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Protective effect of *Zizyphus mauritiana* against oxidative stress induced by silica in Wistar albino rats.

Rudra Prasad Dutta[1], and M. B. Patil[1]*.

[1]University Department of Biochemistry, R. T. M. Nagpur University, Nagpur-440033 (India)

ABSTRACT

Silica (SiO_2) is a chemical compound being used in various industrial applications entailing human exposure and creates adverse health effects. Present study was aimed to investigate the biochemical influence of aqueous extracts of barks of roots, stem and leaves of *Zizyphus mauritiana (ZM)* on silica induced toxicity in model albino rats, with changes in antioxidant markers enzymes in liver and NO, IL-10 level, in blood. To test the protective effect of aqueous extracts of *ZM*, animals were treated with silica by IP administration with simultaneous oral feeding of *ZM* extracts for 21 days. At the end of experiment blood samples were withdrawn for measurement of NO and IL-10 before animals were sacrificed and liver was collected for analysis of endogenous antioxidant enzymes. Silica administration substantially decreases activities of antioxidant enzymes with parallel rise in the level of LPO, NO and IL-10. Oral feeding of *ZM* extracts has shown restoration of studied parameters significantly towards normal level. In conclusion, this study indicated that aqueous extracts of bark of roots and stem and that of leaves of *ZM* could protect experimental animals from deleterious effect of silica and may serve as alternative medicine to protect silica induced oxidative stress in animals.

Keywords: Silicon dioxide, *Zizyphus mauritiana*, oxidative stress, Reactive oxygen species, flavonoids, xenobiotic.

Corresponding author

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Introduction

Silica is a most abundant mineral found on earth in crystalline state, in sand, rocks and soil, etc. Millions of workers are exposed to silica worldwide and increase the mortality and morbidity [1]. Silica exposure occurs mainly during manufacturing and construction processes that use silica as raw materials. During the industrial processes high amount of silica dust and smoke are generated and particles are accidently contacted with workers via different routes such as inhalation, skin penetration and ingestion. After inhalation and ingestion the silica particles are rapidly translocated to the systemic circulation and ultimately they reach different organs in body [2]. Silica could enter the cells through different biological pathways. Cytotoxic effects of silica injure the cell membranes by generating free radicals and causing oxidative damage there by reducing the cell viability [2]. Silica exposure can generate oxidative stress and promote apoptotic signal to induce apoptosis causing cell death. Silica induced ROS generation can lead to release of cytokines and nitric oxide [3]. NO penetrates the surrounding tissues and activates variety of cellular signalling pathway. The released NO reacts with superoxide anions forming peroxynitrite anions, which are highly toxic and reactive anions, bring about variety of cellular damages inducing lipid peroxidation [4]. Interleukin-10 downregulates the expression of Th1cytokines and it also inhibits several functions of macrophages including presentation of antigen to MHC class II molecules. Synthesis of proinflammatory cytokines and production of oxygen free radical and nitric oxide is also governed by IL-10. Several studies have shown that IL-10 also reduces the level of tumour necrosis factor (TNF-α) [5]. Mice injected with repeated exposure of silica form nodules in liver [6]. The liver is major target organ for the biotransformation of toxins, and silica induced cytotoxicity in hepatocytes leads to liver inflammation and creates disturbances in metabolism. International Agency for Research on Cancer (IARC in 1997) has classified silica as a group Group1 carcinogen [7]. Mitochondria play an important role in energy supply to cells, cell differentiation and apoptosis, etc. thus mitochondria are the most sensitive organelles to many exogenous xenobiotic compounds. Silica can get localized in to the mitochondria and induce structural changes through oxidative stress. Mitochondria also play a key role in apoptosis especially with changes in Bcl-2/Bax ratio [2]. Silica can react with lipids, proteins, nucleic acid and form secondary products like malondialdehyde (MDA) and advance oxidation protein products (AOPP). Silica is highly reactive with –SH group and reduces the level of GSH, thereby increasing the level of MDA [8]. Silica exposure can reportedly cause inflammatory responses, hepatotoxicity as well as fibrosis [6].

