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Toxicity Induced Histological Changes in Selected Organs of Male (Wistar) Rats by *Lawsonia inermis* Leaf Extract

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Research Article

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ABSTRACT

Aim: To describe the adverse effects of the leaf extract of *Lawsonia inermis* plant (Henna), extensively used for medicinal benefits in traditional Arab/African culture, on the histology of liver, kidney and spleen of male rats.
Study Design: Cross-sectional study.
Place and Duration of Study: Biology Department, College of Science and Arts at Onaiza, Qassim University, Saudi Arabia, between August 2011 and November 2011.
Methodology: Henna leaf solution was given to male rats at doses of 200 mg and 1000 mg/Kg/day for a period of six weeks/42 days. Rats were sacrificed at the end of the treatment period and their effects were studied specifically focusing on the histology of the liver, kidney and spleen; organs involved in detoxification, hematopoiesis and immune functions.
Results & Conclusion: No pathological changes were evident at 200 mg dose for liver, kidney and spleen sections. However, significant pathological changes were observed in the liver and kidney at 1000 mg dose suggestive of degenerative or apoptotic changes. In view of these pathological relevant results, it is not advisable to use such high doses of Henna leaf extract in clinical formulations. Additional future pharmacokinetics studies are suggested.

Keywords: Lawsonia inermis; henna; histopathology; liver; kidney; spleen; toxicity.

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1. INTRODUCTION

Internationally, the issue of medicinal plants is of great concern in many heath care systems, a survey done by WHO reported that approximately 80% of the global population still depending on traditional plant-based medicines for their basic health care needs (Fransworth, 1993). Globally, there is gradual increase in medicinal plant sales, for example, sales have been increased by 37% from 1993-1996 (Klink, 1997). However, till now no much is known about the dose-related toxicity of medicinal plants, particularly at the histological side. Bioactive compounds derived from medicinal plants can be useful but might have serious dose-related side effects (Taylor et al., 2001). Therefore, in view of their wide spread use of medicinal plants in alternative medicine, toxicological assessment becomes imperative in order to arrive at potencies that can be considered as safe formulations for clinically efficient remedies. *Lawsonia inermis*, commonly known as Henna, is a member of the family *Lythraceae* that was found in the middle-east, North Africa and south-west Asia. It has been used for over 9000 years not only for cosmetic but for medicinal applications to treat diverse ailments, it is now considered as a valuable source unique natural products for development of medicines against various diseases (rheumatoid arthritis, headache, ulcers, diarrhea, leprosy, fever, leucorrhoea, diabetes as well as for diseases involving liver and heart) and also for the development of industrial products (Chaudhary et al., 2010; Abdelgadir et al., 2010). The aim of the present study was to demonstrate the dose related histopathological changes, if any, of Henna on the organs involved in detoxification, hematopoiesis and immune functions; liver, kidney and spleen of male rat.

2. MATERIALS AND METHODS

The leaves of *Lawsonia inermis* (Henna) were collected from Al-Madinah Al-Munawarah region, Saudi Arabia, authenticated by taxonomist at College of Science and Arts, Qassim University (Voucher herbarium specimen number: MEDP 26), dried and crushed into fine powder and an aqueous solutions were prepared. The required doses have been calculated and prepared as mentioned by (Abdallah et al., 2009). Male rats, 2-3 months old (*Rattus norvegicus*) weighing 120 to 200g, were supplied by the Animal House, Faculty of Pharmacy, King Saud University, Saudi Arabia. Rats were housed according to the recommendations of King Saud University Guide for the Care and Use of Laboratory Animals. Animals were divided into three groups of ten rats each. Group one did not receive any treatment (Control). Group two had administered Henna, 200 mg/kg/day per os for 6 weeks. Group three had administered Henna 1000 mg/kg/day per os for 6 weeks. At the end of the treatment period, rats were sacrificed and liver, kidney and spleen were collected in sterile saline. Freshly dissected organs from each animal were cut rapidly and fixed in buffered neutral formalin (10%). The tissues were dehydrated in ascending grades of ethanol (70%, 80%, 90%, 95% and 100%), cleared in 2 changes of Xylene, impregnated with 2 changes of molten paraffin wax, and finally embedded in wax. Tissue sections of 4–5 µm in thickness were cut with a microtome and stained with hematoxylin and eosin (Pearse, 1985).

