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Hepatoprotective effect of kasni against aflatoxin B_1 induced hepatic damage in mice

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SUMMARY

The efficacy of alcoholic extract of Kasni (*Cichorium intybus* L.) to control hepatic damage induced by aflatoxin B_1 was explored in *Swiss albino* mice. Aflatoxin B_1 was administered orally to the mice with a daily dose of 66.6 μg/kg body weight till three months. A signifi-cant increased in thio barbituric acid reactive substances (TBARS) levels with concomitant reduction in enzymatic (glutathione-s-transferase, glutathione peroxidase, superoxide dismutase, and catalase) and non-enzymatic (reduced glutathione) antioxidants were shown in aflatoxin treated group of mice. However, there was a significant reduction in increased TBARS levels and elevation in enzymatic . and non enzymatic antioxidant levels in group of mice which received alcoholic extract of kasni (300 mg/kg bw/day) along with aflatoxin. Histopathological analysis of liver samples also confirmed the hepatoprotective effect of kasni extract. These results suggest that kasni shows hepatoprotective effect against aflatoxin B_1 induced hepatic damage in mice.

Key words: Aflatoxin B_1; Antioxidants; *Cichorium intybus* L.

INTRODUCTION

Aflatoxin B_1 is the most potent toxin frequently present in corn, nuts, cottonseeds and sometimes in meats. Aflatoxin causes aflatoxicosis, a hepatic disease, which leads to clinical illness and death. Clinical signs of aflatoxicosis include gastrointestinal dysfunction, reduced reproductivity and feed utilization, anemia and jaundice. Nursing animals may also be affected as a result of the conversion of aflatoxin B_1 to other endogenously metabolized product (Roebuck and Yulia, 1994). Aflatoxicosis has been reported in India, China, Thailand and several African countries where environmental conditions favor the aflatoxin production and contamination (Muriel and Alex, 1983). Toxicity induced by aflatoxin to human and livestock thus gain importance owing to its long-term ill effects. Use of natural remedies for the treatment of liver diseases has a long history consisting of the Ayurvedhic, Chinese, European and other systems of traditional medicines.

In the Indian system of medicine kasni (*Cichorium intybus* L.) is very commonly used in liver damage (Jindal *et al.*, 1975; Saxena and Garg, 1979) and also afford protection against free radical mediated hepatic damage (Krandikar *et al.*, 1979; Barhan *et al.*, 1985).

In this study we assessed the hepatoprotective effect of kasni against aflatoxin B_1 induced hepatic damage. Our results suggest that kasni extract (KE)

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may show protection due to free radical scavenging properties.

MATERIAS AND METHODS

Reagents

Aflatoxin B_1 (AFB_1, $C_{17}H_{12}O_6$; EC No. 214-603-3), nicotinamide adenine dinucleotide phosphates (NADPH) and glutathione reductase were obtained from Sigma Aldrich (U.S.A.). Thiobarbituric acid was obtained from E-Merck (India) Limited, while the rest were obtained from s.d.Fine Chem Limited.

Preparation of KE

Plants of kasni were collected from the Herbal Garden of Jamia Hamdard, New Delhi. Alcoholic extract of kasni was prepared by soxhlet method using 500 ml absolute alcohol for 100 g (dry weight) of plant material. Extract was concentrated in water bath to semisolid form (Bardhan *et al.*, 1985).

Animals

Fourty eight *Swiss albino* mice (male, 30 - 40 g) bred in Central Animal House of Jamia Hamdard used for the study. The animals were divided in group of twelve and housed under standard laboratory conditions with food and water provided *ad libitum*. The animal ethics committee of Hamdard University approved the study protocol.

Treatment schedule

The experiment includes four groups: (I), control (normal saline); (II), aflatoxin; (III), KE and (IV), aflatoxin + KE. Aflatoxin at a concentration of 66.6 μg/kg bw/day was administered orally to the mice for three months to induce hepatic damage (Nair *et al.*, 2000). KE (0.3 g/kg bw/day) were given after 30 min of aflatoxin administration for three months.