In recent years, plant and plant products have been the main focus in search for natural phytochemical compounds to combat oxidative stress induced diseases. Employment of plant products lowers down the possibility of toxic side effects along with economic burden of treatment. In this context the herbal way to prevent the silica induced toxicity using a wild medicinal plant '*Zizyphus mauritiana' (ZM) was given a thought.* This plant (*ZM*) is found all over the world and has been widely investigated for its medicinal properties. *ZM* is rich with many secondary metabolites and has been reported to contain alkaloids, flavonoids, polyphenols, carotenes, proteins, carbohydrates, vitamins and minerals. Various studies have proved that extract of barks of roots; stem, leaves and seeds are used for treatment of many diseases such as fever [9], diarrhoea [9], arthritis [10], ischemic hippocampus [11], enzymatic deficiencies [12], cancer [13], and used as a free radicals scavenger. Due to its neutraceutical contents, *ZM* has been considered to be a treasure of phytochemicals. With this idea in view the present investigation has been designed to explore the beneficial effects of aqueous extracts of barks of roots, stem and leaves of *ZM* on silica induced toxicity in the animal models using wistar albino rats.

Materials and Methods

Chemicals

Silicon dioxide (SiO_2), ethanol, diethyl ether, chloroform, sodium carbonate, pyrogallol, EDTA, glucose 6 phosphate, phenol, glutathione oxidized, glutathione reduced, NADPH, thiobarbituric acid (TBA), malondialdehyde (MDA), N-(1-naphthyl) ethylenediamine dihydrochloride, sodium citrate, hydrogen peroxid, sodium azide were purchased from Sigma chem. Co. USA.

Animals

Healthy male Wistar albino rats (200-250g) were obtained from National Centre for Laboratory Animal Sciences (NCLAS), Hyderabad, India. Animals were maintained at standard conditions (temperature 25 ± 2^0C) with 12 h light/ 12 h dark cycle and fed ad *libitum* with standard pellet diet (Hindusthan Lever) and purified water, with free access to food and water. All the norms prescribed by Institutional Animal Ethics Committee (IAEC) and COPCEA, Government of India, were critically followed (vide the permission letter from IAEC, dated: 12/3/2015).

Collection of Plant Material

Barks of roots and stem and mature healthy leaves of *Ziziphus mauritiana* (*ZM*) were collected from a single wild tree growing in forest of Gadchiroli district of Maharashtra state (India) in the month of October 2014. The plant was authenticated at University Department of Botany, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur and voucher specimen no. 9138 was deposited in the herbarium. The plant was identified to be *Zizyphus mauritiana Lam.* (Family Rhamnaceae).

Preparation of extracts

Dried barks were crushed in grinder and strained through the strainer to remove any hard part of the bark escaped during grinding. Leaves were air dried under shade in controlled condition and crushed to get powder. The powders were stored in air tight brown glass containers to protect from moisture and direct sunlight. Ten percent aqueous extract, of each powder, was prepared by boiling the powder in water for 30 min and passed through muslin cloth. The aqueous extracts were filtered and subjected to rotary vacuum evaporator (Superfit DB3135S). Complete evaporation of water from extract was achieved by drying the extracts at room temperature under controlled conditions by spreading in clean glass petri plates. The dried scrapings were stored in sterilised airtight brown glass bottles until use. At the time of use the scrapings were carefully weighed on electronic balance and solubilised in 0.1M sod. phosphate buffered saline (PBS, pH 7.0), vortexed for complete solubilisation before oral feeding to animals using gavage at 400 mg/kg body weight.

Acute Toxicity Study

Acute toxicity study of silica was determined by up-and-down method [14], intraperitoneal administration of silica at LD_{50} (lethal dose) at 200mg/kg, and acute toxicity test of *ZM* extracts was determined by using guide lines of Organization for Economic Corporation Development (OECD) [15]. Rats were orally administered daily with extracts of *ZM* and observed for the toxicity symptoms like mortality, loss of body weight and behavioural changes and obtained LD_{50} value at 4000mg/kg.

Experimental Design

Thirty six (36) adult male rats were divided into six groups of six animals each. The animals were administered silica at the dose of 20mg/kg ($1/10^{th}$ of LD_{50}), solubilised in PBS containing Tween 80 (0.01%). To minimize aggregation of silica particles, the suspensions were always sonicated for 10 min and vortexed before intraperitoneal administration (IP) followed by oral feeding of *ZM* extracts at 400 mg/kg ($1/10^{th}$ of the LD_{50} value) for 21 days.

Group 1: Animals were not given any treatment (normal control)
Group 2: Animals received only silica for 21 days (positive control)
Group 3: Animals received silica with extract of root bark (root preventive)
Group 4: Animals received silica with simultaneous extract of stem bark (stem preventive)
Group 5: The animals received silica with simultaneous extract of leaves (leaf preventive)
Group 6: The animals received only PBS with Tween 80 (vehicle control)
The doses were given to animals at particular time schedule daily before 9 AM from 0 to 21 days continuously.