3. RESULTS AND DISCUSSION

Although Henna (*Lawsonia inermis*) is a widely used plant in traditional medicine, little is known about its safety. In the present investigation, an aqueous suspension of henna leaf powder was prepared according to the prescriptions given by traditional healers. In traditional medicine plant extracts are prepared as aqueous suspensions, for example,

infusions, decoctions, poultices (Rabe and Staden, 1997). Our previous study showed that leaves of this plant have antibacterial activity with no toxicity symptoms on male rats based on their hematological and biochemical parameters (Abdallah and Aldamegh, 2011). In this study, as a result of the treatment of rats, no adverse effects were observed on the morphology of the kidneys, liver and spleen of the experimental rat groups. This observation could be a good indicator for safety and non-toxicity (Abdallah et al., 2009). Histological sections of the kidneys, liver and spleen derived from experimental rats treated with Henna leaves at dose 200 mg/kg/day for 42-days appeared normal when compared with the control group whereas serious abnormalities were observed in sections from rats treated with a dose 1000 mg/kg/day.

Histological sections of the kidney derived from rats treated with the dose of 200 mg/kg/day, showed normal appearance of the renal capsules and tubules while epithelial cell degeneration and desquamation were observed in the lining of the renal tubules treated at a dose of 1000 mg/kg/day, compared to the controls (Figure 1), suggestive of some degree of toxicity and adverse effects on circulation at the higher dose. Our result is in agreement with findings of Abdelgadir et al. (2010), who detected scattered lymphocytic infiltration, congestion, heamorrhages, and degeneration or necrosis in the renal tubular epithelia. Kidneys are the main organs in the body susceptible to the toxic effects of drugs (Adekomi et al., 2011).

Degeneration of hepatocytes was observed in histological sections of liver derived from rats treated with 200mg dose, albeit to the same degree as in controls. However, at the dose of 1000 mg/kg/day acute pathological changes were observed in liver histology, viz. severe distortion and degeneration of hepatocytes, hyperplasia of bile duct epithelium, hyalinization of the wall of hepatic arteriolar branch in the portal area, progressive lymphocytic infiltration in the portal area) (Figure 2). These findings are going hand to hand with findings of Abdelgadir et al. (2010), in the liver of Wister rats had taken seed extracts of *Lawsonia inermis*. Liver is the major site of detoxification in the body for all drugs/toxins. Therefore it is an important organ in any toxicological study (Treadway, 1998). Interestingly, many medicinal plants have been found to be toxic to the liver suggestive of a possible toxic effect of Henna leaves at high dose to rats (Sofowora, 1984). It has been found that hyper-bilirubinaemia is associated with periportal liver injury pre-viously induced previously described by Gopinath and Ford (1972) in sheep, Ali and Adam (1978) in goats and by Adam (1999) in rats.

Histopathological investigation of spleen did not exhibit any abnormalities in rats treated with low or high doses of henna implying no effect of this plant on hematopoiesis, and immunologic functions (Figure 3) The present study is in agreement with the study on Henna seed extract where acute histopathological changes in kidneys, liver and intestines were documented at doses 392 and 785.7 mg/kg daily for 4 weeks (Abdelgadir et al., 2010), represented by fatty vacuolation of hepatocytes. 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). Al-Rubiay et al. (2008) reported that Henna leaves alcoholic extract had the highest antibacterial activity with a minimum inhibitory concentrations variable according to the type of microbe. Water extracts of Henna leaves did not show any antibacterial activity compared to alcoholic and oily extracts (Kelmanson et al., 2002).

