*Haryana Vet.* (June, 2024) **63(1),** 57-64 ISSN 0033-4359 (P) **Research Article**

# IMIDACLOPRID INDUCED HISTOPATHOLOGICAL ALTERATIONS, OXIDATIVE STRESS IN LIVER AND HEPATOPROTECTIVE ROLE OF *WITHANIA SOMNIFERA* IN FEMALE ALBINO WISTAR RATS

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## ABSTRACT

Female albino *Wistar* rats were orally administered with 30 mg/kg body weight/day of imidacloprid for 30 days duration to evaluate the histopathological changes and to estimate the oxidative stress parameters in liver. 1 g *Withania somnifera* root powder was mixed in 1 kg of feed to evaluate its ameliorative effects in rats induced with imidacloprid. Total 48 rats in current experiment were grouped into 4 with 12 rats in every group. Group 1 serve as control, group 2 as imidacloprid control, group 3 treated with *W. somnifera* and group 4 treated with both imidacloprid and *W. somnifera* root powder. The relative liver weights were significantly ($P < 0.05$) decreased in imidacloprid control. There was a significant ($P < 0.05$) increase in Thio Barbituric acid Reactive Substance (TBARS) levels and decrease in reduced glutathione (GSH) and superoxide dismutase (SOD) levels in imidacloprid group as compared to control rats. Whereas in group 4, a significant ($P < 0.05$) elevation in GSH, SOD levels and reduction in TBARS levels were noticed compared with the group 2. Histopathological alterations were severe in group 2 and mild in group 4. These results were suggestive of *W. somnifera* acts as a moderate hepatoprotective agent.

**Keywords:** Hepato toxicity, Imidacloprid, Oxidative stress, *Withania somnifera*

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Imidacloprid (IMI) is a neonicotinoid type of insecticide belongs to chloronicotinyl nitroguanidin family (Wismer, 2004; Tomlin, 2006). In 1994, the United States Environmental Protection Agency (USEPA) registered the use of IMI as insecticide in Unites states (Hovda and Hooser, 2002). IMI is used on agriculture crops to kill sucking insects in soil and in veterinary, it is used to treat flea infestation of pets. It is also useful to kill termites. It is applied to structures, crops, soil and also used for seed treatment (Tomlin, 2006; Fossen, 2006). IMI irreversibly binds to nicotinic acetylcholine receptors at post-synaptic junctions in central nervous system of insects and results in inability of neurotransmission (Ware and Whitacre, 2004; Buckingham *et al.*, 1997; Matsuda and Sattelle, 2005; Sheets, 2001).

*Withania somnifera* (WS) roots and leaves were used in therapy of various diseases. The potential of *W. somnifera* in curing hepatotoxicity was proven in various trials (Bhattacharya *et al.*, 2000). Active principles of Ashwagandha have been shown to have significantly reduce the stress (Bhattacharya *et al.*, 1987; Kumar *et al.*, 2013). In present experiment, IMI induced hepatotoxicity and its amelioration with WS was studied. Since the liver is a major organ involved in detoxification of drugs, the current study was planned to investigate the acute liver injury caused by IMI which is the most commonly used pesticide in Indian agriculture. *W. somnifera* due to its well known antioxidant activity it was used as an ameliorative agent to determine its hepatoprotective role against IMI induced toxicity.

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## MATERIAL AND METHODS

200-250 g weighing female rats of wistar strain in forty-eight (48) number were obtained from Sanzyme Private Limited (Pvt. Ltd.), Gagan Pahad, Hyderabad for present study. The solid bottom polypropylene cages were used to house the rats at lab animal house, College of Veterinary Science, Rajendranagar, Hyderabad and they were partly filled with sterile rice husk (bedding material). Temperature was maintained in between 20-22º C by using air conditioner in lab animal house throughout the experiment. The rats were allowed to feed on standard pellet diet (procured from Vyas Labs, Uppal, Hyderabad) and given with adequate amounts of deionized water. Imidacloprid was procured from Tropical Agrosystem India Pvt. Ltd., Chennai, Tamil Nadu and *W. somnifera* was obtained from Herboleaf Organic, Haryana. The rats were grouped into 4 each with 12 rats. Group 1 serve as control, group 2 induced with imidacloprid @ of 30 mg/kg body weight/day orally for 30 days. The IMI dose was calculated as three times to that of NOEL (Kapoor *et al.*, 2010). Group 3 was fed with *W. somnifera* @ of 1 g/ kg feed. Both imidacloprid and *W. somnifera* (dose as above) were given to group 4 for 30 days. W. somnifera dose was calculated as half of the NOAEL (Patel *et al.*, 2016). On 16$^{th}$ day of treatment, half rats from all groups were sacrificed and leftover rats were sacrificed on 31$^{st}$ day by cervical

