



Research Article

Study on hepatic histopathological observations and the ameliorative effects of *Ocimum sanctum* on mancozeb induced toxicity in albino mice

S. Razia* and A. Siddiqui

Department of Zoology, Govt. Holkar Science College, Indore- 452001, India

*Corresponding author, Email: razia22sul@yahoo.co.in

Received: 30/11/2014

Revised: 05/03/2015

Accepted: 15/03/2015

Abstract: The protective effects of *Ocimum sanctum* Linn was studied on liver of albino mice induced by different doses of mancozeb. Three groups of Swiss albino mice were used wherein distilled water at 0.1ml per mice per day orally was used in group I as control while the same volume of mancozeb dissolved in distilled water at 4.2mg/kg/bw and 6.4 mg/kg/bw were administered consecutively for 6 days a week for 4 weeks in group II and III respectively as the treated groups. Aqueous *Ocimum sanctum* extract was given at 400mg/kg/bw after withdrawal of mancozeb for 4 weeks in group II and III in the same way. There was significant decrease in the liver weight. The histopathology of the liver tissues of mancozeb treated group revealed impairment of normal structural organisation. The hepatic lobules and cord-

like arrangements of liver cells were lost. Severe congestion was seen in central and portal vein. In high dose of mancozeb necrosis and cytoplasmic vacuolisation was evident along with pyknotic nuclei. Inflammatory leucocytic infiltrations were also observed. Exposure to *Ocimum sanctum* treatment reduced the lesions and healing was seen with better development in low dose of mancozeb (group I). No cytoplasmic vacuolisation was seen in the hepatocytes and most cells appeared binucleated displaying certain degree of recovery. The results of the present work indicate the ameliorative effect of aqueous extract of *Ocimum sanctum* against hepatic damage induced by mancozeb on albino mice.

Keywords: mancozeb, *Ocimum sanctum*, liver, histopathology.

INTRODUCTION

Pesticides are used extensively throughout the world to control agricultural pests and to protect public health. Although these chemicals are beneficial they can also cause adverse effects in both humans and animals.

One such pesticide is mancozeb, a fungicide of ethylene-bisdithiocarbamate group which is widely used against diseases of field crops, fruits and ornamentals (Worthing, 1991) and is the most commonly used fungicides in commercial use for the last 50

years in India and Madhya Pradesh. Though it has low acute toxicity; yet its breakdown product ethylene thiourea causes major toxicological concern and was found to have toxic effects in number of experimental animals.

Several workers reported histopathological changes in liver and adrenal gland of mice by mancozeb (O'Hara & DiDonto, 1985). Several other workers demonstrated toxicological effects in male rats (Srivastava, et al. 1999) as well as significant histopathological changes such as fibrosis, necrosis, cellular hypertrophy, pyknosis and vacuolization in brain, thymus, spleen, liver kidney in rats when treated with Benzene Hexachloride, monocrotophos, mancozeb, propineb, maneb and mixture of metalaxyl (Hore, 1997; Devenci, 1999 and Kackar, 1999).

About 80% of the world population relies on botanical preparations as medicines to meet their health needs (Ogbera *et.al.*, 2010). One such botanical playing an important role in therapy of different diseases is *Ocimum sanctum* known as "Queen of herbs". It belongs to Lamiaceae family and is commonly known as Tulsi or holy basil in India and is widely available. It has numerous healing properties and has been in use since time immemorial traditionally in treatment of various disorders in Ayurveda. It has been scientifically shown to possess very interesting pharmacological properties relevant to the present study such as anti-inflammatory activity (Singh, *et al.* 1996), antioxidant (Uma Devi, *et al.* 1999) and anti-stress properties (Sood, *et al.* 2006). *Ocimum sanctum* possess antibiotic, immunomodulatory, analgesic, chemopreventive and antipyretic properties proven by various researchers (Vogel, *et al.* 2002, Singh, *et al.* 2007, Joshi, *et al.* 2011, and Mahima, *et al.* 2012). It is widely used in medicine and cosmetics. Hepatotoxicity in

mancozeb exposed Wistar rats was noticed by Zine Kechrid, *et al.*, (2007). The present work was conducted to study the effect of *Ocimum sanctum* on low doses of mancozeb induced hepatic injury in albino mice.

MATERIALS AND METHODS

Test Material

Mancozeb (commercial grade of 75% wettable powder) was made available from Indofil Chemical Company, Mumbai. It was dissolved in distilled water for administration.

