



ISSN: 2230-9926

Available online at <http://www.journalijdr.com>

IJDR

International Journal of  
DEVELOPMENT RESEARCH

International Journal of Development Research  
Vol. 5, Issue, 05, pp. 4489-4495, May, 2015

### Full Length Research Article

## TOXOPATHOLOGICAL EFFECTS OF HENNA (*LAWSONIA INERMIS*) ON RATS ORGANS

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#### ARTICLE INFO

##### Article History:

Received 15<sup>th</sup> February, 2015

Received in revised form

19<sup>th</sup> March, 2015

Accepted 03<sup>rd</sup> April, 2015

Published online 31<sup>st</sup> May, 2015

##### Key words:

Henna,  
Toxicity Histopathology,  
Rats.

#### ABSTRACT

The henna leaf solution is important plant in veterinary medicine for treating of some cases in farm animals like wound healing, constipation and other internal or external conditions, especially when they are resistant to treatment methods. This study included 12 rats where divided into two groups, each group included 6 rats. The first group was considered as a control group, without any treatment. While the second group had been given 500mg/Kg body weight of henna solution which was administered orally by stomach tube for 60 days. The result of the toxicity of henna on the treated rats was represented by dilation of renal tubes, erosion in mucosa of stomach, atrophy of lymphatic tissue in spleen, suppression of spermatogenesis, enlargement of adrenal gland with absence of inflammatory cells and other organs mentioned in the research.

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#### INTRODUCTION

Henna is cultivated commercially in many parts of the world specially the Arab, sub-Saharan Africa and Asia particularly India, Bangladesh and Pakistan (Agabna, Nuha *et al.*, 2014; Muhammad and Muhamad, 2005 and Bharali *et al.*, 2012). Henna (*Lawsonia inermis*) is belonged to family (Lythraceae) it is transmission tree, veteran tree. It is heavy branching (2-hydroxy-1,4 naphthaquinone) is the chief constituent and it is responsible for the dying properties of plant (Rostogi and Mehrota, 1993). Bioactive compounds derived from medical plants can be useful but might have serious dose-related side effects (Taylor *et al.*, 2001 and Uddin *et al.*, 2013). Therefore, *Lawsonia inermis* had been used for ancient times as colorant for medical purpose like skin, hair nail, clothes and leather in many middle eastern counties (Fransworth, 1993 and Rabe and Staden, 1997). The exhibit antimicrobial (Abdallah *et al.*, 2011; Kelmanson *et al.*, 2002; Arun *et al.*, 2010 and Abdul Moneim, 2007), anti-sickling (Clarke *et al.*, 1986 and Chang and Suzuka, 1982), promising activity against Trypanosoma parasite was reported by (Okpekon *et al.*, 2004 and Atawodi *et al.*, 2002) who investigated the traditional use of henna to treat

sleeping sickness especially among the cattle herders anti-inflammatory, antipyretic, anticomplementary, antioxidant, cytotoxic (Taylor *et al.*, 2004) and immunomodulatory property (Ali *et al.*, 1995 and Chun *et al.*, 1998). Henna has been used traditionally to cure burn wounds (Muhammad and Muhamad, 2005; Dikshit *et al.*, 2000 and Mikhaeil *et al.*, 2004). Henna had benefits in treating eczema as paste applied on the affected area 3-5 times and it had also been used to treat psoriasis (Khan *et al.*, 2009). In addition, *Lawsonia inermis* cream gave complete recovery of cervicitis after using for cervical erosion (Hashmi *et al.*, 2011).