dislocation according to the CPCSEA guidelines. Necropsy/ postmortem examination of rats was done according to the standard procedure described by Feinstein (2000). Gross examination of liver was carried out and then they were weighed immediately using electronic balance. The liver samples were collected in 10 % neutral buffered formalin for microscopic examination of tissue alterations and also stored at -20º C to investigate the changes in oxidative stress parameters {(reduced glutathione (GSH), superoxide dismutase (SOD) and thiobarbituric acid reactive substance (TBARS)}. The CCSEA guidelines were followed to conduct the experiment and it was approved from the Institutional Animal Ethics Committee (IAEC-No.7/22/C.V.Sc., Hyd. /IAEC).

**Relative organ weight:** Relative organ weight was expressed as per cent (%) of body weight in relation to body weight (Michael *et al.*, 2007).

$$\text{Relative liver weight} = \frac{\text{Liver weight}}{\text{Body weight}} \times 100$$

**Organ antioxidant profile:** One gram of liver tissue sample with 10 mL of 0.2 M Tris HCl buffer (pH 7.2) was taken into a tissue homogenizer to get 10 per cent homogenate to carry out all the tissue antioxidant parameters. The tissue oxidation was measured by reduced glutathione (GSH), the reaction of the lipid peroxidation end products like malondialdehyde (MDA) with thiobarbituric acid reactive substances (TBARS) and activity of superoxide dismutase (SOD) to know the antioxidant status. GSH, TBARS and SOD were estimated according to the procedures described by Moron *et al.* (1979); Balasubramanian *et al.* (1988); Madesh and Balasubramanian (1998), respectively.

**Histopathology:** The tissues samples of liver were fixed, washed, dehydrated, cleared, embedded in paraffin and blocks were made. The paraffin blocks were sectioned (5 m) and stained with Hematoxylin and Eosin (H&E) for histopathological examination as per the standard protocol (Luna, 1968).

**Statistical analysis:** Statistical Package for Social Sciences (SPSS) version 20.0 was adopted for analyzing the data obtained. One-way ANOVA was applied and Duncan's multiple comparison test was used to test the differences between the means and significance level was set at $P<0.05$.

## RESULTS & DISCUSSION

**Gross pathology:** The liver revealed a normal gross appearance in control and group 3 on 16th and 31st day of experiment (Fig. 1a, b, c & d). Mild congestion of liver was observed in group 2 rats on day 16 (Fig. 1e). Severe congestion of liver was noticed in rats belonging to group 2 on day 31 (Fig. 1f & g). Mild congestion of liver was observed on 16th and 31st day of experiment in group 4 (Fig. 1h & i).

These changes suggest the toxic effect of IMI on liver. Congestion might be due to vascular changes. Similar findings in liver were observed by Wankhede et al. (2017) in Japanese quails and by Babu *et al.* (2015) in chicken. In contrast to this, Palkhade *et al.* (2018) reported non-significant gross lesions in liver of rats treated with IMI.

**Relative liver weight (% of body weight):** In group 2, the mean values of relative liver weights (% of body weight) were significantly ($P < 0.05$) decreased when compared with group 1 on 16th and 31st day of experiment. The mean values of relative liver weights between groups 1, 3 and 4 revealed no significant difference on 16th and 31st day of experiment (Table 1).

**Table 1. Relative liver weight (% body weight) in different groups**

| Group | Day 16 | Day 31 |
|---|---|---|
| Group- 1 | $4.04 \pm 0.10^{a}$ | $4.22 \pm 0.11^{a}$ |
| Group- 2 | $3.43 \pm 0.19^{b}$ | $3.02 \pm 0.07^{b}$ |
| Group- 3 | $4.08 \pm 0.14^{a}$ | $4.10 \pm 0.08^{a}$ |
| Group- 4 | $3.92 \pm 0.08^{a}$ | $4.09 \pm 0.10^{a}$ |

Values are Mean + SE (n = 6); One-way ANOVA
Means with different superscripts in a column differ significantly at $P<0.05$.

