximal convoluted tubular of renal tubules a necrosis, H and E X100

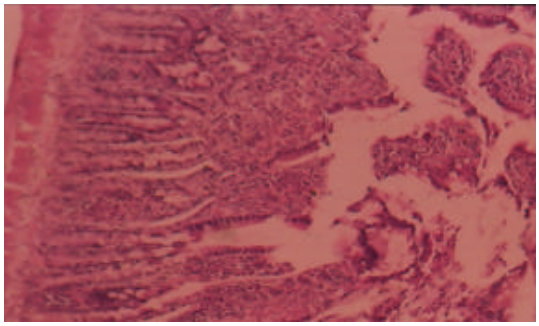

Fig. 3: Intestine of rats receiving daily aqueous extract of *Lawsonia inermis* seeds 392 mg kg^{-1} for 1 week showing vaculation or diqumination of the intestinal epithelium, H and E X200

Table 4: Body weight and body weight gain in rats orally given *L. inermis* extracts for 4 weeks

Treatment groups	Body weight (g) 0 week	Body weight gain (g) 4 weeks
Control (normal diet)	177.5±3.82	2.8±0.33
L. inermis (78.57 mg/kg/day)	171.7±5.27	5.0±5.87*

Values are expressed as Mean±SE; NS: Not significant; *Significant at $p<0.05$

dilatation of the renal tubules in group 2 (Fig. 2). Rats in group 3 showed vacuolation or desquamation of the intestinal epithelium and lymphocytic accumulation (Fig. 3). No significant lesions were observed in the heart and spleen of the tested rats. The tissues of the control rats (group 1) showed no lesions throughout the 1 week treatment in vital organs.

Body weight and body weight gain of rats given daily oral doses of *L. inermis* seeds aqueous extract at 78.57 mg kg^{-1} (group 2) for 4 weeks are presented in Table 4, after 4 weeks, the body weight gain was higher ($p<0.05$) in group 2 than the control (group 1).

Haematological changes for rats given daily oral doses of *L. inermis* seeds aqueous extract at 78.57 mg kg^{-1} (group 2) for 4 weeks are presented in Table 5. Four weeks after treatment, the concentration of Hb and the values of RBC, PCV and WBC were lower ($p<0.05$) than in the control group (group 1).

Serobiochemical changes of rats given daily oral doses of *L. inermis* seeds aqueous extract at 78.57 mg kg^{-1} for 4 weeks are shown in Table 6, 4 weeks after treatment, the activity

Table 5: Haematological analysis of rats given *L. inermis* aqueous extract orally for 4 weeks

	Groups	
Parameters	Control (normal diet)	*L. inermis* (78.57 mg/kg/day)
One week		
Hb (g dL^{-1})	13.5±1.71	10.2±1.75*
RBC ($\times 10^6$ mm^3)	9.0±0.76	6.4±0.65*
PCV (%)	59.0±5.32	41.4±8.2*
MCV (m^3)	65.2±0.54	65.4±0.81^{NS}
MCH (pg)	14.6±1.14	16.7±0.79^{NS}
MCHC (%)	23.4±8.82	25.5±7.25^{NS}
WBC ($\times 10^3$ mm^3)	7.3±0.84	4.2±0.82*
Lymphocytes (%)	61.5±5.79	63.3±3.09^{NS}
Granulocytes (%)	38.5±5.8	36.7±3.09^{NS}

Values are expressed as Mean±SE; NS: Not significant; *Significant at $p<0.05$

Table 6: Serobiochemical analysis of rats given *L. inermis* aqueous extract orally for 4 weeks

