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Oxidative and Histopathological Damage in Liver and Plasma of Rats after Sulphur Dioxide Inhalation

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Abstract: Effect of different concentrations of SO_2 on antioxidant enzymes and histopathology was investigated in liver and plasma of rats of both the sexes under natural and experimental conditions. Naturally exposed, house rat, *Rattus rattus* (Group I) were collected from urban residential areas at Ludhiana, India and acclimatized for one month in the laboratory. Laboratory bred house rats were divided into four groups. Each group was further subdivided into two subgroups having six male and six female rats. Group II (control rats) were exposed to filtered air in one m^3 exposure chamber for five hours day^{-1} for 28 days; Group III, Group IV and Group V were exposed to 5, 10 and 15 ppm of SO_2 respectively. All the three concentrations of SO_2 showed significant increase in lipid peroxidation (LPO) levels followed by significant decrease in the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR) and non-significant decrease in glutathione peroxidase (GPx) and glutathione (GSH) levels in plasma and liver of rats of both the sexes. The increase in LPO levels was more significant at 15 ppm concentration as compared to other two concentrations in all the organs. The results showed that SO_2 is an oxidative damage causing agent to vital organs of rats.

Key words: Sulphur dioxide (SO_2), rats, liver, histopathology, oxidative damage, air pollutant.

In most of the developing countries, air pollution is one of the major problems that arise due to industrial activities and fossil fuel consumption. Sulphur dioxide (SO_2) is colourless, toxic and highly reactive air pollutant formed by the coal fired power plants, industrial processes and motor vehicle operations and present in high concentrations in urban and industrial locations and produces damaging effect on human health (Shen *et al*., 2020). High concentrations of SO_2 can affect functioning of vital organs of animals and humans (Rall, 1974). SO_2 and its derivatives such as sulphites, bisulphites are very toxic to respiratory system and can cause allergic reactions in the bronchus and can also act as carcinogen and mutagen. However, very little information is available on the effect of SO_2 on oxidative stress and antioxidant parameters on various organs of mammals except the effect of SO_2 on brain and RBCs of rats and guinea pigs (Li *et al*., 2019).

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SO_2 may cause oxidative stress to tissues and organs formed by free radical production during oxidation process (Shi and Mao 1994, Meng and Zhang 1999, Meng, 2003). Lipid peroxidation is believed to be involved in several diseased states, such as neurodegenerative, hepatic, reproductive and aging processes (Baynes, 1991; Lester, 1995). SO_2 inhalation can cause toxic symptoms, thickening of wall of respiratory tract, fatigue, diarrhea and changes in sensory organ receptors (Chance *et al*., 2020). Several studies revealed that SO_2 at 10 ppm concentration increased the lipid peroxidation in liver and RBC's in rats (Etlik *et al*., 1995; Gumuslu *et al*., 1998 and Yargicogvlu *et al*., 1999). Normal cell defenses against free radicals include enzymatic and non-enzymatic mechanisms including SOD, GPx, CAT, and GSH (Gumuslu *et al*., 1998) and other antioxidant enzymes. However, oxidative damage might occur when oxidative stress levels are raised or when antioxidant capability is reduced. Numerous injury and disease states have been linked to the pathogenesis of free radical-induced oxidative damage (Freeman and Crapo, 1982; Max, 1987). Exposure to SO_2 can lead to high tissue lipid peroxidation in liver and lungs of rats and this may be due to high concentration of easily per-oxidizable fatty acids in these tissues (Al-Kindi *et al*., 2020).

In the present study attempt was made to find out the effect of increased level of SO_2 on oxidative stress in liver of rats. Liver tissue was selected because it shows high rate of free radical generation. Accordingly three doses of SO_2 i.e., 5, 10 and 15 ppm were taken and these were above the maximum industrial permissible limits of SO_2 present in the atmosphere. The SO_2 concentration of 5 ppm and 10 ppm represents 10 to 20-fold greater values than the typical urban concentration (0.5 ppm) and is known to induce harmful effects to respiratory system of healthy individuals. Third concentration was 15 ppm which is beyond the natural exposure and is used to examine the effects of this higher concentration of SO_2 on the health of individuals.