There are investigations found that crude ethanolic extracts of *Lawsonia inermis* use to treatment diabetes mellitus due to *L. inermis* have antihyperglycaemic effect, protection against most chemical materials which are dangerous that can damage the tissues of the body (Al-Jubory, 2013). Henna is choice factor to fracture healing, tumors, pimples, treatment of uterus diseases, joint diseases (AlferahMosaid, 2012; Ghosh, ? and Endrini *et al.*, 2002), anticarcinogenic potential of henna leaf extract (Abdel-Wahab *et al.*, 2009). But other scientific studies were presented on henna toxic caused allergic, kidney failure and possibly death sometimes with broken red blood cells (Zumrutdal *et al.*, 2008; Burnett and Goldenthal, 1988 and Munday *et al.*, 1991). The present of histopathological examination indicated there are necrosis association with

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inflammatory cells infiltration in interstitial, hemorrhage in the lungs, necrosis of some hepatocytes, hydropic degeneration and edema (Selvanayaki and Ananthi, 2012). Degenerative & necrotic changes, cellular hypertrophy & increased intercellular vacuulations which appear in the stroma of the treatment groups compared to the control result in cell death which is of two types namely apoptotic & necrotic cell death from extrinsic insults to the such as somatic, thermal, toxic & traumatic effects (Bancroft *et al.*, 1996).

## MATERIALS AND METHODS

### Animals

We took 12 healthy *Albino Wister* rats (150-200g) from DhiQar university and placed in the animals house in Basra university, collage of veterinary medicine at adaptation period with giving them diet and water and noted the activity of the rats daily and then began the experiment.

### Plants

We collected leaf of henna from Basra region, dried and crushed into fine powder with help of grinder. The extraction was done by take 5gm of henna powder dissolved in 10ml of distilled water for obtaining the extract.

### Extract Administration

12 *Albino Wister* male rats were selected to be used for the experiment and divided into two groups, each group included 6 rats. The first group had been given 1ml of distilled water while the second group had been 500mg/kg of henna solution orally by stomach tube daily for 60 days.

### Histopathology study

After giving the leaf extract of henna and then death animals with scarified killing & necropsy of the animals, take the organs, fixed by 10% formalin, passed upgrading of concentration alcohol, zylene, embedded in paraffin, cut by microtome 5 $\mu$ m & then staining with hematoxyline & (H&E) eosin and examined by light microscope (H&E).

## RESULTS

The rats of the treated animals in the present study showed clinical signs at final time of experment represented by loss of weight, general weakness, emaciation, nervous signs and convulsions.

### Histopathological Changes

#### Stomach

##### Control Group

The body of the stomach includes mucosa which is made of three components: The epithelium a supporting lamina properia and a thin smooth muscle layers, the muscularis mucosa. There is submucosa which is loose collagenous tissue

supports the mucosa contains the large blood vessels, lymphatic and nerves. There are muscularisproperia consists of smooth muscles which is usually arranged as an inner circular larger and an outer longitudinal layer (Fig. 1).

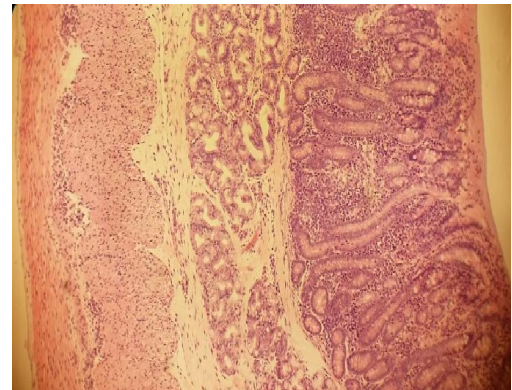


Figure 1. Rat stomach show normal Tissue (H&E, 10X)

##### Treated Group

There are histologic changes in the stomach section of the rats like erosion of the mucosal layer of stomach (Fig. 2). Increase cellularity in lamina properia (Fig. 3). Increase hyperkeratosis of non-glandular layer of the stomach (Fig. 4).

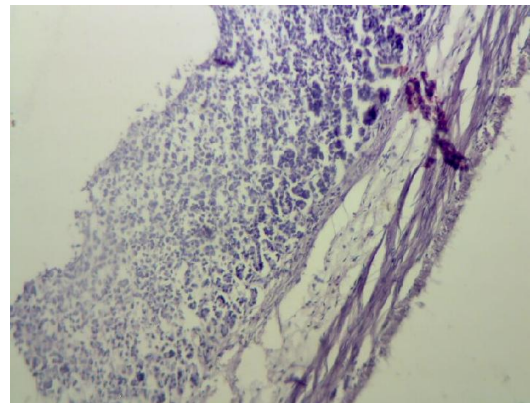


Figure 2. Rat stomach show erosion of mucosal layer (H&E, 10X)