	Groups	
Parameters	Control (normal diet)	*L. inermis* (78.57 mg/kg/day)
4 week		
AST (iu)	22.30±3.0	27.6±2.40*
ALT (iu)	45.0±6.72	42.4±4.41^{NS}
ALP (iu)	232.2±5.50	285.6±5.80*
Total protein (g dL^{-1})	5.4±0.25	7.6±0.47*
Albumin (g dL^{-1})	4.4±0.24	5.6±0.30*
Globulin (g dL^{-1})	1.0±0.39	1.9±0.33^{NS}
Bilirubin (mg dL^{-1})	1.5±0.13	1.2±0.08^{NS}
Cholesterol (mg dL^{-1})	29.5±4.15	30.8±4.63^{NS}
Urea (mg dL^{-1})	101.2±2.10	126.0±2.40*
Sodium (mmol L^{-1})	140.7±1.31	142.2±0.73^{NS}
Potassium (mmol L^{-1})	4.8±0.09	3.9±0.27*

Values are expressed as Mean±SE; NS: Not significant; *Significant at $p<0.05$

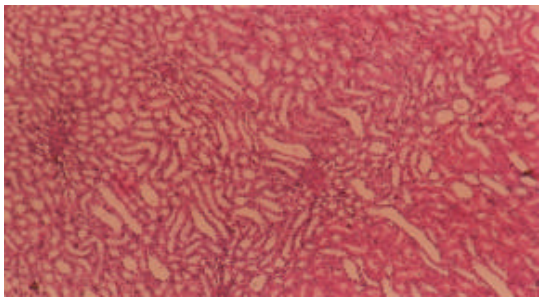

Fig. 4: Kidney of rats receiving daily aqueous extracts of *Lawsonia inermis* seeds 78.57 mg kg^{-1} for 4 weeks showing glomerular alteration, degeneration, dilation and necrosis of renal tubules. H and E X100

of AST was higher ($p<0.05$) and the concentration of Potassium were lower ($p<0.05$) in group 2. The activity of ALP and the concentrations of total protein, albumin and urea were higher ($p<0.05$) in group 2 than in the control group (group 1).

After 4 weeks of treatment with daily oral doses of *L. inermis* seeds extract, rats in group 2 showed dilatation and necrosis of the renal tubules (Fig. 4), rats in group 2 showed intestine catarrhal enteritis with minute erosion of the intestinal epithelium with lymphocytic infiltration (Fig. 5). No significant lesions were observed in the heart and spleen of the test rats. The tissues of the control rats (group 1) showed no lesion throughout the 1 week in vital organs.

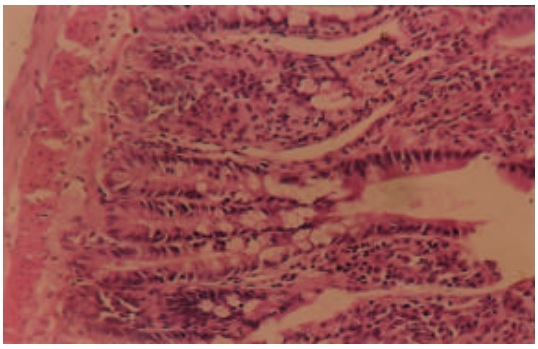

Fig. 5: Intestine of rats receiving daily aqueous extract of *Lawsonia inermis* seeds 78.57 mg kg^{-1} for 4 weeks showing catarrhal enteritis with minute erosion of the intestinal epithelium with lymphocytic infiltration. H and E X200

DISCUSSION

Although, the *Lawsonia inermis* leaves are used in traditional medicine of several countries in Africa and Asia for the treatment of various diseases, little research had been done to investigate the safety of *Lawsonia inermis* seeds on rodents and other species of livestock.

The results of this study, indicated that *Lawsonia inermis* seeds were toxic but not fetal to rats in daily oral doses of 78.57, 392 and 785.7 mg kg^{-1} for 1 week treatment. In rats, damage to the liver and kidneys could explain the development of gradual loss of reflexes. The liver depicted fatty vaculation of the hepatocytes, kidneys had an altered glomeruli and degeneration or necrosis of the convoluted tubules, showed an aggregated lymphocytes in the intestinal lamina propria and other vital organs. The changes in the liver and kidneys probably contributed to the increase in serum AST activity and in urea concentration. The mechanism whereby this plant constituent injured the body tissues can not be elucidated from the present study. The mechanism whereby, the plant constituents injured body tissues can not be derived from the present study but the injury to these organs probably contributed to the fluctuating serum AST and ALT activities and cholesterol and urea concentrations and albumin concentration and ALP activity. The haemoglobin parameters showed no significant differences in all experimental groups. Previous investigations showed macrocytic anemia in rats which had been fed a diet containing 10 or 50% of Rhazya stricta leaves (Adam, 1999) or in chickens which had received a diet consisting of 10% Cassia italica seeds (Bakhiet and Adam, 1996).