Materials and Methods

Rats of both the sexes in the weight range of (100-150 g) were considered for study. Naturally exposed (Group I), house rat, *Rattus rattus* were collected from urban residential areas of Ludhiana, India and acclimatized for 1 month in the laboratory. Laboratory bred rats were divided into four groups. Each group was further subdivided into two subgroups having six male and six female rats. Group II (control rats) was exposed to filtered air in one m^3 exposure chamber for five hours day^{-1} for 28 days; Group III, Group IV and Group V were exposed to 5, 10 and 15 ppm of SO_2 respectively (SO_2 gas cylinders of required ppm were purchased from the local market, Ludhiana, Punjab, India). SO_2 gas was provided to treated rats through a tube located at the top of each chamber and was distributed with the help of a fan in each chamber. Rats were kept in cages under standard conditions of humidity and temperature with light-dark cycle. Food (loose mixture of cracked wheat grains, powdered sugar and edible vegetable oil in ratio 96:2:2) and water were provided to control and treated rats *ad libitum*. The weight of individual rat was recorded weekly. After 28 days of exposure and deprivation of food for 18 hours rats were dissected. The experiment was performed after the approval of Institutional Animal Ethical Committee, Guru Angad Dev Veterinary and Animal Sciences University Ludhiana, India under protocol No. (GADVASU/2022/IAEC/64/17) dated March 11, 2022.

Assay of antioxidant enzymes

Blood plasma and liver were removed and weighed after dissecting the test animals. The tissues were sheared in 0.9% saline (chilled) and homogenization was done in 0.1 M phosphate buffer (pH 7.4). The homogenates were centrifuged for 30 min at 1000 rpm at 4°C to obtain supernatants. The blood plasma and tissue supernatants were used for the assay of different antioxidant enzymes like superoxide dismutase (SOD), glutathione S-transferase (GST), catalase (CAT), glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR) and lipid peroxidation (LPO) following the standard methodology given by various scientists for estimation of these enzymes Marklund and Marklund (1974), Habig *et al.* (1974), Hafeman *et al.* (1984), Carlberg and Mannervik (1985), Aebi (1983), Stocks and Dormandy (1971) and Jollow *et al*. (1974) respectively. In the estimations of all the antioxidant enzymes the total soluble protein content was estimated by Lowry *et al*.

Table 1. Effect of SO_2 on net body weight and weight of liver of male and female rats

Group	Treatment	Male rats			Female rats		
		Initial weight (g)	Final weight (g)	Liver weight (g 100 g^{-1} body weight)	Initial weight (g)	Final weight (g)	Liver weight (g 100 g^{-1} body weight)
I	Naturally exposed field rats	103.66 ± 1.85	-	4.23 ± 0.11[a]	107.66 ± 3.84	-	4.35 ± 0.008[a]
II	Control	118.33 ± 7.26	110.33 ± 6.38	4.28 ± 0.11[a]	115.00 ± 2.88	106.66 ±1.66	4.28 ± 0.11[a]
III	5 ppm SO_2	111.66 ± 9.27	106.66 ± 9.27	3.70 ± 0.40[a]	121.66 ± 1.66	116.00 ± 3.05	4.25 ± 0.09[a]
IV	10 ppm SO_2	105.66 ± 3.48	100.00 ± 3.78	3.95 ± 0.44[a]	114.00 ± 3.05	109.33 ± 1.76	3.95 ± 0.44[a]
V	15 ppm SO_2	105.33 ± 1.76	102.33 ± 1.45	4.28 ± 0.11[a]	125.66 ± 6.98	120.00 ± 5.77	3.70 ± 0.40[a]

Values are shown as mean ± SE

(1951) taking BSA (Bovine Serum Albumin) as standard.