Collection of Blood & Serum Samples

At the end of the experimental schedule, animals were anesthetised by deep di-ethyl-ether (Merck) and blood was immediately collected from retro orbital plexus through capillary tube and allowed to clot for 30 min at room temperature followed by centrifugation using simple table-top centrifuge (Remi CM 12 Plus) at 3500 rpm for 10 min to obtain the serum. The colourless serum samples were stored at -80^{0}C until use. Blood samples were preserved at 4^{0}C until analysis.

Collection of Liver from Experimental Rats

For collection of liver rats were sacrificed by cervical dislocation, using butterfly needle and syringe to wash blood from the vein by using cold PBS. The liver tissue was immediately transferred into the cold PBS and used for experimental analysis.

Preparation of tissue homogenate

Liver tissue was washed and cleaned three to four times with cold PBS solution, blotted with the help of filter paper, weighed and rinsed in ice cold PBS before it was homogenized with motor driven teflon coated pestle glass homogenizer (RemiRQ 127 A). The tissue homogenate was centrifuged at 10,000 rpm for 10 min at 4^{0}C in a cooling centrifuge (Remi C24). Supernatant was immediately processed for analysis of endogenous antioxidants.

Determination of endogenous antioxidant status

Determination of Lipid Peroxidation (LPO)

The LPO was determined by the thiobarbituric acid (TBA) method. The assay mixture consisted of 0.1 ml tissue extract, 1 ml TBA (0.67%), 1.5 ml TCA (20%), and 1.5 ml BHT (0.04%). The mixtures were kept in a boiling water bath for 20 min. After cooling to room temperature, the reaction mixture was centrifuged at 4000*g* for 10 min and the absorbance of supernatant was measured at 532nm. The concentration of TBARS was calculated using tetraethoxypropane as a reference standard and expressed as nmol MDA formed/mg protein [16].

Determination of advance oxidation of protein products (AOPPs)

Tissue AOPP assay was measured spectrophotometrically as described by Witko-Sarsat *et al.,* 1996. Each well of 96 well microtitre plate received 200 µl of tissue extract diluted at a ratio of 1:5 in PBS or chloramine T standard solution (0-100µmol/L), 10 µl of potassium iodide (1.16M), 20µl acetic acid. The absorbance of reaction mixture was immediately recorded at 340nm (Thermo electron Corp. 358 ELISA plate reader) against blank. The results of AOPP assays are expressed as µmol/mg protein [17].

Determination of Catalase Activity

Catalase activity was measured by the method of Aebi (1984). The assay mixture consists of 200 µl of sodium phosphate buffer (50mM, pH 7.0), 50 µl of tissue extract and 250 µl of H_2O_2 (30 mM in sod. phosphate buffer) and the decrease in absorbance was measured at 240 nm for 120 seconds in a UV spectrophotometer (eppendorf AG 6135). The results are expressed as U/mg protein [18].

Determination of Superoxide Dismutase Activity (SOD)

The SOD activity was measured by the method of Marklund and Marklund (1974). The assay mixture consisted of 2.875 ml Tris-HCl buffer (50 mM, pH 8.5), 25 µl pyrogallol (24 mM in 10 mM HCl) and 100µl of tissue extract in a total volume of 3 ml. The enzyme activity was measured at 420nm and was expressed as U/mg of protein [19].

Determination of Gluthione Peroxidise Activity (GPx)

Glutathione Peroxidase catalyzes reduction of hydrogen peroxide (H_2O_2), oxidizing reduced glutathione (GSH) to form oxidized glutathione (GSSG). The activity of GPx was calculated by the method of Mohandas, *et al*.(1984). A total 2 ml reaction mixture consisted of 0.1 ml EDTA (1mM), 0.1 ml sodium azide (1mM), 1.49 ml sod. phosphate buffer (0.1 M, pH 7.4), 0.05 ml glutathione reductase, 0.05 ml of reduced glutathione (1mM), 0.1 ml NADPH (0.2 mM) and 0.01 ml H_2O_2 (0.25 mM) and 0.1 ml of tissue extract. After complete reaction the decrease in absorbance was measured at 340nm. Enzyme activity was calculated as nmol NADPH oxidized/min/mg protein [20].

Determination of Tissue Glutathione Reductase (GSSG)

Glutathione reductase activity was measured by the method of Carlberg and Mannervik (1975). Assay mixture contained 1.65 ml sod. phosphate buffer (0.1 M, pH 7.6), 0.1 ml EDTA (0.5mM), 0.05 ml oxidized glutathione (1 mM), 0.1 ml NADPH and 0.1 ml tissue extract and the disappearance of NADPH were measured at 340nm after complete reaction. Enzyme activity was expressed in nmol NADPH oxidized/min/mg protein [21].