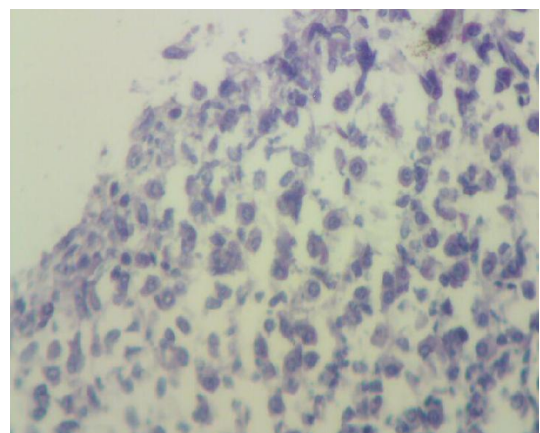


Figure 3. Rat stomach show increase cellularity in lamina properia, H&E, 40X

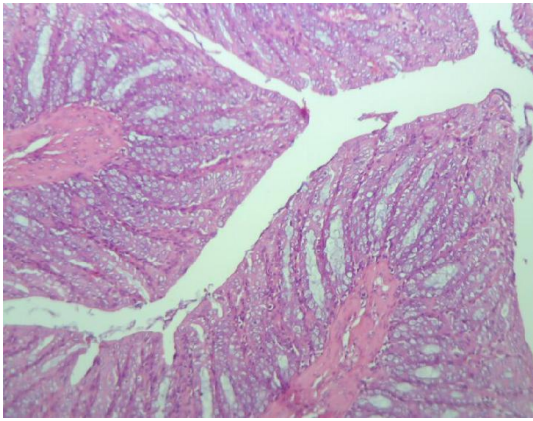


Figure 4. Rat stomach give 1 ml of henna show hyperkeratosis in non -glandular layer H&E,10x

## Liver

### Control Group

The histological examination of the liver Tissue of the Albino Wister rates showed normal portal triad area with no abnormality (Fig. 5).

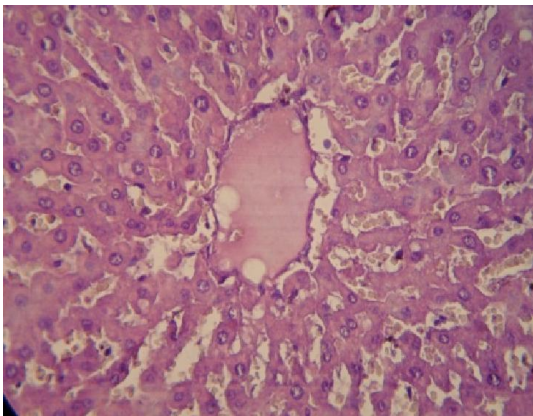


Figure 5. Central vein sinusoid. H&E 10X

### Treated Group

After examination of the liver section, there were congested central vein and sinusoids, (Fig.7) accumulation fibrosis and mononuclear cells around central vein of the liver of the treated group (Fig. 6).

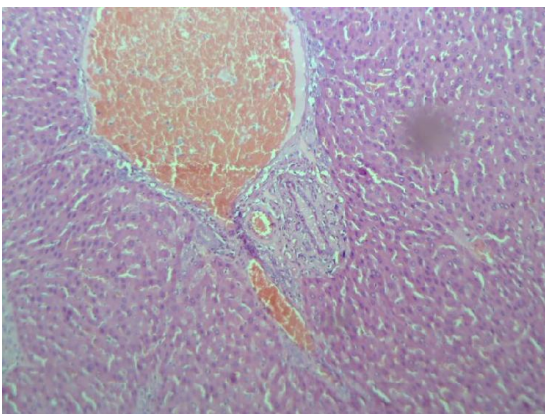


Figure 6. Rat liver give 1 ml of henna, show minimal fibrosis & mononuclear around central vein H&E 10X