Histological Studies

Liver was cleared and fixed in 10% formaline for 24 hours. Then the tissues were dehydrated in different grades of ethanol, clearing was done in xylene and embedding was done in paraffin wax for the preparation of blocks. The 5-7 µm thick sections were cut and stained in haematoxylin-eosin stain and mounted in DPX and slides were seen under microscope.

Statistical analysis

Statistical analysis software (SPSS) was used to analyse the data. The data was subjected to analysis of variance (ANOVA), One way analysis of variance (ANOVA) with post hock Tukey's t-test was used for making comparisons between control, naturally exposed and treated group of rats. A value of $P< 0.05$ indicated significant differences.

Results and Discussion

Mean body weight and organ weight did not vary significantly between rats of control group and treated group (5, 10 and 15 ppm). A non-significant decrease in body weight was observed in male and female treated rats as compared to control rats. No significant difference was observed in the weights of liver of rats of male and female among all the treated and control groups (Table 1).

In the plasma of rats of both the sexes, SO_2 inhalation at all the tested concentrations caused significantly decreased activities of antioxidant enzymes and significantly increased lipid peroxidation. SO_2 at low concentration (5 ppm) in plasma of male rats, caused significant decrease in SOD, CAT, GST and GR activity and non-significant decrease in GPx, and GSH activity and at higher concentration (10 and 15 ppm) caused significant decrease in SOD, CAT, GST and GSH activity and non-significant decrease in GPx and GR activity as compared

Table 2. Effect of SO_2 on antioxidant parameters of plasma in male rats

Group	Treatment	Antioxidant Parameters						
		Superoxide dismutase (SOD)	Catalase (CAT)	Glutathione-S-transferase (GST)	Glutathione Peroxidase (GPx)	Glutathione (GSH)	Glutathione reductase (GR)	Lipid peroxidation (LPO)
I	Naturally exposed field rats	14.85 ±0.17[b]	10.49 ± 0.35[c]	0.61 ± 0.004[b]	4.31 ± 0.06[a]	46.78 ± 0.34[a]	0.05 ± 0.004[a]	6.36± 0.19[b]
II	Control	17.88 ± 0.13[b]	11.53 ± 0.62[c]	0.78 ± 0.01[b]	4.91 ± 0.02[d]	48.65 ± 0.16[b]	0.05 ± 0.003[c]	6.53 ± 0.09[a]
III	5 ppm SO_2	5.85 ± 0.09[a]	8.91 ± 0.27[b]	0.41 ± 0.003[a]	0.84 ± 0.007[a]	41.60 ± 0.39[a]	0.04 ± 0.0006[b]	7.52 ± 0.17[c]
IV	10 ppm SO_2	5.37 ± 0.09[a]	7.87 ± 0.24[a]	0.43 ± 0.01[a]	2.25 ± 0.05[a]	42.17 ± 0.32[a]	0.02 ± 0.005[a]	6.69 ± 0.26[b]
V	15 ppm SO_2	5.56 ± 0.18[a]	3.76 ± 0.018[a]	0.41 ± 0.009[a]	3.04 ± 0.09[c]	42.62 ± 0.21[a]	0.03 ± 0.002[b]	1.87 ± 0.45[a]

Values are expressed as mean ± SE of six animals in each group

Words in super scripts represents significant difference between treatments at P≤0.05 as compared to control.