Biochemical assays

On 30, 60 and 90 days during treatments, the animals were sacrificed by cervical dislocation. Liver of all controls and treated groups of animals were quickly isolated, blotted free of blood and utilized for biochemical analysis. Post mitochondrial supernatant (PMS) was prepared using method of Mohandas *et al.* (1984) with some modifications. The tissues were homogenized in chilled phosphate buffer (0.1 M, pH 7.4) using a potter-elvejem homogenizer. The homogenate was filtered through muslin cloth and centrifuged at 800 × *g* for 5 min at 4°C to separate the debris. The supernatant was centrifuged at 1,200 × *g* for 30 min at 4°C to obtain the PMS used for the biochemical analysis.

Activities of antioxidant enzymes were analyzed in order to assess their status during hepatic damage and to investigate the role of KE on their levels. Glutathione-s-transferase (GST) activity was measured by the method of Habig *et al.* Glutathione peroxidase (GPX) activity was determined by the method of Mohandas *et al.* (1984). Superoxide dismutase (SOD) activity was measured by the method of Dhindsa *et al.* (1981) and catalase activity was measured by the method of Claiborne *et al.* (1985). Reduced glutathione (GSH) level was determined by the method of Jollow *et al.* (1974) and oxidative damage (thio barbituric acid reactive substances; TBARS) was determined by the method of Utley *et al.* (1967).

Histopathological analysis

Histopathological studies of liver tissues were carried out in pathology department of Maulana Azad Medical College, New Delhi, India to assess the visible hepato-protective effect of kasni on aflatoxin induced hepatic damage in mice.

Statistical analysis

Data are presented as mean ± S.E. The differences among different groups were analyzed using one-way analysis of variance (ANOVA) followed by Student '*t*' test. A *P* value of less than 0.05 was considered statistically significant.

RESULTS

In our study oral administration of aflatoxin B_1

showed decrease in antioxidant enzymes such as GST, GPX, SOD and catalase (CAT) and GSH as compared to control (group I). However there is elevation in TBARS levels in aflatoxin treated group of mice (II) as compared to control group.

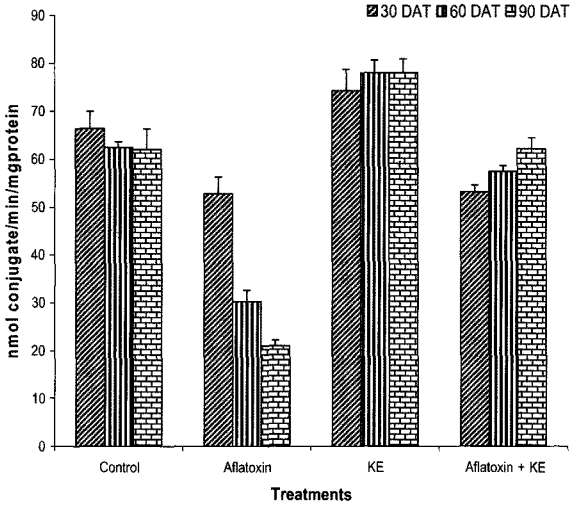

Fig. 1. Effect of KE on GST activity in aflatoxin treated liver of mice. All values in graphs expressed as mean ± S.E. $P < 0.05$ as compared with the control group and as compared with the aflatoxin treated group (one way ANOVA).