Al- Dabbagh and Al-Bahadyli (2015) was also reported the similar findings. The decreased liver weight in current study may be due to hepatotoxicity induced by IMI as degeneration of hepatocytes was evidenced in histopathology in group 2 rats. Contrary to this, a significant increase in relative liver weight was reported by earlier researchers (Bhardwaj *et al.*, 2010; Arfat *et al.*, 2014; Chakroun *et al.*, 2017; Lohiya *et al.*, 2017). Whereas, no significant difference in relative liver weights was documented by Bagri *et al.* (2013); Preeti *et al.* (2014); Vohra *et al.* (2014); Vohra and Khera (2015); Palkhade *et al.* (2018). In group 4 rats, the relative liver weights did not differ significantly from control group animals. This might be due to potential hepatoprotective (Saxena et al., 2007) role of WS due to its ability to scavenge free radicals (Sumathi *et al.*, 2007). Repair and regeneration of hepatocytes in group 4 rats was evidenced in light microscopy is a strong indication of WS as a hepatoprotective compound.

**Tissue anti oxidant profile**

**Reduced Glutathione Concentration:** GSH (nmol/mg tissue): The mean values of GSH concentrations (nmol/mg tissue) in liver tissues in group 2 and group 4 were significantly

**Table 2. Liver antioxidant profile in different groups**

| Group | GSH (n mol/mg tissue) | | TBARS (n mol/g tissue) | | SOD (U/mg protein) | |
|---|---|---|---|---|---|---|
| | Day 16 | Day 31 | Day 16 | Day 31 | Day 16 | Day 31 |
| Group 1 | 170.95±0.79[a] | 169.03±0.71[a] | 169.55±2.41[c] | 173.40±2.69[c] | 20.45±0.55[a] | 21.00±0.63[a] |
| Group 2 | 139.77±0.88[c] | 129.92±0.72[c] | 184.35±1.12[a] | 186.93±0.83[a] | 14.58±0.60[c] | 11.15±0.33[c] |
| Group 3 | 169.48±1.38[a] | 169.62±0.54[a] | 168.68±2.67[c] | 175.68±1.47[c] | 20.28±0.55[a] | 20.85±0.60[a] |
| Group 4 | 159.85±0.79[b] | 152.52±1.13[b] | 176.70±1.36[b] | 182.12±0.38[b] | 17.58±0.67[b] | 15.83±0.28[b] |

Values are Mean + SE (n = 6); One way ANOVA; Means with different superscripts in a column differ significantly at P<0.05.