Units: SOD (U mg^{-1} protein), CAT (µmole of H_2O_2 decomposed min^{-1} mg^{-1} protein), GPx (U mg^{-1} protein), GST (µmoles of GSH-CDNB conjugate formed min^{-1} mg^{-1} protein), GSH (nmol mg^{-1} protein), GR (µmoles of NADPH oxidized min^{-1} mg^{-1} protein), Lipid peroxidation (nmol MDA 100 mg^{-1} tissue)

Table 3. Effect of SO_2 on antioxidant parameters of plasma in female rats

Group	Treatment	Antioxidant Parameters						
		Superoxide dismutase (SOD)	Catalase (CAT)	Glutathione-S-transferase (GST)	Glutathione Peroxidase (GPx)	Glutathione (GSH)	Glutathione reductase (GR)	Lipid peroxidation (LPO)
I	Naturally exposed field rats	15.94 ± 0.13^{b}	12.85± 0.30^{c}	0.71± 0.004^{c}	4.09± 0.06^{c}	46.94 ± 0.23^{c}	0.05± 0.001^{c}	1.84 ± 0.35^{a}
II	Control	17.95 ± 0.10^{b}	13.32 ± 0.09^{c}	0.80 ± 0.03^{c}	4.87 ± 0.007^{c}	48.57 ± 0.19^{c}	0.06 ± 0.001^{c}	1.62 ± 0.07^{a}
III	5ppm SO_2	5.89 ± 0.29^{a}	7.32 ± 0.11^{b}	0.46 ± 0.008^{b}	3.86 ± 0.02^{a}	40.46 ± 0.12^{a}	0.04 ± 0.001^{b}	6.56 ± 0.12^{b}
IV	10ppm SO_2	5.57 ± 0.15^{a}	5.77 ± 0.07^{b}	0.46 ± 0.01^{b}	4.95 ± 0.12^{c}	41.42 ± 0.14^{b}	0.02 ± 0.001^{a}	7.77 ± 0.23^{c}
V	15ppm SO_2	5.49 ± 0.20^{a}	3.6 ± 0.14^{a}	0.32 ± 0.005^{a}	2.81 ± 0.003^{b}	40.24 ± 0.23^{a}	0.03 ± 0.001^{b}	6.61± 0.13^{b}

Values expressed as mean ± SE
Words in super scripts represents significant difference between treatments at p≤0.05 as compared to control.
Units: SOD (U mg^{-1} protein), CAT (µmole of H_2O_2 decomposed min^{-1} mg^{-1} protein), GPx (U mg^{-1} protein), GST (µmoles of GSH-CDNB conjugate formed min^{-1} mg^{-1} protein), GSH (nmol mg^{-1} protein), GR (µmoles of NADPH oxidized min^{-1} mg^{-1} protein), Lipid peroxidation (nmol MDA 100 mg^{-1} tissue)

to control and naturally exposed rats. SO_2 at low concentration (5 ppm) in plasma of female rats, caused significantly decrease in SOD, CAT, GSH, GPx, GST and GR activity and at higher concentration (10 and 15ppm) caused significant decrease in SOD, CAT, GR and GSH activity and non-significant decrease in GST, GPx activity as compared to control and naturally exposed rats. SO_2 inhalation at all the three tested concentrations (5, 10 and 15 ppm) showed significantly increased lipid peroxidation (LPO) in plasma from rats of both the sexes (Tables 2 and 3)

In the liver of male and female rats SO_2 at low concentrations (5 and 10 ppm) showed significant decrease in SOD, CAT, GST, GSH and GR and non-significant decrease in GPx activity and significant increase was seen in LPO activity as compared to control and naturally exposed rats. At higher concentration (15 ppm) there was significant decrease in SOD, CAT, GST, GSH and GR activity and non-significant reduction in GPx activity. Non-significant decrease was seen in the glutathione peroxidase (GPx) activity in all the concentrations (5, 10 and 15 ppm) of treated rats as compared to control rats and a significant increase in LPO activity in liver from male and female rats as compared to control rats (Tables 4 and 5). According to Chance *et al.*, 2020 se-dependent GPx and CAT are the main hydrogen peroxide scavengers. The working of SOD and CAT is interrelated as superoxide molecule is dismuted to hydrogen peroxide by the enzyme SOD followed by removal of hydrogen peroxide molecule by GSH-Px and CAT (found in peroxisome). The functions of GPx are performed in cytosol which limits its role in comparison to other antioxidative enzymes. Unexpectedly, the activity of these