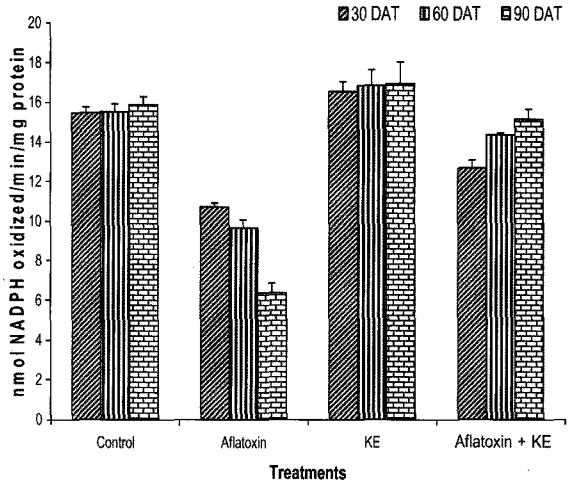

Fig. 2. Effect of KE on GPX in aflatoxin-treated liver of mice. All values in graphs expressed as mean ± S.E. $P < 0.05$ as compared with the control group and as compared with the aflatoxin treated group (one way ANOVA).

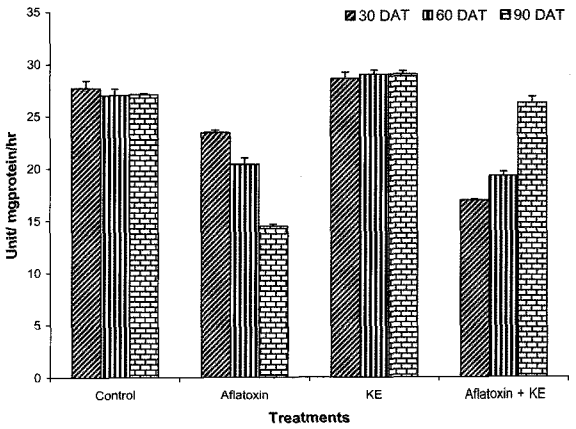

Fig. 3. Effect of KE on SOD activity in aflatoxin treated liver of mice. All values in graphs expressed as mean ± S.E. $P < 0.05$ as compared with the control group and as compared with the aflatoxin treated group (one way ANOVA).

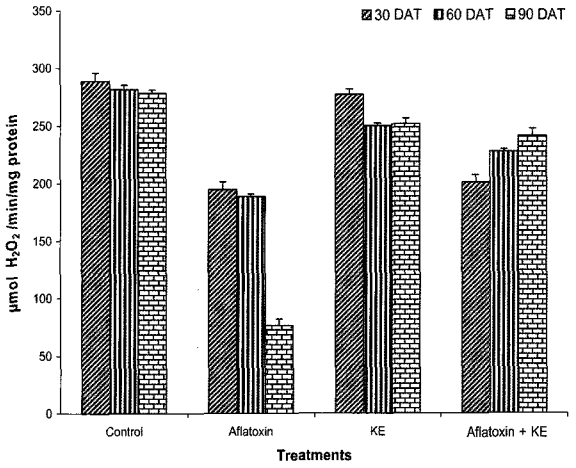

Fig. 4. Effect of KE on CAT activity in aflatoxin treated liver of mice. All values in graphs expressed as mean ± S.E. $P < 0.05$ as compared with the control group and as compared with the aflatoxin treated group (one way ANOVA).

Animals of group IV treated with KE along with aflatoxin showed significant ($P < 0.05$) increase in levels of enzymatic and non enzymatic antioxidants as compared to group II animals which received only aflatoxin (Fig. 1 - 5).

The present study showed that when mice were treated with alcoholic extract of kasni along with aflatoxin (group IV) showed time dependent decrease

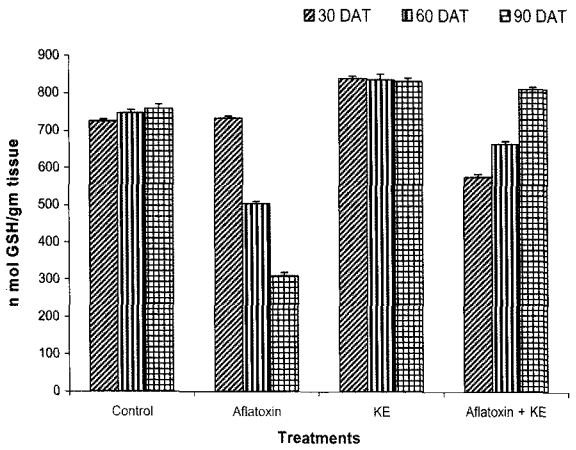

Fig. 5. Effect of KE on GSH levels in aflatoxin treated liver of mice. All values in graphs expressed as mean ± S.E. $P < 0.05$ as compared with the control group and as compared with the aflatoxin treated group (one way ANOVA).