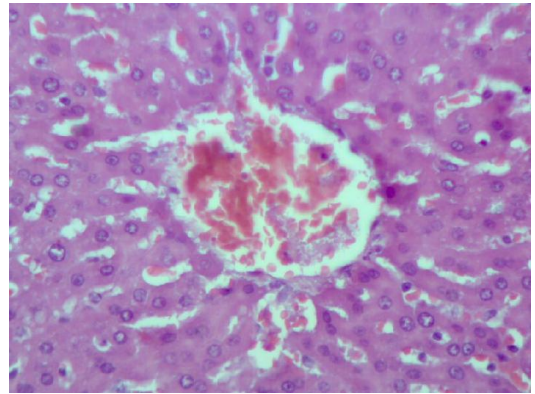


Figure 7. Rat liver give 1 ml of henna, show congestion of central vein & sinusoid H&E 10x

## Kidney

### Control Group

The histopathological examination of the kidney in *Albino Wister* rates of the control group showed the glomeruli with normal size and normal renal tubules (Fig. 7).

### Treated Group

The histological examination of the kidney of the treated group showed hemorrhage in the interstitial tissues with dilated renal tubules (Fig. 8) and vacuolation of the cells of the renal tubules (Fig. 9). The glomerulus with high cellularity of prominent Jakssta glomerulus (Fig. 10).

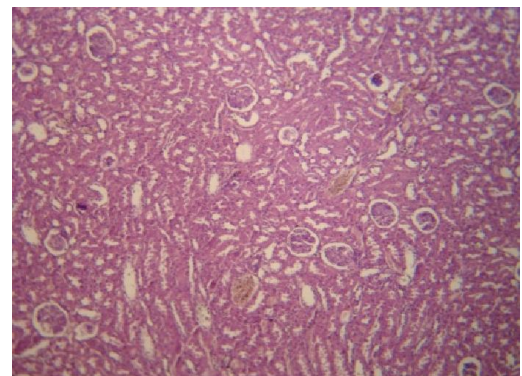


Figure 8. Glomerulus is normal size and renal tubules H&E10x

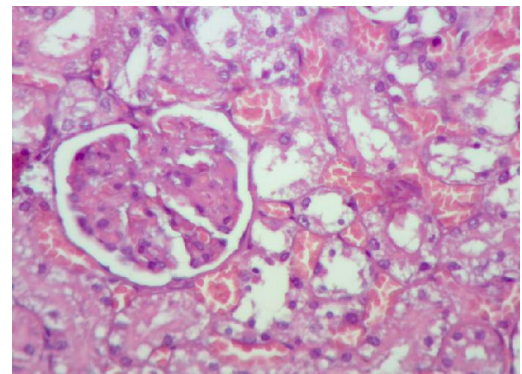
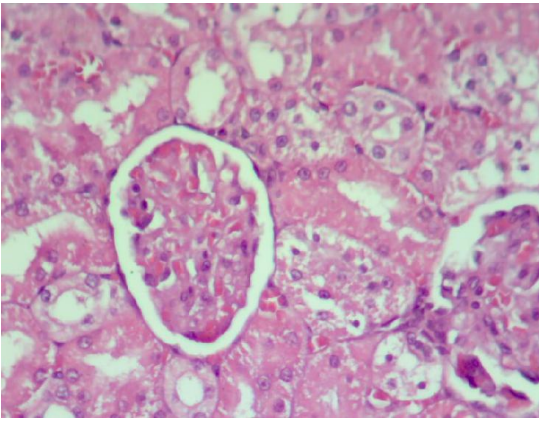


Figure 9. Rat kidney give 1 ml of henna show haemorrhage in interstitial tissue, dilation of renal tubules with vacuolated cells. H&E, 10x