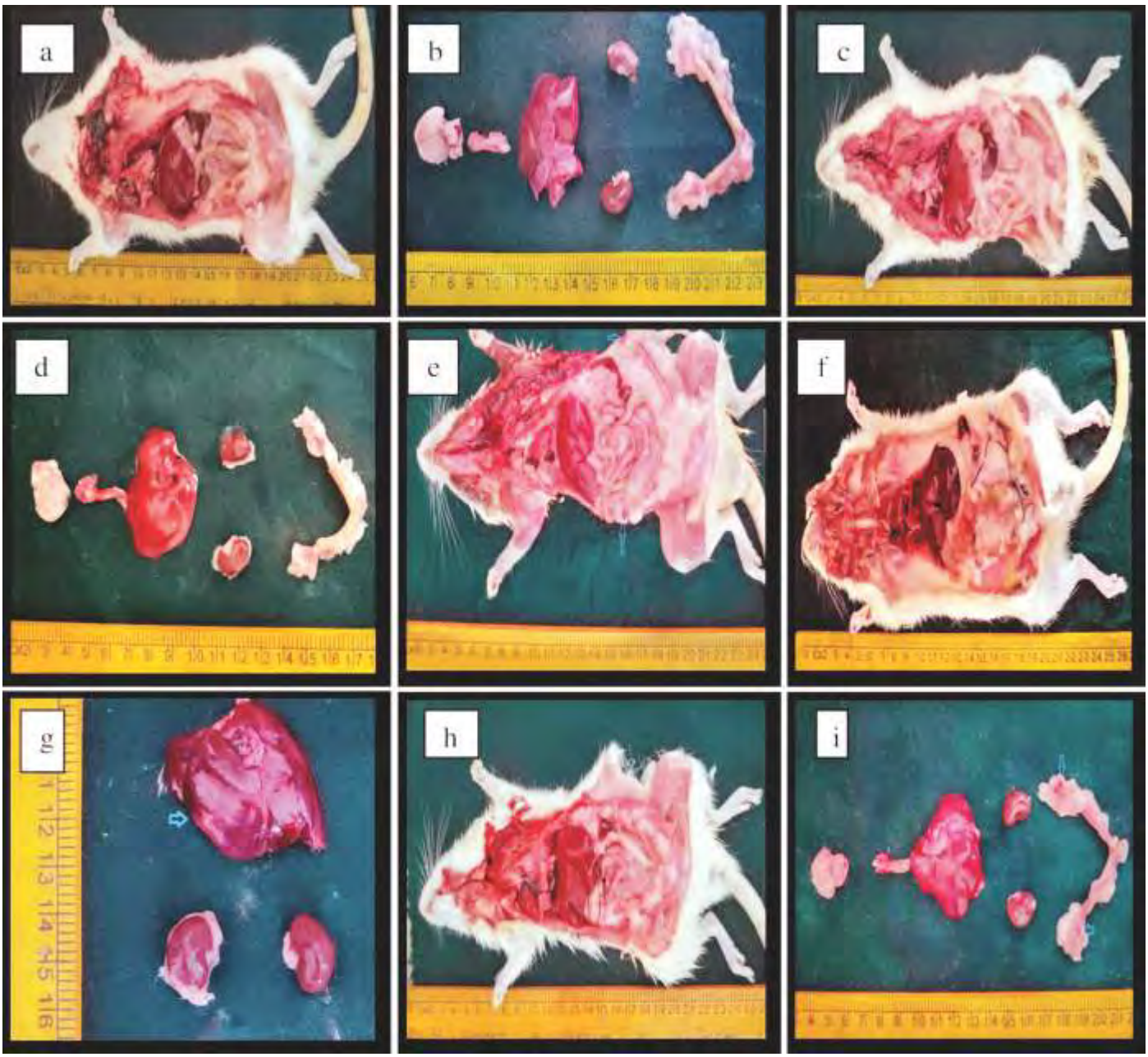


Fig 1. Photomicrograph showing
[a. grossly normal appearance of liver (Group 1, Day 16); b. grossly normal appearance of liver (Group 1, Day 31);
c. grossly normal appearance of liver (Group 3, Day 16); d. grossly normal appearance of liver (Group 3, Day 31);
e. mild congestion of liver (Group 2, Day 16); f. severe congestion of liver (Group 2, Day 31);
g. severe congestion of liver (Group 2, Day 31); h. mild congestion of liver (Group 4, Day 16);
i. mild congestion of liver (Group 4, Day 31)

