

Ocimum sanctum aqueous leaf extract provides protection against mercury induced toxicity in Swiss albino mice

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HgCl₂ (5.0 mg/kg body weight) induced toxicity led to significant elevation of lipid peroxidation (LPO) level but decline in the glutathione content in liver of Swiss albino mice. In serum of HgCl₂ treated mice there was significant elevation in serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) activities but significant decline in the alkaline phosphatase activity. Animals treated with *O. sanctum* extract (10 mg/kg body weight, po) before and after mercury intoxication showed a significant decrease in LPO level, SGOT and SGPT activities and increase in serum alkaline phosphatase activity and glutathione (GSH) content. *Ocimum* treatment alone did not alter SGOT, SGPT and alkaline phosphatase activities but significantly enhanced reduced glutathione. The results suggest that oral administration of *Ocimum* extract provides protection against HgCl₂ induced toxicity in Swiss albino mice.

Mercury is a transition metal, it promotes the formation of reactive oxygen species (ROS) such as hydrogen peroxides. These ROS enhances the subsequent iron and copper-induced production of lipid peroxides and the highly reactive hydroxyl radical¹⁻³. These lipid peroxides and hydroxyl radical may cause the cell membrane damage and thus destroy the cell. Mercury also inhibits the activities of the free radical quenching enzymes catalase, superoxide dismutase, and glutathione peroxidase⁴. Exposure to mercury cannot be avoided since it is being widely used⁵ in the industrial, medical, agriculture and other fields. Thus it is important to develop an effective drug to provide protection against mercury induced toxicity.

Several naturally occurring dietary or non-dietary constituents, and parts of several species of edible plants having pharmacological activity, influence the antioxidant enzymes and provide protection against free radical induced damage.

Ocimum sanctum (sacred basil; green tulsi; Family: Labiatae) is a traditional medicinal plant. The oil of *O. sanctum* possesses antibacterial⁶, antifungal⁶, antistress⁷, immunostimulatory⁸, anticarcinogenic⁹, antioxidant⁹ and radioprotective¹⁰ properties.

Keeping in view the pharmacological properties of *Ocimum*, present investigation has been undertaken to assess the protective effect of *Ocimum* extract on HgCl₂ induced toxicity in Swiss albino mice.

Animals—Adult male Swiss albino mice (6-8 weeks old, weighing 23 ± 2g) from an inbred colony

(procured from IVRI - Izatnagar) and maintained at the animal house of the department were used. The animals were maintained on standard mice feed and water *ad libitum*. Once a fortnight tetracycline water was given as a preventive measure against infection.

***Ocimum* extract**—*Ocimum sanctum* is an annual herb. Fresh leaves of *O. sanctum* collected locally, were air dried, powdered mechanically and extracted with double distilled water at 60°C for 36 hr. The aqueous extract was vacuum evaporated to powdered form. The extract was re-dissolved in double distilled water just before oral administration.

Mercuric chloride—Mercury in the form of HgCl₂ was obtained from Merck India Ltd. It was dissolved at different dose levels in 0.9% NaCl and administered ip.

Experimental design—Swiss albino mice (120) were divided into the following 4 groups of 30 each.

- Group I : No treatment was given to these animals. (control)
- Group II : The animals were given *Ocimum* leaves extract (10 mg/kg body weight in distilled water po) for 30 consecutive days.
- Group III : These animals were administered HgCl₂ 5.0 mg/kg body weight in 0.9% NaCl, ip.
- Group IV : In this group of animals, *Ocimum* extract (10 mg/kg body weight) was given orally for 10 consecutive days, before. HgCl₂ (5 mg/kg body weight) administration and until 30 days of HgCl₂ administration

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Six animals from each group were autopsied at 1, 3, 7, 15 and 30th days after HgCl₂ administration. The liver was excised and processed for GSH¹¹ and LPO¹² estimation. Blood from autopsied animals was collected by cardiac puncture and serum was separated. The serum was processed for SGOT¹³, SGPT¹³ and alkaline phosphatase¹⁴ activity.

Histological studies in liver have been done as per methods reported earlier¹⁵. These studies were approved by the ethical committee of the department.

Statistical analysis—The values are expressed as mean \pm SE. The data were subjected to Student's *t* test for comparison between the groups.

Results are presented in Figs. 1 and 2. Mercuric chloride induces pathological changes in the liver such as cytoplasmic vacuolization, karyorrhexis, karyolysis, pycnosis and centrilobular necrosis. However, in combination group (Group IV) reduced cytoplasmic vacuolization and centrilobular necrosis were observed¹⁵ (Fig. 2).