Preparation of extract

The leaves of *Ocimum sanctum* (Linn) was collected, washed and colourless shade dried. It was powdered in electric blender and refluxed. The solvent was lyophilised into powder.

Treatment in animals

Adult male Swiss albino mice (*Musculus albinus*) weighing 22-28 gm were used for the experiment. Animals were kept in the laboratory under constant temperature $22 \pm 2^\circ\text{C}$, RH $50 \pm 10\%$ and 12 hr photoperiod. Commercial pellet diet and water were fed *ad libitum*. Animals were divided into 5 groups in polypropylene cages. Group I was used in control and given 1ml of distilled water orally. Group II and Group III were administered same volume of mancozeb dissolved in distilled water at 4.2 and 6.7 mg/kg/body weight respectively for 6 days a week continuously for 4 weeks. Group IV was given mancozeb at 4.2 mg/kg/body weight for 30 days and withdrawn thereafter and aqueous extract of *Ocimum sanctum* was administered for another 30 days. Group V treated similar to group IV except dose of mancozeb was 6.7 mg/kg/body weight.

After 4 weeks mice from each group were sacrificed by cervical dislocation and dissection performed quickly to remove liver for histological observations. The

remaining mice of group II and III were administered *Ocimum sanctum* at 250 mg/kg/body weight in the same way. After 30 days animals were sacrificed and liver removed after dissecting the animal. The livers were weighed in all the respective treatments and immediately fixed in 10% formalin solution after washing in saline. After 24 hrs liver were washed with distilled water and dehydrated with graded series of alcohols, cleaned in xylene and finally embedded in paraffin wax. Sections were cut at 5 μ thickness and stained in Haematoxylin and Eosin for histopathological analysis. Microscopic observations were carried out after staining & microphotographs were taken using light microscope Axioscope 2 plus (Carl Zeiss, Germany).

RESULTS AND DISCUSSION

In the present study, the overall body weight of the exposed mice showed slight decrease in the body weight while the liver weight slightly increased in the mice treated with mancozeb as compared to control after 30 days of exposure. This finding is in agreement with those of Hore, *et al.*, (1997), Kackar, *et al.*, (1999) and Adjrah, *et al.*, (2013). A significant increase in weight of liver was seen in Group III while Group II showed insignificant increase. Kechrid, *et al.*, (2007) observed decrease in body weight in male rats exposed to 3.5 g/l of mancozeb. In the *Ocimum* treated group the weight of liver decreased slightly towards the control value.

Histological Observations

Microscopic examinations of the liver sections after 4 weeks from the control group showed normal architecture with no signs of infiltration. The central veins, portal areas and sinusoids appear normal (Fig.1).

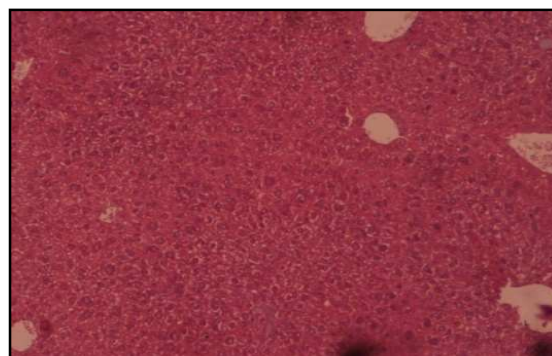


Fig.1: Liver section of control group showing normal architecture (10X)

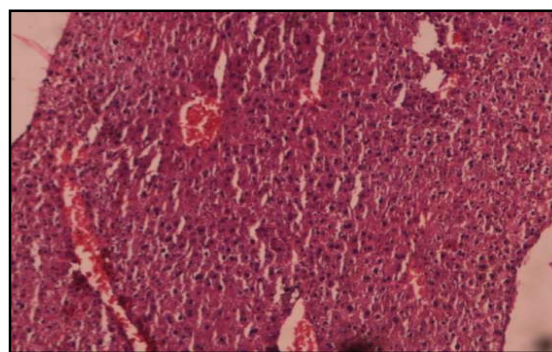


Fig. 2: Liver section of Group II (10X) showing enlargement and congestion in central veins sinusoids and, infiltration and inflammation