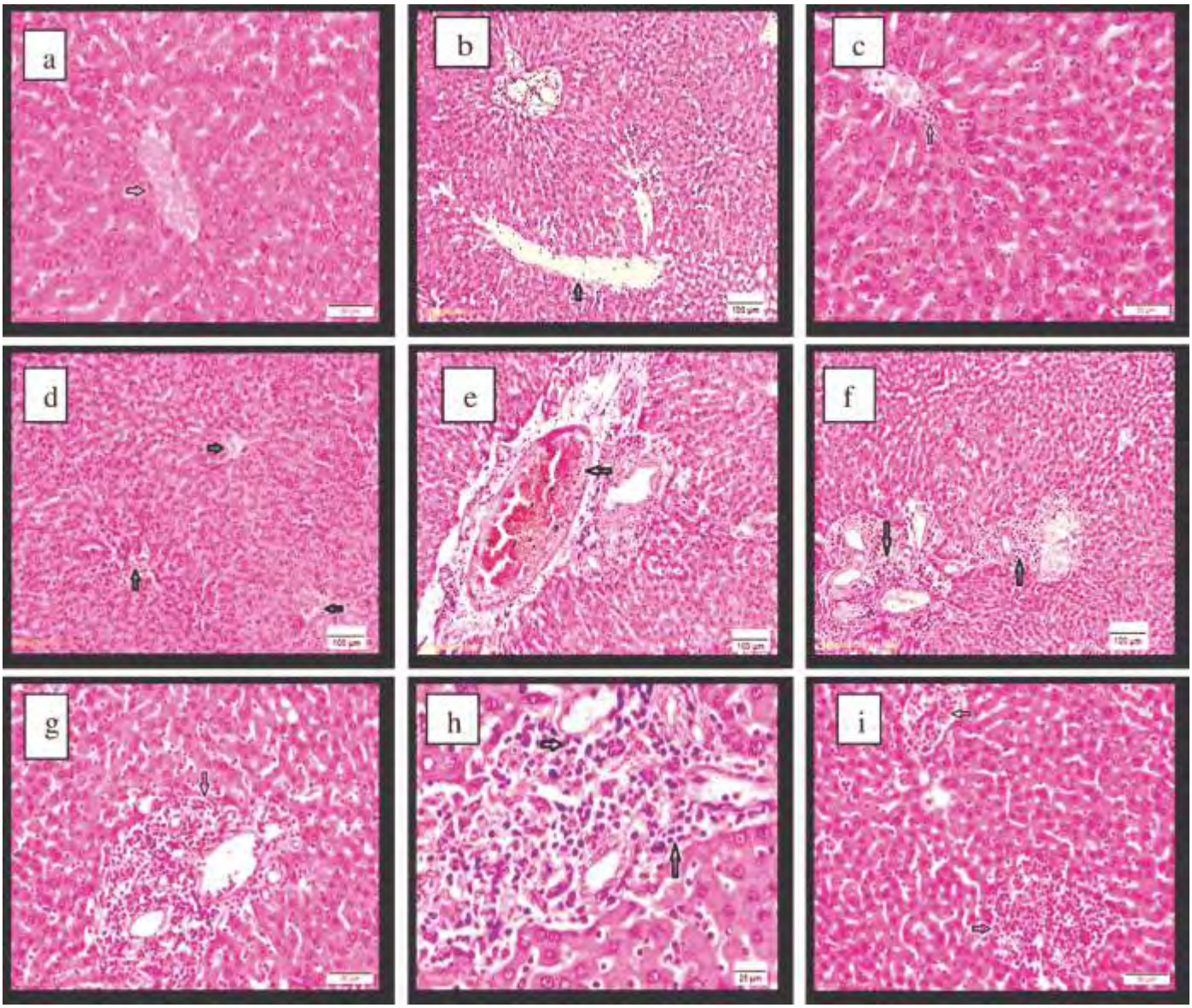


Fig 2. Photomicrograph of liver showing
[a. moderate dilation and congestion of the central vein and cloudy swelling of hepatocytes (Group 2, Day 16): H&E ×200
b. moderate dilation of sinusoids and vacuolation of hepatocytes (Group 2, Day 16): H&E ×100
c. mild proliferation of Kupffer cells around the central vein and hyperchromatic nuclei in hepatocytes (Group 2, Day 16): H&E ×200
d. moderate sinusoidal haemorrhage (Group 2, Day 16): H&E ×100
e. severe congestion of portal vein and hyperplasia of bile duct (Group 2, Day 31): H&E ×100
f. moderate infiltration of mononuclear cells around the portal triad and proliferation of bile duct epithelium (Group 2, Day 31): H&E ×100
g. moderate infiltration of mononuclear cells around the portal triad (Group 2, Day 31): H&E ×200
h. moderate infiltration of mononuclear cells around the proliferating bile ducts (Group 2, Day 31): H&E ×400
i. moderate infiltration of mononuclear cells in the sinusoids (Group 2, Day 31): H&E ×200]

($P < 0.05$) reduced in comparison with groups 1 and 3 on 16$^{th}$ and 31$^{st}$ day of experiment. In group 4, a significant ($P < 0.05$) increase in liver GSH concentration was observed as compared to imidacloprid group and however, group 3 values were non-significant as compared with control (Table 2).

Similarly decrease in GSH levels in liver due to IMI administration were also reported by Babu *et al.* (2014); Chakroun *et al.* (2017); Kapoor *et al.* (2010); Lohiya *et al.* (2017). These results are suggestive of oxidative stress induced by IMI. The decreased GSH levels may be associated with excess production of ROS released from damaged mitochondria. The ROS might have enhanced the lipid peroxidation of cell membranes and led to increased consumption of GSH for detoxification of peroxides, thereby led to exhaustion of the GSH stores in liver of group 2 rats. In group 4 rats, liver showed a significant ($P < 0.05$) increased in GSH levels in comparison with group 2 rats on 16$^{th}$ and 31$^{st}$ day of experiment. The WS might have interfered in restoration