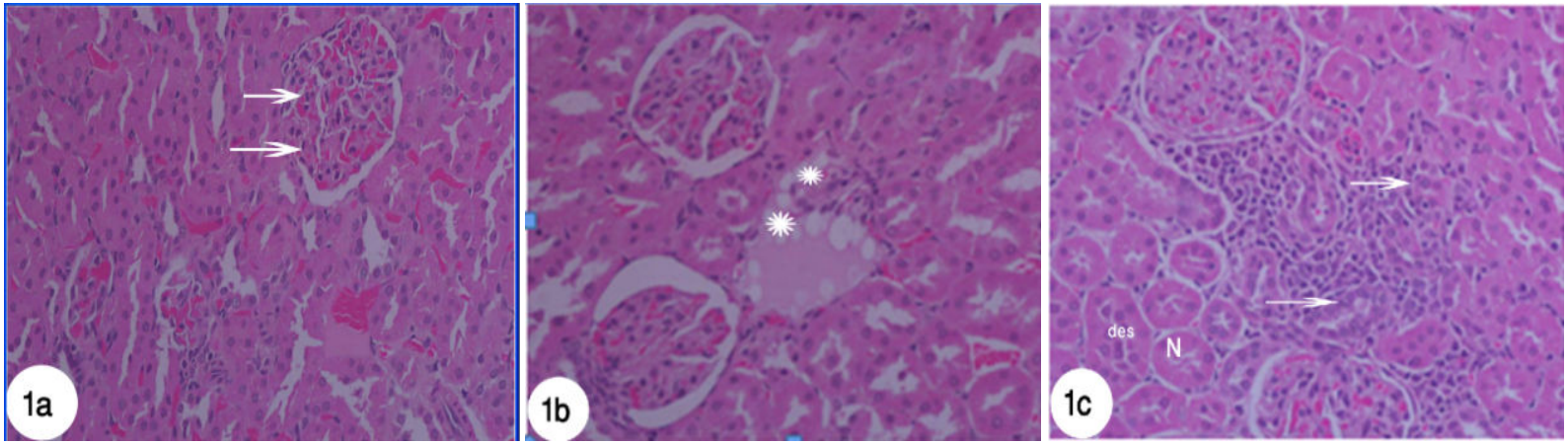

Fig. 1. H&E stained sections of kidneys at 400X magnification
(1a) Kidney sections of control rat showing normal histology. (Arrows depict normal glomerulus and renal tubules);
(1b) Kidney sections of treated animals of dose 200 mg/kg/day, showing moderate degenerative changes in the epithelium lining the renal tubules
(1c) Kidney sections of treated animals of dose 1000 mg/kg/day, showing severe changes and death of the epithelial cells lining the convoluted tubules. (Arrows depicts apoptosis in epithelial cells in tubules, N; normal tubule, des; desquamated epithelium in the lumen of the tubule)

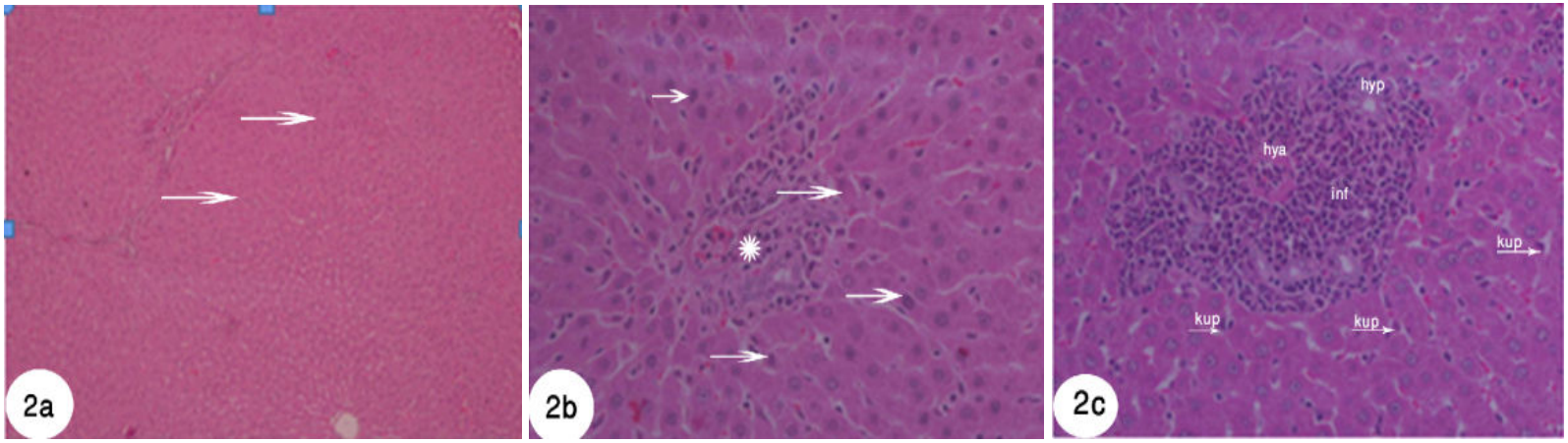

Fig. 2. H&E stained sections of Liver at 100X magnification
(2a) Liver sections of control animal showing normal histology (Arrows depict normal hepatocytes)
(2b) Liver sections of treated animals of dose 200 mg/kg/day, showing moderate distortion and degeneration of the hepatocytes. (Arrows depicts moderate distortion and degeneration of hepatocytes, *; lymphocytic infiltration in the portal area)
(2c) Liver sections of treated animals of dose 1000 mg/kg/day, showing distortion and degeneration of the hepatocytes. (hyp, hyperplasia of bile duct epithelium, hya, hyalinization of the wall of hepatic arteriolar branch in the portal area, in progressive lymphocytic infiltration in the portal area)