Table 4. Effect of SO_2 on liver antioxidant parameters of male rats

Group	Treatment	Antioxidant Parameters						
		Superoxide dismutase (SOD)	Catalase (CAT)	Glutathione-S-transferase (GST)	Glutathione Peroxidase (GPx)	Glutathione (GSH)	Glutathione reductase (GR)	Lipid peroxidation (LPO)
I	Naturally exposed field rats	12.66 ± 0.008^{c}	11.48 ± 0.22^{d}	0.48 ± 0.001^{c}	0.68 ± 0.006^{a}	46.77 ± 0.10^{c}	0.58 ± 0.003^{c}	0.46 ± 0.01^{a}
II	Control	13.58 ± 0.008^{c}	12.98 ± 0.21^{d}	0.49 ± 0.001^{c}	0.69 ± 0.006^{a}	48.73 ± 0.10^{c}	0.61 ± 0.001^{c}	0.47 ± 0.01^{a}
III	5 ppm SO_2	8.69 ± 0.70^{a}	8.79 ± 0.28^{b}	0.41 ± 0.003^{a}	0.69 ± 0.008^{a}	41.32 ± 0.55^{ab}	0.049 ± 0.001^{b}	0.75 ± 0.01^{b}
IV	10 ppm SO_2	10.66 ± 0.30^{b}	10.02 ± 0.21^{c}	0.43 ± 0.001^{b}	0.67 ± 0.005^{a}	41.86 ± 0.45^{b}	0.05 ± 0.0008^{b}	0.52 ± 0.006^{a}
V	15 ppm SO_2	9.85 ± 0.25^{ab}	8.28 ± 0.19^{ab}	0.40 ± 0.003^{a}	0.65 ± 0.01^{a}	41.14 ± 0.59^{ab}	0.05 ± 0.0008^{b}	0.82 ± 0.01^{c}

Values expressed as mean ± SE
Words in super scripts represents significant difference between treatments at p≤0.05 as compared to control.
Units: SOD (U mg^{-1} protein), CAT (µmole of H_2O_2 decomposed min^{-1} mg^{-1} protein), GPx (U mg^{-1} protein), GST (µmoles of GSH-CDNB conjugate formed min^{-1} mg^{-1} protein), GSH (nmol mg^{-1} protein), GR (µmoles of NADPH oxidized min^{-1} mg^{-1} protein), Lipid peroxidation (nmol MDA 100 mg^{-1} tissue)

Table 5. Effect of SO_2 on liver antioxidant parameters of female rats

Group	Treatment	Antioxidant Parameters						
		Superoxide dismutase (SOD)	Catalase (CAT)	Glutathione-S-transferase (GST)	Glutathione Peroxidase (GPx)	Glutathione (GSH)	Glutathione reductase (GR)	Lipid peroxidation (LPO)
I	Naturally exposed field rats	12.16 ± 0.007^{d}	11.48 ± 0.16^{c}	0.48 ± 0.0006^{d}	0.71 ± 0.008^{a}	46.77 ± 0.44^{d}	0.06 ± 0.003^{b}	0.39 ± 0.02^{a}
II	Control	13.86 ± 0.009^{d}	12.74 ± 0.17^{c}	0.49 ± 0.0007^{d}	0.70 ± 0.007^{a}	48.59 ± 0.48^{d}	0.06 ± 0.003^{b}	0.38 ± 0.022^{a}
III	5 ppm SO_2	6.07 ± 0.11^{a}	8.52 ± 0.50^{b}	0.43 ± 0.003^{c}	0.67 ± 0.009^{a}	40.77 ± 0.13^{ab}	0.03 ± 0.001^{a}	0.93 ± 0.008^{d}
IV	10 ppm SO_2	10.71 ± 0.14^{c}	9.57 ± 0.15^{b}	0.42 ± 0.002^{c}	0.66 ± 0.08^{a}	42.28 ± 0.29^{c}	0.042 ± 0.0006^{a}	0.48 ± 0.02^{b}
V	15 ppm SO_2	7.05 ± 0.13^{b}	8.43 ± 0.17^{a}	0.41 ± 0.003^{a}	0.67 ± 0.01^{a}	41.26 ± 0.40^{bc}	0.043 ± 0.0009^{a}	0.90 ± 0.04^{d}