Mercury intoxication showed a significant increase in SGOT and SGPT activities. This confirms our earlier report on histopathological changes in liver induced by HgCl₂ (ref. 15). The increase in SGOT and SGPT in serum may be due to hepatocellular necrosis, which causes increase in the permeability of the cell membrane resulting in the release of transaminases in the blood stream^{16,17}.

Further, there was a decrease in the serum alkaline phosphatase activity after HgCl₂ intoxication. In the liver, it is closely connected with lipid membrane in the canalicular zone, so that any interference with the bile flow, whether extra hepatic or intra hepatic leads to decrease in serum alkaline phosphatase activity¹⁶. Mercury causes cell membrane damage (lipid peroxidation), which leads to the imbalance between synthesis and degradation of enzyme protein¹⁸, thus lowering, the enzyme activity. Present findings are in agreement with the findings of El-Demerdash¹⁹ who showed that HgCl₂ (0.5 μ mol/ml) intoxication significantly decreases the alkaline phosphatase activity in rats.

GSH is the major thiol, which binds electrophilic molecular species and free radical intermediates. It plays a central role in the antioxidant defence system, metabolism and detoxification of exogenous and endogenous substances.^{20,21} Mercury has high affinity for GSH and causes the irreversible excretion of up to two GSH tripeptides²². The metal-GSH conjugation process is desirable in that it results in the excretion of

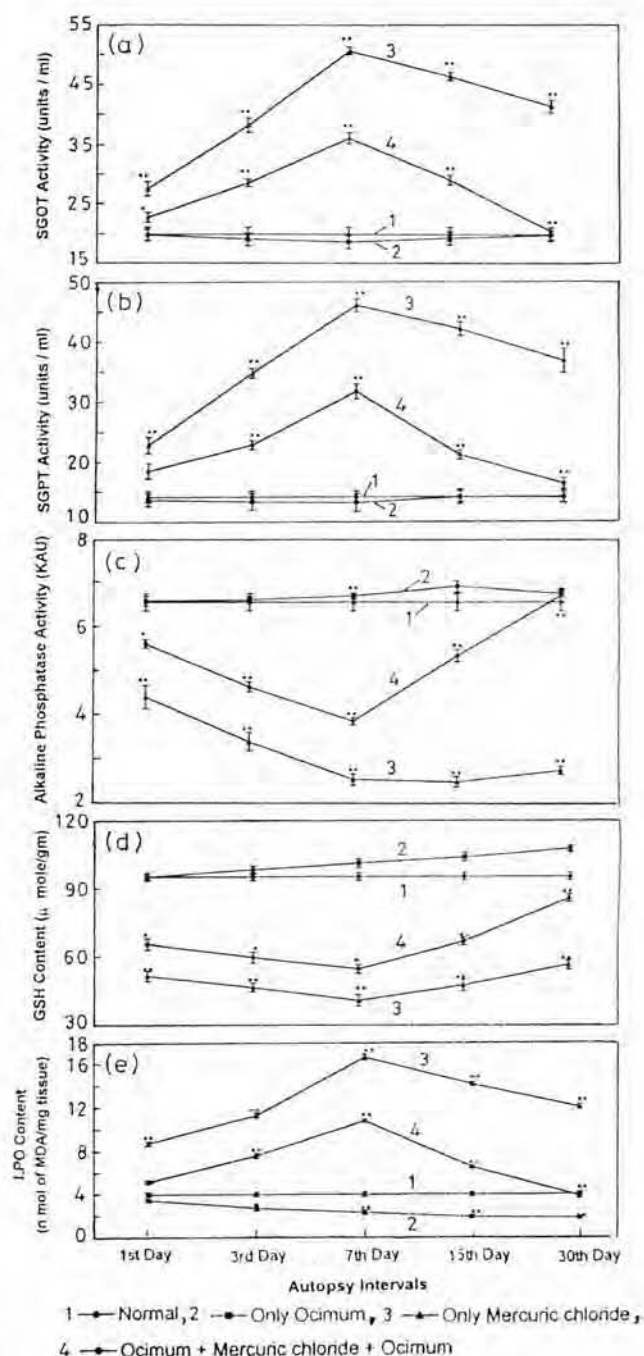


Fig. 1—Effect of *O. sanctum* aqueous leaf extract on (a) SGOT, (b) SGPT, (c) alkaline phosphatase activities and (d) GSH and (e) LPO content in Swiss albino mice (Values are mean \pm SE of 6 mice; $P^* < 0.01$; $^{**} < 0.001$)

the toxic metal into the bile. However, mercury can deplete the GSH from the cell and decrease the antioxidant potential. In the present investigation it was observed that HgCl₂ treatment significantly reduced the GSH content thus reducing the antioxidant potential and accelerating the lipid peroxidation, resulting in cellular damage.