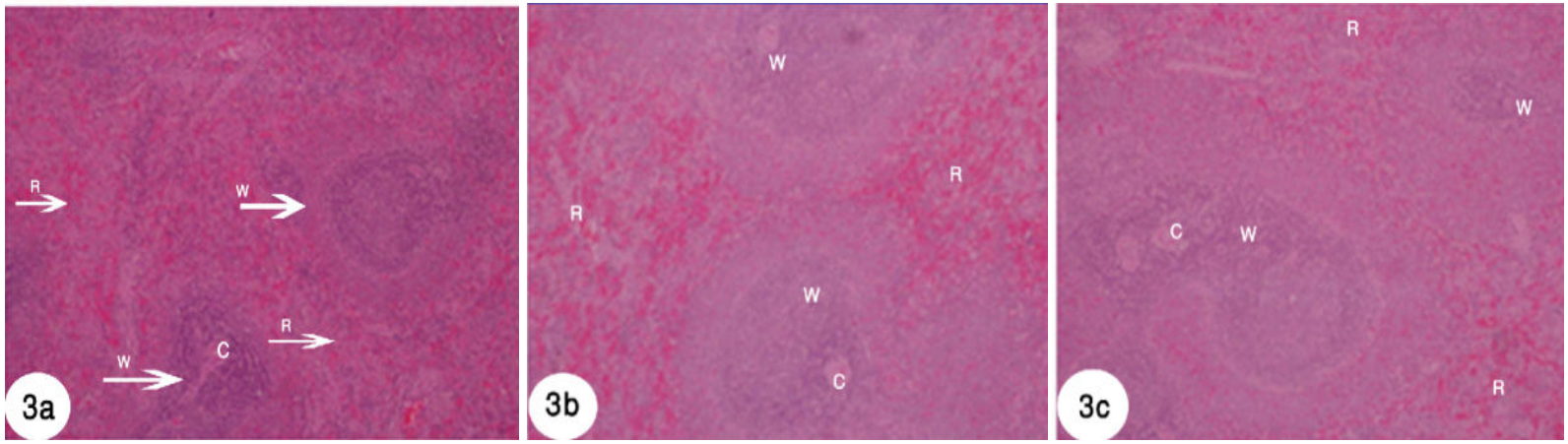

Fig. 3. H&E stained sections of the Spleen at 100X magnification
(3a) Spleen sections of control animal showing normal histology. (W, white pulp, R, red pulp, C, central arteriole)
(3b) Spleen sections of treated animals of dose 200 mg/kg/day, showing normal histology.
(It is not varied from the control, R, red pulp, W, white pulp, C, central arteriole)
(3c) Spleen of treated animals of dose 1000 mg/kg/day, showing somewhat normal histology, so revealed more expanded white pulp on the account of red pulp, R; red pulp, W; white pulp, C, central arteriole.

Moreover, *Lawsonia inermis* appeared to contain substances that had antimicrobial properties because of, the methanolic extract of *Lawsonia inermis* leaves were active against five different bacterial strains. Five concentrations of the extract were used (1000 µg/ml, 500µg/ml, 250µg/ml, 125µg/ml and 62.5 µg/ml). It is estimated that if an inhibition is obtained by 250µg/ml-1000µg/ml of test solution, the extract can be considered worthy for further investigations. According to Munday et al. (1991), Lawsone is considered to be the active component in henna, responsible for its haermotoxicty (Sauriasori et al., 2007).

The effect of 200 and 400 mg/kg body weight of 80% ethanolic extract of the fresh leaves of *Lawsonia inermis* were examined by Dasgupta et al. (2003), in mice, where they found that anti-carcinogenic potential of Henna leaf extract was adopting the protocol of benzo(a)pyrene induced forestomach and 7,12 dimethylbenz(a)anthracene (DMBA)-initiated and croton oil-promoted skin papillomagenesis. Also, they denoted 'duel-acting' nature of henna leaf as deduced from its potential to induce only the phase-II enzyme activity, associated mainly with carcinogen detoxification in liver of mice and inhibit the phase I enzyme activities. The hepatic glutathione S-transferase and DT-diaphorase specific activities were elevated above basal ($p<0.005$) level by *Lawsonia innermis* extract treatment.

4. CONCLUSION

The results obtained in this investigation are in accord with the safety claims of the applications of Henna in traditional and folk medicine, at doses up to 200 mg/kg/day, for rats. Health risk may increase at high doses. More studies are recommended in order to confirm that leaves of henna plant are free of cytotoxic effects on short and long term treatment at lower doses used in medicinal formulations. However, the most important fact to be determined is the documentation of the clinical efficacy of Henna treatment of diverse symptoms that it claims to cure before it can be accepted or allowed to be continued.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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