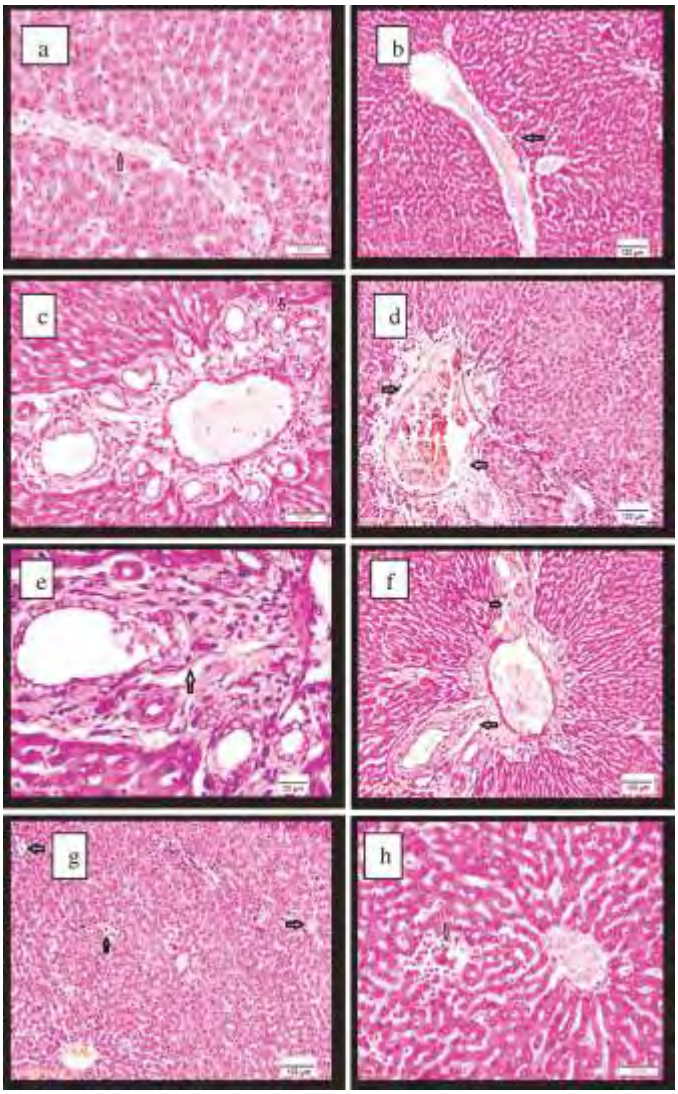


Fig 3. Photomicrograph of liver showing
[a. severe dilation and congestion of sinusoids (Group 2, Day 31): H&E ×200; b. severe dilation and congestion of central vein and mild infiltration of mononuclear cells around the central vein (Group 2, Day 31): H&E ×100; c. bile duct hyperplasia and degenerating hepatocytes with pyknotic nuclei (Group 2, Day 31): H&E ×200; d. mild fibrous tissue proliferation in the portal triad and severe congestion of portal vein (Group 2, Day 31): H&E ×100; e. mild fibrosis of the portal triad (Group 2, Day 31): H&E ×400; f. degenerating bile ducts, mild fibrosis in the portal area and shrunken hepatic cords around the portal triad (Group 2, Day 31): H&E ×100; g. focal areas of necrosis (Group 2, Day 31): H&E ×100; h. focal necrosis with loss of hepatocytes and their replacement with inflammatory cells (Group 2, Day 31): H&E ×200]

of GSH levels in group 4 rats due to neutralization of ROS. Hypothetically, there are two reasons for restoration of GSH, first reason could be due to decrease in GSH degradation and second reason might be due to increased biosynthesis of GSH by transcriptional induction of genes responsible for GSH synthesis. This hypothesis is supported by Babu *et al.* (2014) who also reported increased GSH levels in liver tissue of layer birds given with WS along with IMI.