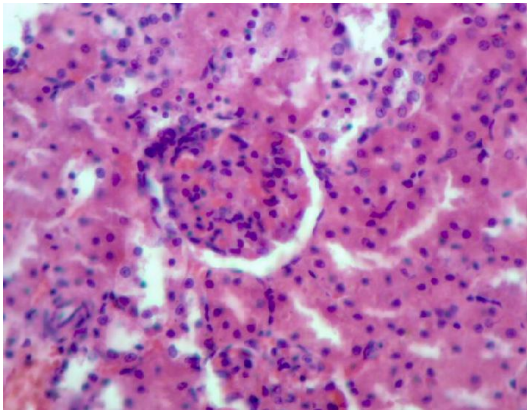


**Figure 10. Rat kidney give 1 ml of henna show vacuolated of renal tubules cells, H&E, 10X**

## Testes

### Control Group

Seminiferous tubules had been cut in transverse section. The processes of spermatogenesis and spermiogenesis are synchronized, undifferentiated germ cells were found in the basal compartment of the seminiferous tubules are called spermatogonia type A, spermatogonia type B, produce primary spermatocytes, spermatozoa (Fig. 11).



**Figure 11. Rat kidney give 1 ml of henna show glomerulus with high cellularity and prominent juxtaglomerular apparatus (H&E,X10)**

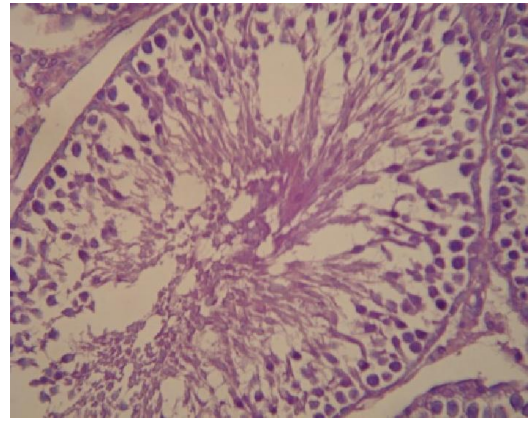
### Treated Group

The histopathological changes of testicular tissue showed suppression of spermatogenesis, giant spermatogonia and no stages or absence of primary, secondary spermatogonia (Fig. 12) as well as formation of multinucleated spermatid giant cells (Fig. 13).

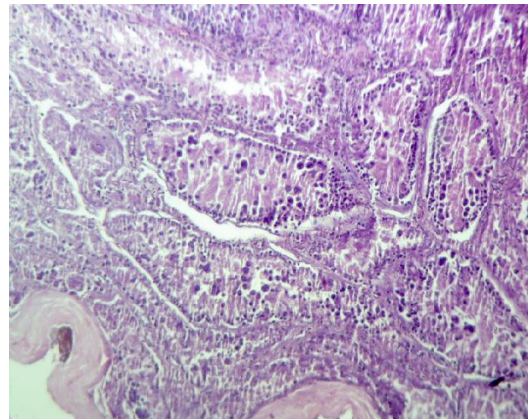
### Adrenal Gland

#### Control Group

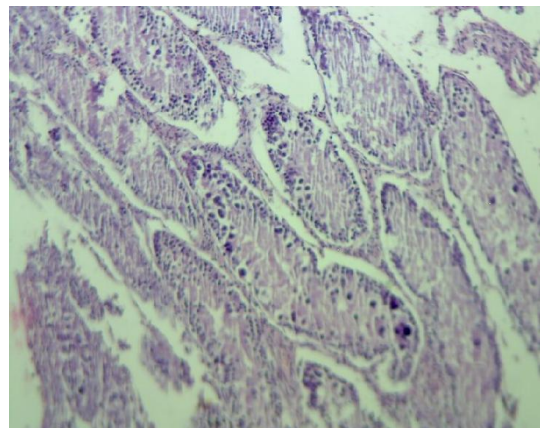
The adrenal gland had two parts; outer cortex and medulla. The cortex consisted of three histological zones which are named according to the arrangement of the secretory cells: Zona glomerulosa, zona fasciculata and zona reticularis. The medulla was a pale stained inner layer which is a dense fibrous tissue capsule (Fig. 14).



**Figure 12. Seminiferous tubule with normal tissue. (H&E,X10)**



**Figure 13. Rat testes give 1 ml of henna show testicular suppression of spermatogenesis (H&E,X10)**



**Figure 14. Rat testes give 1 ml of henna show formation of multinucleated spermatid giant cells. (H&E,X10).**

### Treated Group

The histopathological changes of the adrenal gland were represented by presence of enlargement of tissue especially in zona glomerulosa, zona fasciculata & zona reticularis (Fig.15).