Determination of Reduced Glutathione Level (GSH)

Determination of Glutathione level in tissues is based on the development of relatively stable yellow colour when 5, 5'-dithio bis-2 nitro benzoic acid (DTNB) reacts with GSH to produce a coloured ion. The assay mixture consisted of 1 ml tissue extract mixed with 1ml of sulphosalicylic acid (4%). The samples were incubated at 4^0C for 30 min and centrifuged at 1500 rpm for 10 min at 4^0C. Total 3 ml of assay mixture contained 0.4 ml filtered aliquot, 2.2 ml of sodium phosphate buffer (0.1 M, pH 7.4) and 0.4 ml DTNB (10mM). The yellow colour developed was read immediately at 412nm. The GSH content was calculated as nmol DTNB conjugate formed/g of tissue [22].

Determination of Glucose 6 Phosphatase (G-6-Pase)

Glucose-6-Phosphatase activity was determined according to Freedland and Harper (1957). The reaction mixture consisted of 0.5 ml tissue extract, 0.1 ml of G-6-Phosphate (30mM), 0.2 ml citrate buffer (50mM, pH 6.5) incubated at 37^0C for 15 min. Reaction was terminated by addition of 2 ml 10% TCA and centrifuged at 5000*g* for 10 min and the supernatant was used for inorganic phosphate estimation (Fiske and Subbarow 1925). Results are expressed as µmol Pi/min/g/liver [23].

Determination of Nitric Oxide (NO) Level

Nitric oxide in the serum was evaluated by the measurement of nitrite, the stable product of decomposition, was measured by employing the Griess reaction. The assay was performed by adding 100µl of each test sample to 96 well microtitre plate, 50 µl of Griess reagent 1(0.1% naphthalene diamine dihydrochloride), 50µl of Griess reagent 2 (1% sulphanilic acid in 2% o-phosphoric acid) and mixed thoroughly by tapping the sides. After 10 min incubation in room temperature, absorbance was read at 540nm. The results of NO levels were expressed as µmol/L [24].

Determination of Interleukin-10 level (IL-10)

Level of anti-inflammatory cytokines including IL-10 in serum was evaluated using commercially available multi-analyte ELISA kit (Qiagen). Absorbance was read at 450nm using Thermo electron Corp. 358 ELISA plate reader. Results of IL-10 levels are expressed as pg/ml.

Determination of Protein Content

Protein content of the tissue was estimated by the method of Lowry et al., (1951), using BSA as a standard protein [25].

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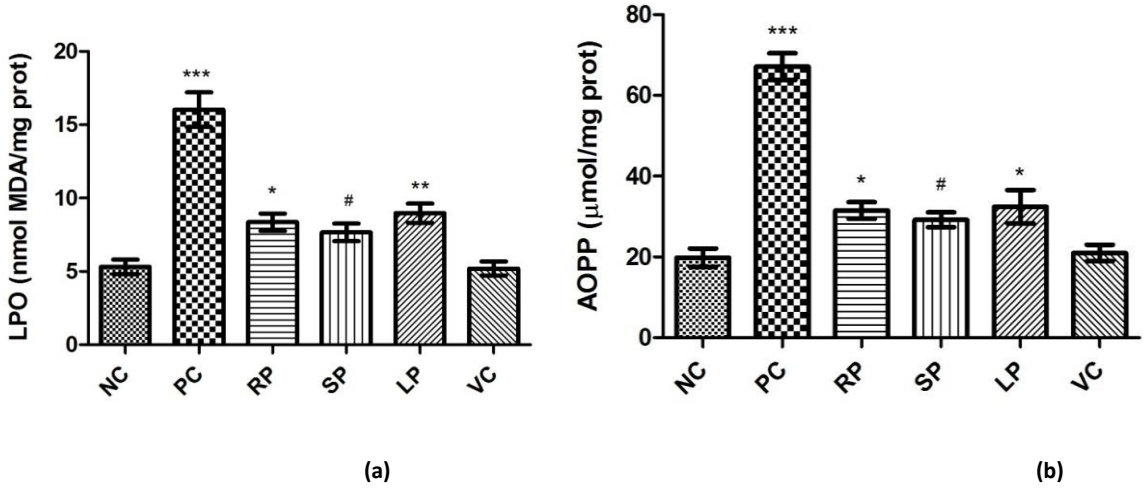
Statistical Analysis

The data obtained were expressed as Mean ± Standard error of mean (n=6) and analyzed with one way ANOVA followed by Dunnett test by comparing all experimental readings vs. control reading using GraphPad Prism version 5.03. P< 0.05 was considered as statistically significant.