**Thiobarbituric Acid Reactive Substance Concentration:** TBARS (nmol/g tissue): Significantly ($P < 0.05$) increased

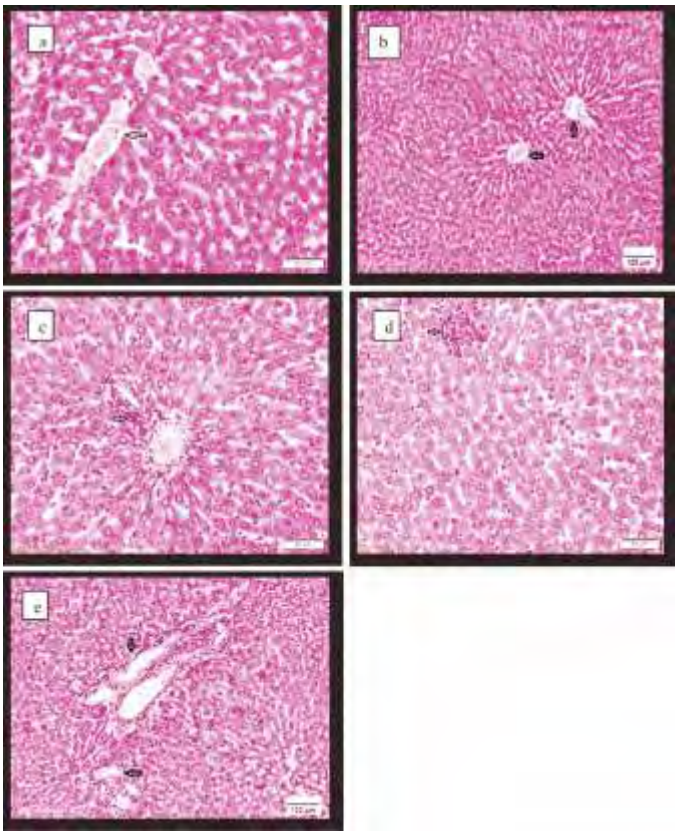


Fig 4. Photomicrograph of liver showing
[a. mild dilation and congestion of the sinusoids (Group 4, Day 16): H&E ×200; b. mild congestion of the central vein and mild degeneration of the hepatocytes (Group 4, Day 16): H&E ×100; c. mild congestion of portal vein and infiltration of mononuclear cells around the portal triad (Group 4, Day 31): H&E ×200; d. mild infiltration of mononuclear cells in the sinusoids (Group 4, Day 31): H&E ×200; e. bile duct proliferation and mild infiltration of mononuclear cells in the portal area (Group 4, Day 31): H&E ×100]

mean values of TBARS concentrations (nmol /g tissue) in liver tissues were recorded in group 2 and group 4 when compared with group 1 and group 3 on 16$^{th}$ and 31$^{st}$ day of experiment. The mean values of liver TBARS were significantly ($P<0.05$) decreased in group 4 when compared with group 2 and there was no significant difference between groups 1 and 3 was observed (Table 2).

Kapoor *et al.* (2010); Mohany *et al.* (2011); Mohany *et al.* (2012); Babu *et al.* (2014); Chakroun *et al.* (2017); Lohiya *et al.* (2017) also reported a significant ($P < 0.05$) increase in TBARS levels in liver tissue due to IMI induced toxicity. The increased TBARS levels suggests the excessive ROS production by IMI and its interaction with cell membranes led to enhanced peroxidation of poly unsaturated fatty acids (PUFA) in cell membrane which can be measured by using malondialdehyde (MDA) (Tsikas, 2017). The increased MDA levels may also be a consequence of depletion of GSH and SOD levels, because the main defense to alleviate oxidative stress and repair the damaged macromolecules in the cells is by enzymatic (SOD) and non-enzymatic antioxidants (GSH), which are involved in scavenging of

the ROS (Birben *et al.*, 2012). In present study, the GSH and SOD levels were significantly decreased in group 2 rats which led to a significant increase in TBARS levels in IMI treated rats. The TBARS levels in liver were significantly ($P<0.05$) decreased in group 4 rats when compared with group 2 rats on 16th and 31st day of experiment. This might be due to antiradical and antioxidant properties of WS due to the presence of alkaloids and steroidal lactones which are potent free radical scavengers (Saleem *et al.*, 2020). Similarly, the decreased levels of TBARS in liver due to WS administration were noticed by Babu *et al.* (2014) against the IMI induced toxicity in layer chicken.

**Superoxide Dismutase Activity:** SOD (U/mg protein): The mean value of SOD activity (U/mg protein) in liver tissue in group 2 and group 4 were significantly ($P < 0.05$) decreased when compared with group 1 and group 3 on 16th and 31st day of experiment. In group 4, a significant ($P < 0.05$) increased in liver SOD activity in comparison to group 2 was recorded and group 3 values was non-significant from control group (Table 2).

Similar observations were reported by Kapoor *et al.* (2010); Chakroun *et al.* (2017); Lohiya *et al.* (2017). SOD constitutes the first line of defense against the deleterious effect of oxygen derived free radicals in the cell by catalyzing the dismutation of superoxide radicles, led to formation of hydrogen peroxide and oxygen (Yasui and Baba, 2006). The reduction in SOD activity in present study might be due to the excess production of superoxide radicles by IMI resulted in increased utilization of SOD in conversion of superoxide radicles to hydrogen peroxide, led to depletion of SOD and oxidative damage to liver tissue in group 2 rats. SOD activity was significantly ($P < 0.05$) increased in group 4 rats in comparison with group 2 rats on 16th and 31st day of experiment. This might be a consequence of antioxidant activity of WS that might have terminated the free radical chain reaction.