Values expressed as mean ± SE

Words in super scripts represents significant difference between treatments at $p \leq 0.05$ as compared to control.

Units: SOD (U mg^{-1} protein), CAT (µmole of H_2O_2 decomposed min^{-1} mg^{-1} protein), GPx (U mg^{-1} protein), GST (µmoles of GSH-CDNB conjugate formed min^{-1} mg^{-1} protein), GSH (nmol mg^{-1} protein), GR (µmoles of NADPH oxidized min^{-1} mg^{-1} protein), Lipid peroxidation (nmol MDA 100 mg^{-1} tissue)

anti-oxidative enzymes decreased in the livers of rats at various SO_2 concentrations did not follow a linear dose curve (Avci *et al*., 2017).

Superoxide dismutases (SOD) helps in protecting the cells from reactive oxygen species (ROS) and also removes superoxide radicals and decreased SOD level in liver may lead to free radical damage at large scale (Van loon *et al*., 1986). Decreased level of SOD will decrease the production of hydrogen peroxide and it also reduces the protection against free radicals. Bakurt *et al*. (1994) also reported decreased SOD level in the liver and erythrocytes of rats after SO_2 exposure. The findings of Medeiros *et al*. (1983) while exposing the rats to SO_2 also showed reduction in anti-oxidative enzymes and increased lipid peroxidation which is in agreement with our results. Decrease in the concentration of CAT will decrease the H_2O_2 concentration which in turn will decrease the free radicals. Glutathione peroxidase is known for the reduction of various hydro peroxides to H_2O through oxidation of reduced GSH into Glutathione disulphide (GSSH). Lovati *et al*. (1996) also reported similar decrease in levels of GST, GSH and GPx in their studies on effect of SO_2 inhalation on rats. Decreased level of GSH after SO_2 inhalation was also shown by some other studies on rats (Lovati *et al*., 1996). Under normal conditions, cells cope with free radicals with the help of antioxidant enzymes like SOD, CAT, GSH, GPx (Ceballos-Picot *et al*., 1992; Singh and Pathak, 1990). Studies conducted by Haider *et al*. (1981) and Etlik *et al*. (1995) showed increased lipid peroxidation in liver of rats and erythrocytes of guinea pigs exposed to SO_2. Tyler (1975) also accounted for increased lipid peroxidation in mitochondria after exposure to derivatives of SO_2 in laboratory conditions. Increased lipid peroxidation seems to be indicator of several disorders in cells, tissues or organs and it might be involved in different diseased state of cell such as aging, nervous disorders etc. (Meng *et al*. 2002 a & b). Increased lipid peroxidation may have harmful effects on composition and function of biological membranes (Gupta *et al*., 1991). A number of studies have been reported to show the increased lipid peroxidation in the liver, plasma of rats and RBCs of guinea pigs (Etlik *et al*., 1995; Haider *et al*., 1981; Gümüslü *et al*., 1998 and Yargicogvlu *et al*., 1999). SO_2 toxicity may involve oxidative stress to cells and tissues because of the production of free radicals during oxidation process (Shapiro, 1977 and Shi and Mao, 1994).