RESULTS

Effect on Lipid peroxidation and AOPP level:

Fig.1 (a, b) shows that the silica treated positive control group of rats have markedly increase in the level of LPO and AOPP as compared to the normal control group. Simultaneous supplementation with *ZM* extracts progressively decreased the LPO and AOPP level. Stem bark treated group had reduced level of LPO and AOPP than the group treated with extract of roots and leaves. There were no significant changes in **Lipid peroxidation and AOPP level** as observed in vehicle control group.



(a) **(b)**

Figure 1. Effect of *Z. mauritiana* extracts on LPO (a) and AOPP (b) levels in liver tissue of silica treated rats. Results represent in mean ± S.E.M of six animals per group *P< 0.05, **P<0.01, ***P<0.001 and #P> 0.05 compared with normal control group.

Effect on Catalase and SOD activity: Rats treated with silica showed significant decrease in activity of catalase and SOD in liver tissue compared to the rats in the control group. However, rats treated with *ZM* extracts showed a significant increase in the activity of catalase and SOD. There were no significant changes observed in vehicle control group **Fig.2 (a, b)**.

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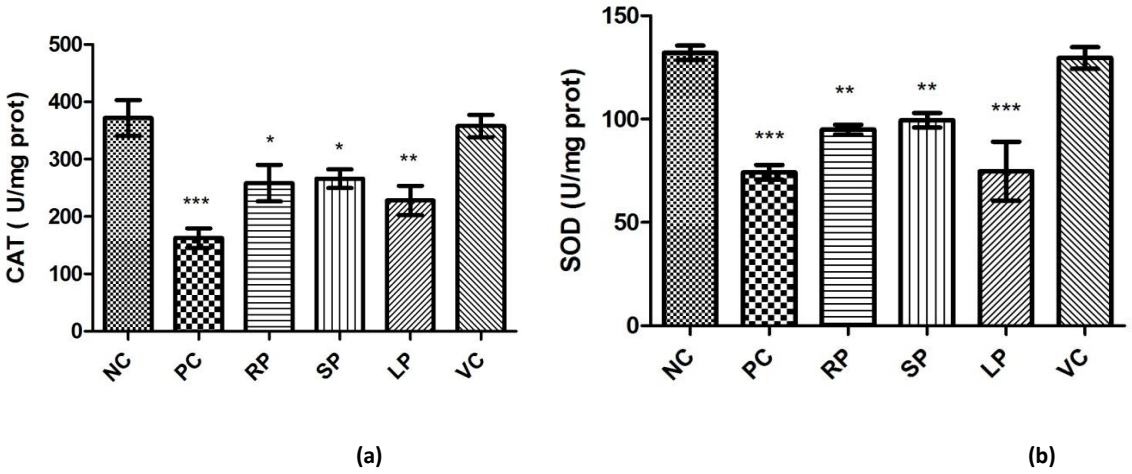

Figure 2. Effect of treatment with *Z. mauritiana* extracts on Catalase (a) and SOD (b) liver tissue enzyme activity of silica treated rats. Results represent in mean ± S.E.M of six animals per group *P< 0.05, **P<0.01 and ***P<0.001compared with normal control group.

Effect on GPx and GR activity: Fig.3 (a, b) depicts the effect of the *ZM* extracts on the GPx and GR activity levels in silica treated rat. A significant decrease in GPx and GR activity level was recorded in silica treated rat liver in positive control group as compared with normal control group. Treatment with *ZM* extracts (root bark, stem bark and leaves) significantly reversed their level towards control group. There is no significant change observed in vehicle control group as compared with normal group of rats.