It was observed that an aqueous extract of *Ocimum* leaves when given alone or in combination significantly enhances the GSH content and declines lipid peroxidation and reduces the mercury toxicity which in turn, is reflected by significant decrease in SGOT and SGPT and increase in alkaline phosphatase activity.

The protective activity of *O. sanctum* aqueous leaves extract against mercury induced toxicity may to some extent, be mediated through the release of intracellular antioxidants which, in turn, will scavenge the free radicals and may also help in repair of biochemical lesions. *In vitro* studies^{23,24} further indicate that metal chelation may also have a role in the anti-lipid peroxidative effect of *Ocimum* leaves. In addition, immune stimulation was suggested as a mechanism contributing to the adaptogenic action of plant²⁵.

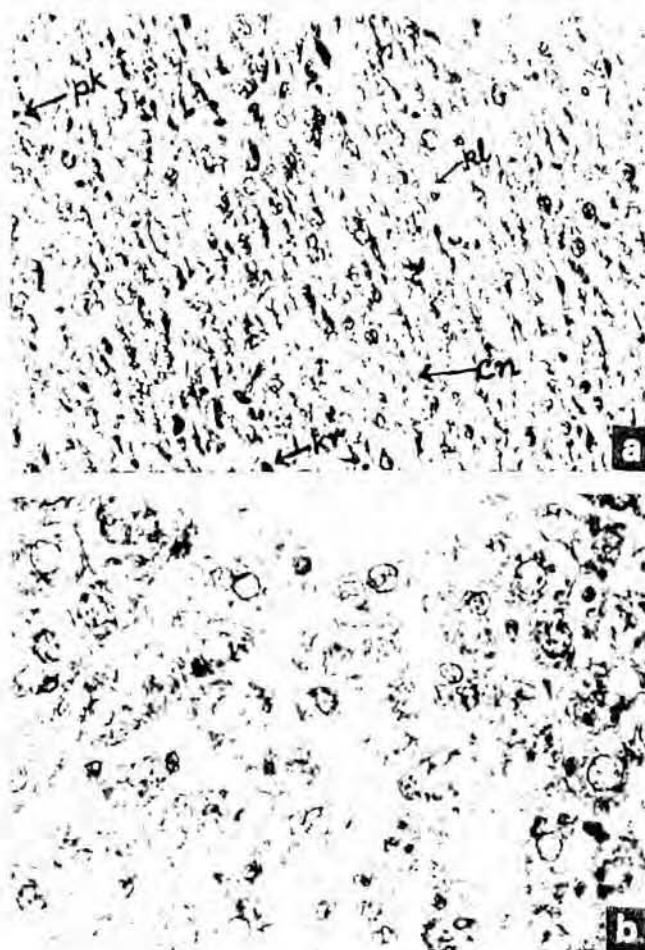


Fig. 2—Photomicrograph of liver (a) showing various pathological alterations in HgCl_2 treated group such as, centrilobular necrosis (cn), karyolysis (kl), karyorrhexis (kr) and occasional pyknosis (pk). HE X 100, and (b) showing less damage in the combination group (*Ocimum* + HgCl_2 + *Ocimum*) as compared to HgCl_2 treated group HE X 200.

Some of the compounds found in the *Ocimum* plant have been reported to possess strong antioxidant activity. Eugenol and ursolic acid from *O. sanctum* have been reported to induce protection against free radical induced cellular damages²⁶⁻²⁸. The *Ocimum* flavonoids orientin and vicenin also exhibited strong inhibitory effect on the fenton reaction generated OH radical activity. They have strong antioxidant activity *in vitro*^{24,25,29} and anti lipid peroxidative effect *in vivo*^{25,30} which strongly suggest free radical scavenging as a major mechanism by which *Ocimum* products protect against cellular damage. Thus it may be speculated that several potentially confounding substances present in *Ocimum* leaves extract e.g. eugenol, flavonoids and ursolic acid, contribute effectively in a synergistic manner to provoke the activity of free radical scavenging enzyme system and prevent the free radical induced cellular damages^{24,31}.

To conclude, the present study demonstrated that deleterious reactive oxygen species or lipid peroxides responsible for HgCl_2 induced toxicity may be alleviated by aqueous extract of *Ocimum* leaves, which in turn reflected in decline in LPO, SGOT and SGPT and elevation in GSH content and alkaline phosphatase activities as compared to HgCl_2 treated group in Swiss albino mice.

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