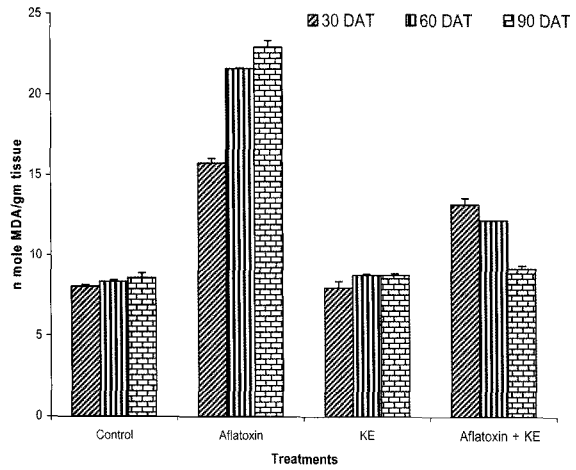

Fig. 6. Effect of KE on oxidative damage (TBARS) in aflatoxin treated liver of mice. All values in graphs expressed as mean ± S.E. $P < 0.05$ as compared with the control group and as compared with the aflatoxin treated group (one way ANOVA).

in TBARS levels significantly ($P < 0.05$) (Fig. 6).

Histopathological analysis of aflatoxin treated mice liver showed congestion, inflammation and hydropic changes in hepatocytes as compared to normal saline treated mice liver (Fig. 8). While, mice liver treated with aflatoxin and KE simultaneously showed kupfer cells hyperplasia and regeneration

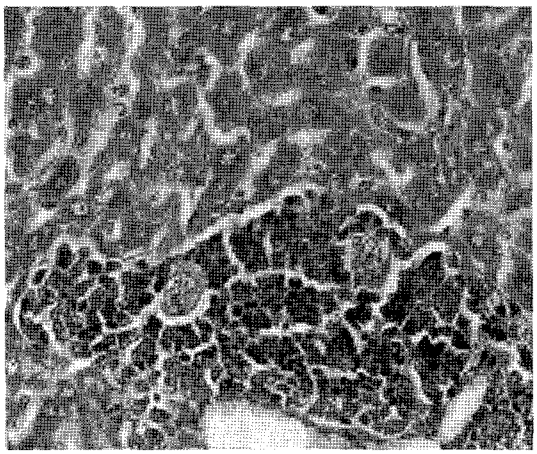

Fig. 7. Histopathological analysis of mice liver showing effect of normal saline.

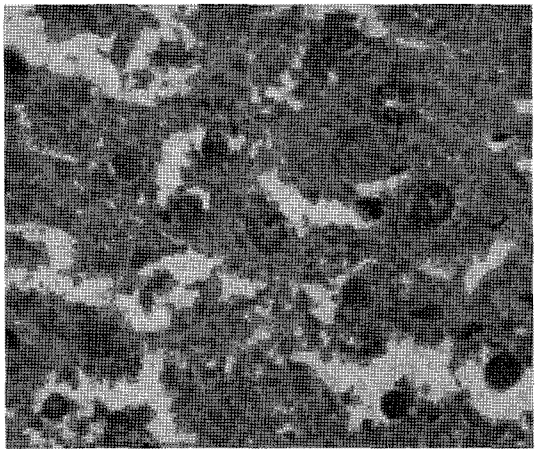

Fig. 8. Histopathological analysis of mice liver showing effect of aflatoxin.