Histologically, liver tissue section of control rats and naturally exposed rats showed normal central vein, hepatic cord and sinusoids whereas SO_2 exposed groups showed haemorrhagic spots or haemorrhage on the surface of liver, number and diameter of blood vessels was increased. Infiltration of leucocytes was also observed in liver section of 15 ppm SO_2 exposed group (Figure 1). Our findings are in agreement with Liu *et al*. (2022).

Conclusion

Decreased antioxidant enzymes influence the liver to increase free radical damage which in turn affects the defense system of body. The main target organ for SO_2 intoxication is the liver as it is shown by oxidative damage and histopathological alterations in liver, besides

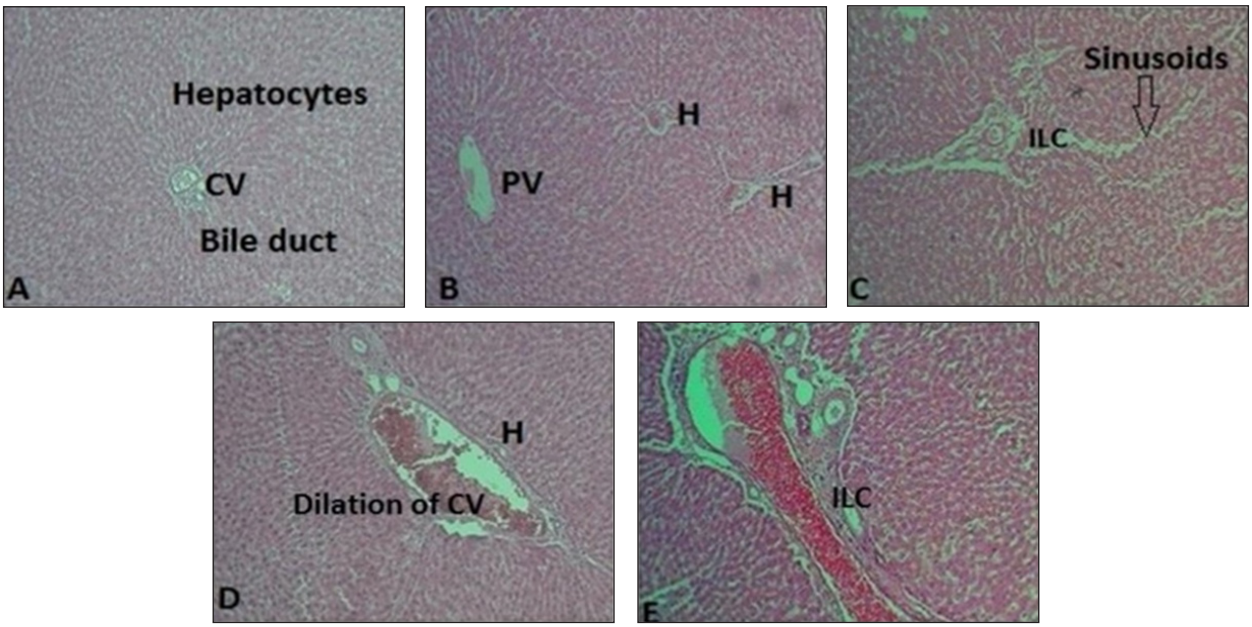

Fig. 1. Liver tissue section of control and naturally exposed rats (Fig 1 A,B) showed normal central vein(CV), hepatic cord and sinusoids whereas 5 and 10 ppm (Fig. 1. C,D) SO_2 exposed groups showed haemorrhagic spots or haemorrhage on the surface of liver, number and dilation of central veins. Infiltration of leucocytes (ILC) was observed in liver section of 15 ppm SO_2 exposed group (Fig. 1. E) observed by light microscopy with 400X magnification.

liver, it also affects other organs like lungs, brain, heart and reproductive organs. From the present study, it can be concluded that SO_2 inhalation have toxic effects in liver and plasma of rats of both the sexes. Further work is needed to understand the toxicological role of SO_2 on multiple organs of mammals.

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Conflicts of Interest

There is no conflict of interest

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