Despite the therapeutic uses of the two plants in traditional medicine, their toxicity in rats or other animals has not been evaluated.

It is clear from the results of the present investigation that the liver and kidneys are the sensitive organs to the toxic action of the active constituents of the plant products utilized.

The hepatic changes comprised focal necrosis and fatty cytoplasmic vacuolation of the centrilobular hepatocytes, congestion, haemorrhage and lymphocytic accumulation. The renal lesions consisted of scattered lymphocytic infiltration in the cortex, congestion, haemorrhage and degeneration or necrosis of the epithelial cells of the convoluted tubules. However, these plants seeds contain lawsone, 2-hydroxy-naphthoquinone ($C_{10}H_6O_3$) which is known to be the major bioactive constituent in addition to flavonoids and triterpenes (Ageel *et al*., 1987).

The organ damage caused by a dose of (78.57 mg/kg/day) of *Lawsonia inermis* seeds aqueous extract for 4 weeks was less intense. The erythrocytic series have not been seriously altered and the granulocytes seemed to decrease probably due to infiltration in the vital organs. It is well known that the susceptibility of animals to feeding plant material is dependent on the type of the active constituents and concentrations in the amount added to the diet as well as on the rate of their metabolic conversion in the liver to metabolites and consequent excretion (Adam, 1999; Ibrahim *et al*., 2004).

The development of cytoplasmic fatty vacuolation or necrosis of the centrilobular hepatocytes, degeneration or necrosis of the epithelial cells of the renal tubules segmentation, packing or necrosis of the glomerular tufts with lymphocytic infiltration could explain the toxic effect. However, the mechanism whereby the plant constituents damage body tissues can not be defined from the present study. The decrease in serum AST and ALT activities could have been due to enzyme excretion. It has been found that hyperbilirubinemia is associated with periportal liver injury previously described by Gopinath and Ford (1972) in sheep, Ali and Adam (1978) in goats and by Adam (1999) in rats and the increase in urea concentration indicates a renal malfunction.

There were no significant haematological differences in all experiments which disagreed with the results of Adam (1998), who observed anemia of a macrocytic hypochromic type as indicated by increases in MCV and decreases in MCHC. Previous investigations showed normocytic normochromic anemia in rats on 100 g kg^{-1} *F. crispa* leaves for 8 weeks or normocytic hypochromic anemia in rats fed a diet containing 100 g kg^{-1} of cuminum cyminum fruits for 6 weeks (Adam, 1998).

It is well known that a plant or drug may interact with another plant or drug and as a consequence modification in activity and/or toxicity can be observed. For example, simultaneous feeding of Citrullus colocynthis and cassia senna resulted in an increased toxic effect on rats (Adam *et al*., 2001). On the other hand, paracetamol-induced hepatonephrotoxicity in rats was reduced by feeding the seeds of *Nigella sativa* (El-Habib *et al*., 2007).

CONCLUSIONS

From this study, It can be concluded that there would be a significant toxicity for male Wister rats administered orally with *Lawsonia inermis* seeds at concentrations of (78.57, 392 and 785.7 mg/kg/day) and damage of vital organs exemplified by necrosis, fatty changes, hemorrhage and congestion. However, it is not fetal when given at these concentrations.

While, a dose of 78.57 mg/kg/day of the extract caused less organ damage and obvious changes on serobiochemical parameters.

We suggest that further research might be carried out with different doses to detect the exact lethal dose of the seeds extract.

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