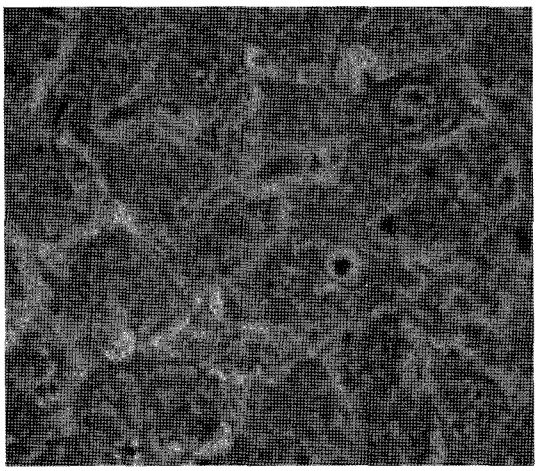

Fig. 9. Histopathological analysis of mice liver showing effect of aflatoxin with KE.

activities in cells (Fig. 9) as compared to control group, which was normal (Fig. 7).

DISCUSSION

Aflatoxins are toxic metabolites of fungal origin, most commonly present in food stuffs. Aflatoxin B_1 is biologically reactive compound altering a number of biochemical systems (Keeler, 1983). It causes oxidative stress in animals, which implies that cells have intact pro-oxidant/antioxidant systems that continuously generate and detoxify oxidants during normal aerobic metabolism. When additional oxidative events occur, the prooxidant systems outbalance the antioxidants, potentially producing oxidative damage to lipids, proteins, carbohydrates and nucleic acids, ultimately leading to the cell death in severe oxidative stress conditions (James, 1999). It has been assured that aflatoxinB_1 with the help of microsomal cytochrome - p450 mediated oxidation is biotransformed into afalatoxin 8, 9 epoxide which is a reactive intermediate and gives toxic properties (Lin *et al.*, 1977; Iyer *et al.*, 1994). In our study, the results obtained showed that GST, GPX, SOD and CAT activities declined in aflatoxin B_1 treated group of mice as compared to the control group. On the other hand, there was an increase in the levels of these enzymes in group, which received aflatoxin along with alcoholic extract of kasni (IV). In previous study, it has been reported by Narasimhan *et al.* (2000) that *Amrita bindu* (mixture of spices) alleviate aflatoxin induced oxidative stress in case of fishes.

Under oxidative stress conditions, the concentration of glutathione is considerably diminished through conjugation to xenobiotics, and by secretion of both the glutathione conjugates and glutathione disulfides from the affected cells. Our results showed that GSH levels decreases in aflatoxin treated group of mice as compared to control group. However these levels showed an increase in group of mice which received KE along with aflatoxin. TBARS levels also showed increase in group IV as compared to group II.

Histopathological analysis of liver was also carried out to study the hepatoprotective role of alcoholic extract of kasni in aflatoxin induced hepatic damage. Aflatoxin treated mice liver showed congestion, inflammation and hydropic changes in hepatocytes as compared to normal saline treated mice liver. While, mice liver treated with aflatoxin and KE simultaneously showed kupfer cells hyperplasia and regeneration activities in cells as compared to control group, which was normal.

Previous study reported the hepatoprotective efficacy of kasni against carbon tetrachloride induced liver injury (Karandikar, 1963; Bardhan *et al.*, 1985). It may be proposed that their efficacy may be due to their free radical scavenging ability. The extract of this plant have reported to contain many polyphenolics compounds mainly flavonoids. The antioxidant activity of KE may, therefore, be due the presence of polyphenolics constituents (Rastogi and Mehrotra., 1991). It may be suggested that antioxidant property of extract due to the presence of polyphenolic compounds might be responsible for its hepatoprotective property. Thus, our results suggests that alcoholic extract of kasni may be useful in hepatic damage caused by aflatoxin.

CONCLUSION

The present study has demonstrated that kasni exerts hepatoprotection against aflatoxin induced hepatic damage by suppressing the formation of reactive oxygen species, detoxifying aflatoxin induced free radical generation and by enhancing the recovery and repair process. However, further studies are required to elucidate the biochemical mechanisms of protection by alcoholic extract of kasni against aflatoxin induced hepatic damage in mice.

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