**Histopathology:** The liver sections of group 2 rats on 16th day of experiment showed moderate dilation and congestion of the central vein (CV), cloudy swelling and vacuolation of hepatocytes, moderate dilation and haemorrhage of sinusoids, mild proliferation of Kupffer cells around the CV and hyperchromatic nuclei in hepatocytes (Figs. 2a, b, c and d). On 31st day, severe congestion of portal vein (PV), hyperplasia of bile duct (Fig. 2e), mild to moderate infiltration of mononuclear cells (MNCs) around the portal triad, CV, proliferating bile ducts and also in the sinusoids (Figs. 2f, g, h and i) were observed. Other sections showed severe dilation and congestion of sinusoids and CV (Figs. 3a and b). Degenerating hepatocytes with pyknotic nuclei and mild fibrosis of the portal triad (Figs. 3c, d and e) were also observed. Some other sections revealed degenerating bile ducts, shrunken hepatic cords around the portal triad, focal areas of necrosis with loss of hepatocytes and their replacement with inflammatory cells (Figs. 3f, g and h). Group 4 rat liver sections revealed mild dilation and congestion of the sinusoids, mild degeneration of the hepatocytes and mild congestion of the CV (Figs. 4a and b) on 16th day of experiment. Mild congestion of PV, infiltration of MNCs around the portal triad and in the sinusoids and bile duct proliferation (Figs. 4c, d and e) were noticed on 31st day.

The histopathological lesions in IMI treated rats were in accordance with the findings of El-Feki *et al.* (2008); Bhardwaj *et al.* (2010); Mohany *et al.* (2011); Vohra and Khera (2015), Chakroun *et al.* (2017); Lohiya *et al.* (2017); Mehmood *et al.* (2017); Hassan *et al.* (2019) in rats and also with the observations of Arfat *et al.* (2014); Kumar *et al.* (2014); Al-Dabbagh and Al-Bahadyli (2016) in mice. Similar lesions were reported by Kammon *et al.* (2010); Komal *et al.* (2016); Ravikanth *et al.* (2018) in chicken and by Eissa (2004); Wankhede *et al.* (2017) in Japaneese Quail. These changes could be due to local concentration of IMI metabolites in liver and the liver is the major site of metabolism including detoxification as well as activation of many foreign compounds (Guyton, 1995). The liver is first organ to be exposed to ingested toxins due to its portal blood supply (Popp and Cattley, 1991). Sheets (2001) reported that the liver is the principal target organ in IMI toxicity. The vacuolation of hepatocytes might be due to the retention of fluid inside the cell resulting in cloudy swelling which might be due to reduction of energy necessary for regulation of ion concentration of the cells or due to hypoxia or may be related to oxidative stress (Elwi, 1967). The haemorrhages are suggestive of damage to endothelial lining of microcapillaries by IMI. Infiltration of MNCs suggest the process of inflammation in liver parenchyma as a part of defense mechanism against the irritation caused by accumulated toxic material and necrosed tissue, which may have activated the proliferation of Kupffer cells (El-Feki *et al.*, 2008). Hepatic necrosis might be due to oxidative stress induced by IMI, which further involved in cellular protein degradation. The sinusoidal spaces were dilated due to shrinkage and necrosis of hepatic cords (Wankhede *et al.*, 2017). These findings are supported by increased levels of TBARS and decreased levels of GSH and SOD in liver of IMI treated rats in the present experiment. Minimal lesions were observed in group 4 liver sections in comparison to toxic group rats. These findings are in line with the findings of Babu *et al.* (2015) in layer chicken. It

might be due to alleviation of inflammation, enhancement of antioxidant enzymes and thiols by WS that resulted in mild repair and regeneration of damaged hepatocytes.

It could be concluded that severe alterations in relative organ weight, gross pathology, histopathology and oxidative stress parameters were noticed in liver of rats administered with IMI which indicates the hepatotoxicity due to production of reactive oxygen species by IMI. Whereas, simultaneous administration with WS resulted in moderate improvement in antioxidant parameters and liver histology due to free radical scavenging activity of WS. Hence WS can be used as ameliorating agent to alleviate the hepatotoxicity induced by IMI.

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