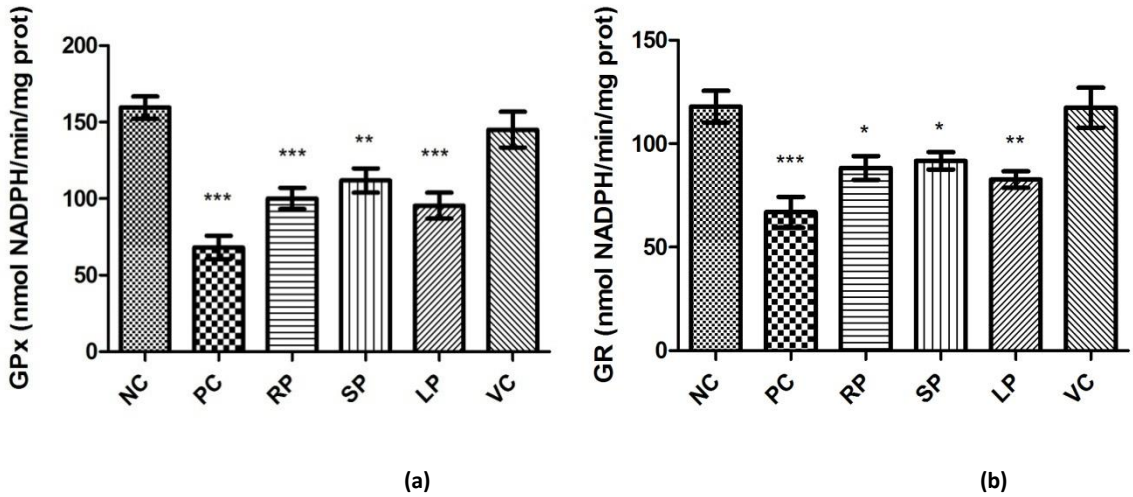

Figure 3. Effect of treatment with *Z. mauritiana* extracts on GPx (a) and GR (b) liver tissue enzyme activity of silica treated rats. Results represent in mean ± S.E.M of six animals per group *P< 0.05, **P<0.01 and ***P<0.001compared with normal control group.

Effect on reduced glutathione (GSH) level: Fig.4 depicts the significant decrease the GSH level as compared with normal control group. However rats receiving silica with simultaneous *ZM* extracts show an increase the GSH level as compared to positive control group. There is no significant change in GSH level as observed in vehicle control groups.

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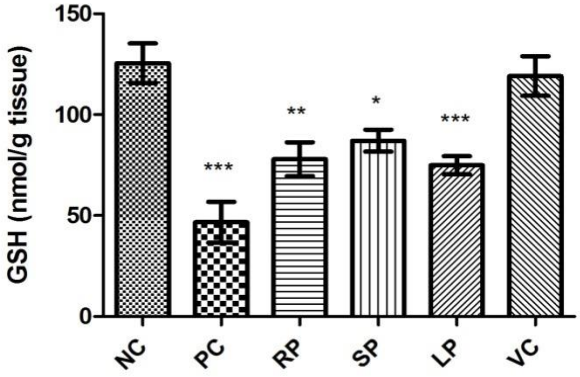

Figure 4. Effect of treatment with *Z. mauritiana* extracts on GSH level in liver tissue of silica treated rats. Results represent in mean ± S.E.M of six animals per group *P< 0.05, **P<0.01 and ***P<0.001 compared with normal control group.

Effect on G-6-Pase activity: Fig.5 shows that the silica treated positive control group have significantly decreased G-6-Pase activity as compared with normal control group. Simultaneous treatment with *ZM* extracts helps to recover the G-6-Pase activity towards normal. There is no significant change observed in G-6-Pase activity in vehicle control group.

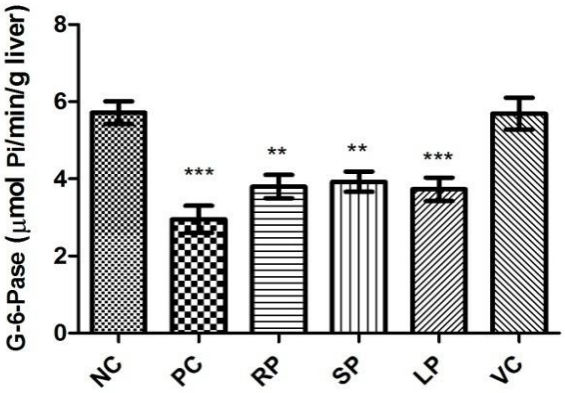

Figure 5. Effect of treatment with *Z. mauritiana* extracts on G-6-Pase liver tissue enzyme activity of silica treated rats. Results represent in mean ± S.E.M of six animals per group **P<0.01 and ***P<0.001compared with normal control group.

Effect on serum NO and IL-10 level: Fig.6 (a, b) depicts that the NO and IL-10 level increases with continuous exposure of silica generating more oxidative stress as seen in positive control group. Treatment with extracts of *ZM* significantly decreased level of NO and IL-10. Stem treated group of rats exhibited more reduced level of NO and IL-10 than extracts of root bark and leaves.