Histopathological examination of group II samples treated with 4.2 mg/kg/bw mancozeb revealed slight enlargement and congestion of central vein, hypertrophy of hepatocytes with pyknotic nuclei and enlargement of sinusoids was observed along with erythrocyte accumulation in portal veins and vacuolization. The radial arrangement of hepatocytes was absent (Fig. 2). In Group III, histopathological observations treated with 6.7 mg/kg/bw mancozeb revealed severe dilation of central veins, sinusoids, and hypertrophied hepatocytes. Erythrocyte accumulation and congestion in central and portal veins along with mononuclear cell infiltration was observed. Degeneration, necrosis and hemorrhages along with increased

vacuolisation of hepatocytes in comparison to untreated normal controls were seen. The infiltration of inflammatory cells was also observed. These observations could be due to its toxic effects primarily by the generation of reactive oxygen species causing damage to the various membranous components of the cell. This finding is in accordance to Pandey, *et al.*, (1990); Dikshith, *et al.* 1972; Subramaniam, *et al.*,(1991) who reported that ethylenethiourea, the breakdown product of mancozeb and ziram was found to have hepatotoxic effects in male rats.

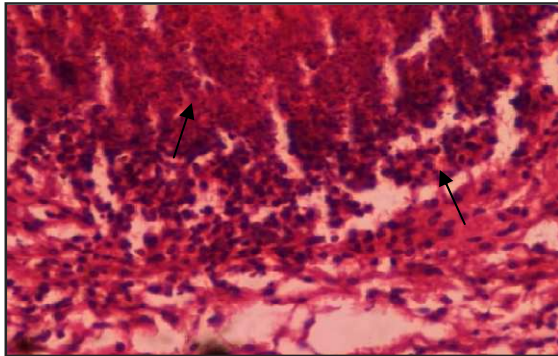


Fig.3: Liver section of Group III (40X) showing inflammatory cells infiltrate, severe showing hemorrhages, loss of radial arrangement, necrosis and vacuolization.

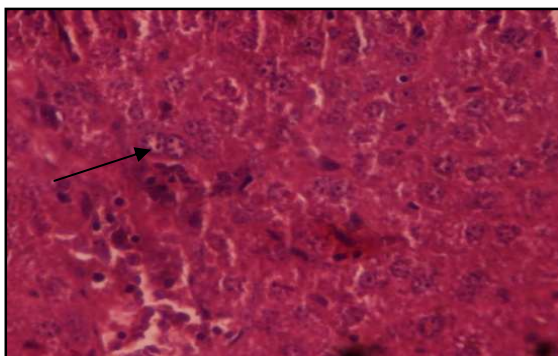


Fig.4: Liver section of Group III (20X) hypertrophy of hepatocytes with pyknotic nuclei.

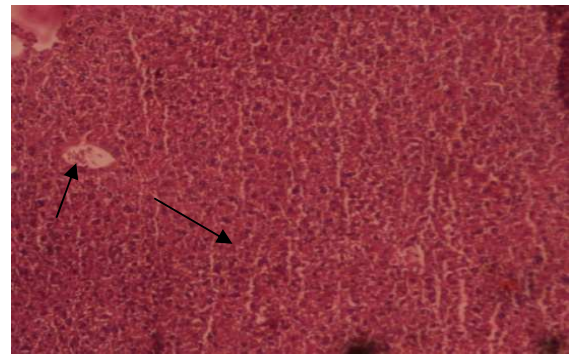


Fig.5: Liver section of Group IV (10X) showing restoration of healing of tissue, showing normal arrangement of hepatocytes, some congestion in central vein

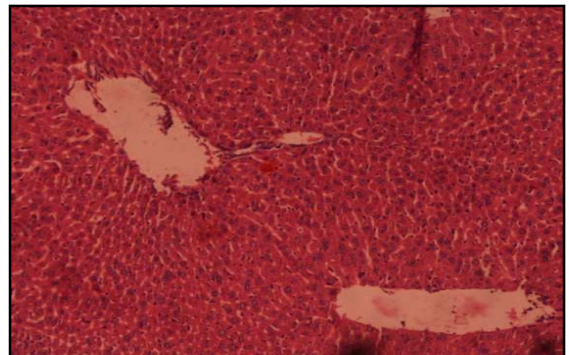


Fig.6: Liver section of Group IV (10X) binucleated cells were identified

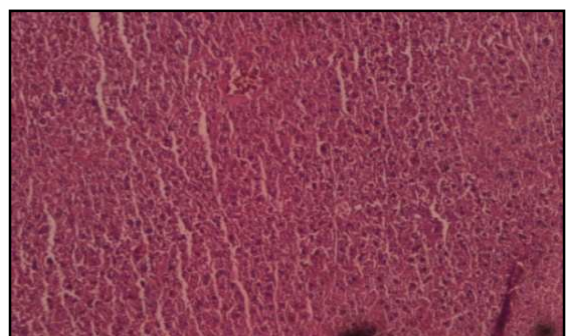


Fig.7: Liver section of Group V (10X) showing ruptured hepatocytes, less histological lesions.