### Cardiac Muscle

#### Control Group

The longitudinal section of the cardiac muscle cells showed containing of one or two nuclei and extensive cytoplasm

which branches to give the appearance of a continuous dimensional network and presence a specialized intercellular muscle cells which are intercalated discs between the ends of the adjacent cardiac muscle cells (Fig. 16).

### Treated Group

There were a swelling of cardiac muscle cells due to vacuolated appearance in the cytoplasm (Fig.17).

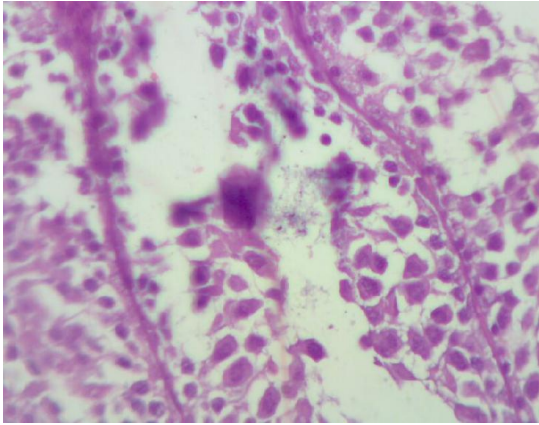


Figure 15. Rat testes give 1 ml of henna show formation of multinucleated spermatid giant cells. (H&E,X40)

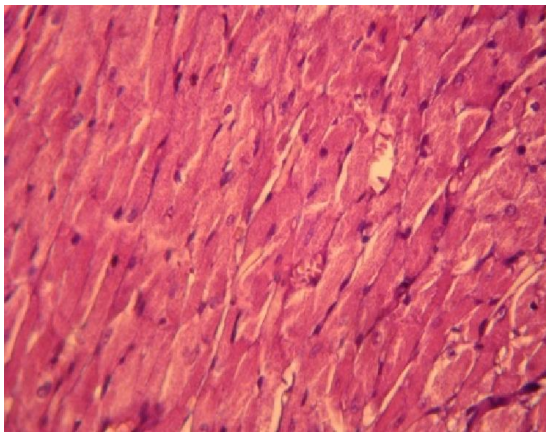


Figure 16. Cardiac muscle cells with intercellular junctions. (H&E,X10)

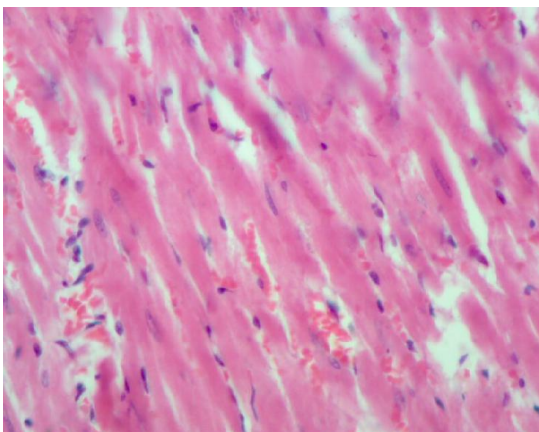


Figure 17. Rat heart muscle give 1 ml of henna vacuolation of myocardial cells. (H&E,X10)

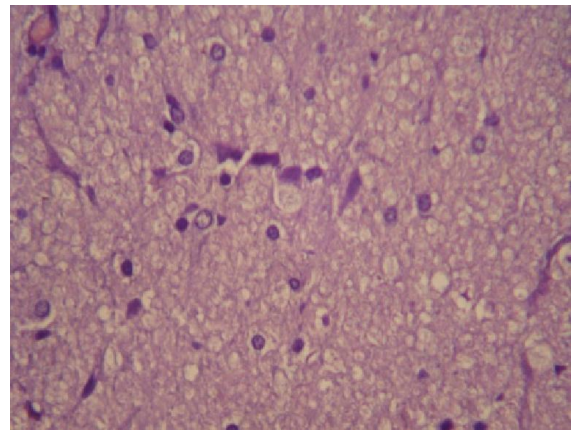


Figure 18. Sciatic nerve fiber with normal structure.(H&E,X10)