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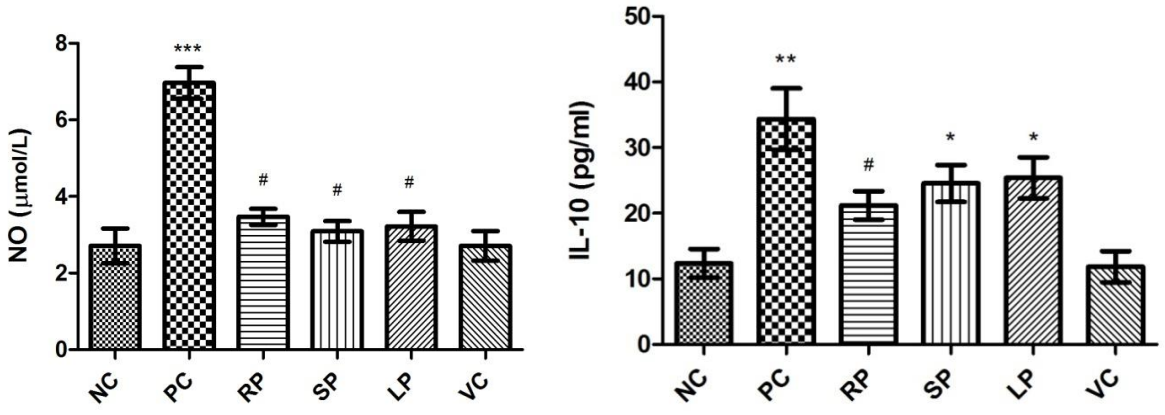

(a) (b)

Figure 6. Effect of treatment with *Z. mauritiana* extracts on NO (a) and IL-10 (b) levels in serum of silica treated rats. Results represent in mean ± S.E.M of six animals per group *P< 0.05, **P<0.01, ***P<0.001 and #P> 0.05 compared with normal control group.

DISCUSSION

Silica is one of the minerals used for various applications. People working in silica mine and industry are more prone and susceptible silica induced adverse health effects. The present study focuses on the preventative measure of silica induced toxicity. The plant in this study is a folk medicinal plant used since ages to cure various diseases. Present study has demonstrated that silica induced toxicity mainly affects liver tissues. Silica as it occurs in various industrial applications. Previous studies have demonstrated that after inhalation silica particles they get into the systemic circulation and reach different organs. Accumulation of silica in liver is far greater than the other organs. The results of the present study revealed that silica induced oxidative stress as evidenced by increase in the level of lipid peroxidation has altered the antioxidant enzyme status in liver of silica treated rats. However, supplementation of *ZM* extracts simultaneously with silica treatment has reversed to some extent the LPO and antioxidant status towards normal control rats. Disruption of various biochemical processes has been proposed rather than a single mechanism involved in silica induced toxicity. Oxidative stress is one of the more important mechanisms of toxic effect of silica. MDA and AOPP levels in the tissues have been used as biomarkers of oxidative stress. In this study MDA levels were found elevated with continuous exposure of silica for 21 days, because silica induced oxidative damage in different tissues occurs due to enhanced peroxidation of membrane lipids and has altered the antioxidant defence system of the cells because liver contained more amount of unsaturated lipids. Present study demostrated not only increased in LPO level but also decrease in the activity of catalase, SOD, GPx and GR. Oxidative stress leads to formation of AOPP. Silica induced toxicity increases the level of AOPP, suggesting that there are positive correlation between the level of MDA and AOPP on exposure to silica. Therefore AOPP is a good oxidative stress marker and its measurement reflects the degree of protein oxidation. Several studies have reported that H_2O_2 and NO production leads to generation of AOPP. Silica induced oxidative stress probably progressively decreased the level of catalase activity due to high accumulation of hydrogen peroxide in the tissues, and it favoured to more peroxidation. This could be the reason for increased LPO levels observed in continuous silica exposure. Present study reflects the significant decrease in catalase activity, which may be due to inactivation by super oxide radicals or inefficient scavenging of the H_2O_2 due to oxidative inactivation of enzyme because silica can interfere with iron which is essential metal ion to maintain the integrity of catalase activity [8]. SOD is an important antioxidant enzyme that inhibits the formation of oxygen radical and this enzyme has been used as a biomarker related to oxidative stress. A large amount of SOD was consumed to maintain the ROS level and disturbed the balance between the oxidant and antioxidant process. Present study concluded that the damages in liver tissues may be related to significant loss of activity of SOD caused due to silica administration. GPx activity was again significantly decreased in liver with continuous exposure of silica, because impairment in GSH