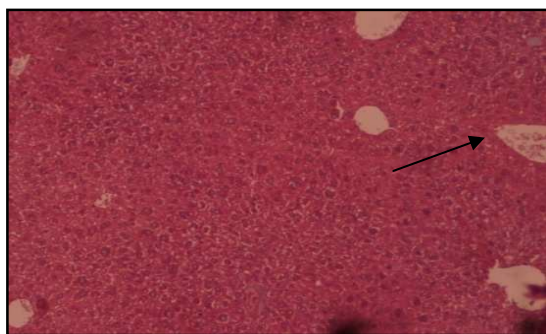


Fig. 8: Liver section of Group V (10X) showing some congestion and inflammation and normalcy of architecture.

The liver sections of mice post treated with aqueous *Ocimum sanctum* leaf extract after withdrawal of mancozeb revealed restoration and improvement of tissues and significantly prevented further damage to the liver tissue. In Group IV the normal arrangement of hepatocytes was restored though some congestion was seen in around central vein and binucleated cells were identified. In Group V after treatment with aqueous *Ocimum sanctum* leaf extract some ruptured hepatocytes was seen though the histological lesions were less and healing of tissue was evident. Eugenol present in the *O. sanctum* extract has significant ability as antioxidant and provides cure from body ailments. The effect of toxins on liver may have been supplemented by these antioxidants.

Similar protective role of *Ocimum* was reported by Sharma, *et al.*, (2002) who found that oral administration of *O. sanctum* provides protection against mercuric chloride induced toxicity in Swiss albino mice. Yamamoto, *et al.*, (2005) proved that *Ocimum* suppressed hepatic fibrosis and protected liver against parenchymal damage. Dasgupta, *et al.*, (2007) found that *O. basilicum* increased the activity of xenobiotic metabolizing phase I and phase II enzymes, elevating antioxidant-enzyme response by increasing significantly the

hepatic glutathione reductase, superoxide dismutase, and catalase activities, increasing glutathione content and decreasing lipid peroxidation and lactate dehydrogenase activity in the liver of mice.

Conclusion: Liver is one of the main metabolic organs of higher vertebrates which is highly affected by toxins. In the present study histopathological changes were observed in the liver of mancozeb exposed albino mice. Severe alterations from the normal group were observed in Group III than in Group II. When aqueous extract of *Ocimum sanctum* at 250 mg/kg/body weight was administered in Group II and III after withdrawal of mancozeb, restoration of histopathological changes was seen towards the normal histological structure. Better healing was observed in Group II than Group III, suggesting curative properties of *Ocimum sanctum* against mancozeb induced hepatic toxicity.

Thus, it could be used safely as a drug of choice during fungicidal intoxication with no untoward side effects. The results of the present study shall be helpful in the field of chemotherapy as herbal treatment has fewer side effects and pronounced effective results are also seen.

Acknowledgement: The authors are thankful to Dr. R. K. Tugnawat, Principal, Govt. Holkar Science College, Indore for providing necessary facilities and Dr. Mishra, Veterinary college, Mhow for providing animals for the research work.

REFERENCES

Worthing C. R. (1991) Mancozeb. In the pesticide manual, C.R. Worthing, ed. Lovensham, Suffolk, Great Britain, pp. 529-530.

O'Hara G.P. and L.J. DiDonto (1985) Dithane M-45 & ethylene thiourea: 3 month dietary toxicity study in mice. *J. Environment. Health.* 10 (2), 32-38.

Srivastava M. K., and Raizada R. B. (1999) Assessment of toxicological effects of mancozeb in male rats after chronic exposure. *Indian J. Exp. Biol.* 37(6), 553-559.

Hore S. K., Maitis S. K., Chauhan H.V.S., Gupta N., Koley K. M. (1997) Effects of long term exposure of mancozeb on clinico-haemato biochemical and pathological changes in rats. *Indian Vet. J.* 74, 20-28.

Deveci E.C., Guven K., Bashan, M., Onen A. and D. de-pomerai (1997) The accumulation and histological effects of organometallic fungicides propineb and maneb in the livers of pregnant rats and their offspring. *J. Toxicol. Sci.* 24(2), 79-85.