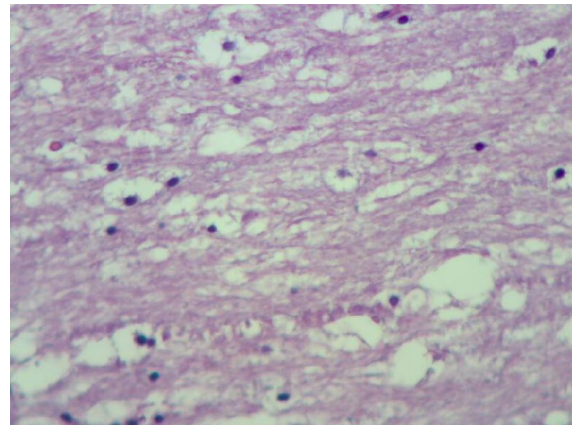


Figure 19. Rat nerve fiber give 1 ml of henna show vacuolated of nerve cells. (H&E,X10)

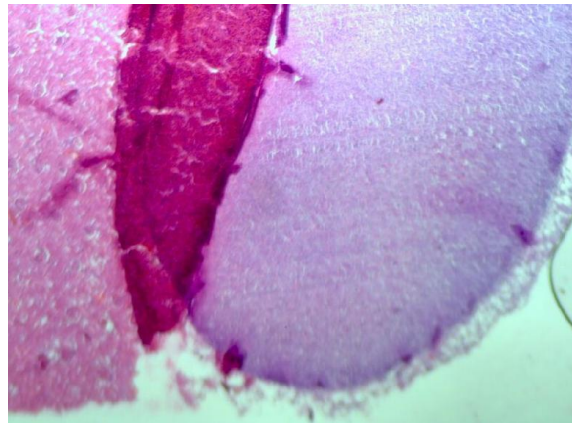


Figure 20. Rat adrenal gland give 1 ml of henna enlargement of adrenal gland. (H&E,X10)

### DISCUSSION

In this study observed clinical signs after repeated administration of henna (*lawsoniainermis*) at dose 500 mg/kg for 60 days which observed into lating period of treated agree with (Agabna *et al.*, 2014; Khanet *al.*, 2009 and Selvanayakiand Ananthi, 2012) and in the histological features of rat tissue due to their wide spread use of medicinal plants in alterative medicine toxicological assessment becomes

imperative in order to arrive at potencies that can be considered as safe formulations for clinically efficient remedies (Chang and Suzuka, 1982 and Taylor *et al.*, 2004). The result of all scientific studies of henna as plant or flowers have effects on human or animals with notable exceptions (Dikshit *et al.*, 2000), histopathological effect of 500 mg/kg of henna in stomach for 60 days due to saponinos, tannins and flavinoids are known ulcer productive agents or materials of henna especially phenolic content (Chun *et al.*, 1998 and AlferahMosaid, 2012) some researchers were signal there are somatic, thermal, toxic and traumatic effect of administration of henna in stomach layer (AwekaAdjene, 2013). As well as 2-hydroxyl 1,4-naphthoquinone was not only haemolytic agent but anephrotoxin caused enlargement elevated plasma levels of urea and necrosis of renal tubules.

The relationship between the *in vivo* toxic effects of these naphthoquinones and previously reported data on their *in vitro* cytotoxic action (Munday *et al.*, 1991) the greater chemical reactivity of nanomaterials can result in increased production of reactive oxygen species (ROS) including free radicals, which is one of the primary mechanism of nanoparticle toxicity. It may result in oxidative stress, inflammation and consequent damage to proteins, cell membranes and DNA as well as have steroids and haemolytic effect that is mean caused degree of toxicity which effect on circulation lead to degeneration in muscle and sciatic nerve with characteristic features of severe angioneurotic edema (Sauriasori *et al.*, 2007). Liver and kidney were important organs of metabolic, detoxification, storage and excretion of xenobiotics and their metabolites and especially vulnerable to damage.

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