homeostasis in liver tissue occurs due to free radical dependent inactivation of enzyme or depletion of GSH and NADPH at cosubstrate level. GPx mainly catalyzes the direct reaction of GSH with free radical and formed GSSG. Thiol redox system majorly contributes to detoxify reactive electrophiles and toxic oxygen metabolites generated during metabolism on exposure to xenobiotics [26]. Peroxide radicals are reduced by GPx at the expense of GSH and oxidized glutathione reduced by GR by using NADPH. Redox cycle always maintains the intracellular GSH: GSSG ratio and protects the cells against oxidative damages. Silica exposure affects the liver and decreased the production GSH that insults to maintain the interorgan glutathione redox cycle. Present study witnesses that the IP administration of silica decreases the level of endoplasmic reticulum bound enzyme G-6-Pase because LPO leads to the damage of the cellular membrane. Studies demonstrate that Vanadate and Tungstate are well known inhibitor of phosphatase and inhibited phosphohydrolase and phosphotrasferae activities of G-6-Pase [27]. Studies have shown that kupffer cells are nonparenchymal cells in the liver and it acts as resident macrophages and play a major role in defence against invading particles via phagocytosis. Kupffer cells are a primary responder to a toxicant and released some bioactive substances when hepatic damages occur [28]. Kupffer cells get stimulated by silica and release harmful bioactive mediator like NO, which is harmful for hepatic cells. In this study elevated level of serum NO may be correlated with inhibition of the proper functioning of antioxidant enzymes due to silica exposure. Several studies reported that NO downregulate functioning of cytochrome P_{450} and suppress liver protein and DNA synthesis [29]. NO and H_2O_2 interaction may form singlet oxygen that can also initiate lipid peroxidation and decrease the blood cell glutathione level. High levels of blood NO are responsible for nitrification of the phenol groups of tyrosine in blood proteins and elevate the level of nitrotyrosine that can alter the pathological functions. IL-10 is an important immunoregulatory cytokine and it plays a major role in liver cirrhosis. IP administration of silica continuously increases the IL-10 protein level in blood. IL-10 protects the body from excessive cellular and organ damage. Regulatory T cells, monocytes, hepatocytes and kupffer cells also release the IL-10 to protect the cell from chemical exposure. In chemical hazard by CCl_4 and thioacetamide induced liver fibrosis. IL-10 therapy reduces this along with cell apoptosis and necrosis [30]. Present study demonstrates that fibrotic reaction is not only dependent on inflammatory reaction but also involved in lymphocyte response by IL-10. After supplementation of *ZM* extracts for 21days simultaneously it appears to attenuate hepatotoxic effect of silica exposure. *ZM* extracts might be playing an important role in preventing the peroxidation of membrane lipids by inhibiting the free radical attack on the membranes thereby decreasing the production of AOPP. Previous phytochemical studies on this plant demonstrated the presence of various types of polyphenolic compounds such as alkaloids, flavonoids, tritepenoids, tannins, ascorbic acid and minerals and vitamins [31]. These secondary metabolites help in scavenging the free radicals and also help to maintain the proper functioning of antioxidant enzymes. In this study it was found that stem bark treated groups can attenuate the harmful effect of silica as compared to animals receiving extracts of root bark and leaves. Presence of –OH and –H groups of its polyphenolic components bind directly with free radical and maintain the redox equilibrium within the body. Alkaloids and flavonoids are widely used for development of medicine. Stem bark and root bark of *ZM* contains higher amounts of alkaloids and flavonoids [32], on the other hand leaves of *ZM* contained excessive amount of ascorbic acid which also helps to neutralizes the excessive free radicals and play a major role to cure the disease of silica induced toxicity in male reproductive organs [33]. Nitrogenous compounds are comprised in alkaloids and that are used as therapeutic agents for cancer treatment. Polyphenolic compounds inhibit nitrosation of liver and kidney cells. The present study reveals that oral administration of extracts of root, stem and leaves significantly decrease the NO level in blood and ability to maintain normal functioning of antioxidant enzymes such as catalase, SOD, GPx and GR activity and increase the GSH level. Studies have also reported that *ZM* contains catechin and epigallocatechin [34] which can scavenge the superoxide and peroxy radicals, NO, and peroxynitrite and prevent the nitration of tyrosine. IL-10 has an ability to protect cells from free radical damage when repeated exposure of silica administration increases the level of IL-10 and failed to repress the cyclooxygenase activity [35]. The phytochemical content of stem bark, root bark and leaves of *ZM* may inhibit the activity of cyclooxygenase (COX-I and COX-II) enzyme [36] and help to maintain the proper functioning of IL-10. *ZM* contains flavonoids which inhibit the NF-kβ activity helping IL-10 to regulate the JAK-STAT signalling pathway [37].

CONCLUSION

From the results of the present study it can be interfered that aqueous extracts of stem, root bark and leaves of *ZM* partially protected liver of rats from Silica induced injury, probably due to presence of various

polyphenolic compounds which help to scavenge silica generated free radical attack and maintained the structural and functional integrity of the cells. An anti inflammatory effect of *ZM* observed in present study opens up new promising area for the development of selective immune therapy. These finding would encourage pharmacological significance of using plant extracts as alternative medicines for treatment of silica induced toxicity.

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