Kackar R., Srivastava M. K. and Raizada R. B. (1999) Assessment of toxicological effects of mancozeb in male rats after chronic exposure. *Ind. J. Expt. Biol.* 37, 553-559.

Ogbera A.O., Dada O., Adeyeye F. and Jewo P.I. (2010) Complementary and alternative medicine use in diabetes mellitus. *West Afr. J. Med.* 29(3), 158-162.

Singh S., Majumdar D. K., Rehan H. M. S. (1996) Evaluation of anti-inflammatory potential of fixed oil of *Ocimum sanctum* (Holybasil) and its possible mechanism of action. *J. Ethnopharmacol.* 54, 19-26.

Uma Devi P., Ganasoundari A., (1999) Modulation of glutathione and antioxidant

enzymes by *Ocimum sanctum* and its role in protection against radiation injury. *Ind. J. Exptl. Biol.* 37, 262-26.

Sood S., Narang A., Dinda S. and Maulik K. (2005) Chronic oral administration of *Ocimum sanctum* Linn. augments cardiac endogenous antioxidants and prevents isoproterenol-induced myocardial necrosis in rats. *J. Pharm. Pharmacol.* 57(7), 127-133.

Vogel H.G., Vogel W. H., Scholkens B. A., Sandow J., Muller G. and Vogel, W. F. (2002) Analgesic, anti-inflammatory and antipyretic activity. In: *Drug discovery & evaluation Pharmacological Assays*, 2nd edition, New York; Springer. pp. 759-767.

Singh S., Manish T., Dipak M. (2007), Biological activities of *Ocimum sanctum* L. fixed oil – An overview. *NISCAIR Online Periodicals Repository.* 45 (5), 403-412.

Joshi B., Sah G. P., Basnet B. B., Bhatt M. R., Sharma D., Subedi K., Pandey J., Malla R. (2011) Photochemical extraction and antimicrobial properties of different medicinal plants: *Ocimum sanctum* (Tulsi), *Eugenia caryophyllata* (Clove), *Achyranthes bidentata* (Datiwan) and *Azadirachta indica* (Neem). *J. Microbiol. Antimicrobials.* 3(1), 1-7.

Mahima Rahal A., Deb R., Latheef S.K., Samad H.A., Tiwari R., Verma A.K., Kumar A., Dhama K., (2012) Immunomodulatory and therapeutic potentials of herbal, traditional / indigenous and ethnoveterinary medicines. *Pak. J. Biol. Sci.* 15 (16), 754-774.

Kechrid Z., Naima L., El-hadjla derai, Lamia M. and Noureddine B. (2007) Effect

of mancozeb on the metabolism and enzymatic activities of transaminases and alkaline phosphatase in albino wistar rats. African Crop Science Conference Proceedings. 8, pp. 1709-1711.

papillomagenesis. *Phytomedicine*. 11(2-3), 139-151.

Adjrah Y., Karou S. D., Agbonon A., Eklu-Gadegbeku K., De Souza C. and Gbeassor M. (2013) Toxicological assessment of effect of mancozeb-treated lettuce (*Lactuca Sativa*) on Wistar rat liver. *Ethiopian Journal of Environmental Studies and Management*. 6 (1), 67-73.

Pandey B. P., Anita, (1990) In: *Economic Botany*. Ramnagar, New Delhi; Chand and Company Ltd. pp. 294.

Dikshith T. S. S., Raizada R. B. and Kumar S. N. (1988) Effect of repeated dermal application of endosulfan to rats. *Vet. Hum. Toxicol.* 30, 219-224.

Subramaniam A., Deepa A., Srivastava S. P., Seth P. K. (1991) Influence of mancozeb on mitogenetically responsive lipids in rat cerebrum and liver. *Ind. J. Expt. Biol.* 29, 943.

Sharma M. K., Kumar M., Kumar A. (2002) *Ocimum sanctum* aqueous leaf extract provides protection against mercury induced toxicity in Swiss albino mice. *Indian J. Exp. Biol.* 40(9), 1079-1082.

Yamamoto J., Yamada K., Neemura A., Yamashita T. and Arni R., (2005) Testing various herbs for antihelminthic effect. *Nutrition*. 21(5), 580-587.

Dasgupta T., Rao A. and Yadava P. (2007) Chemomodulatory efficacy of basil leaf (*Ocimum basilicum*) on drug metabolizing and antioxidant enzymes, and on carcinogen-induced skin and fore stomach