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Antitumorigenic action of fenugreek seeds

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1,2-dimethylhydrazine (DMH) is a toxic environmental pollutant. Humans are exposed to DMH through rocket fuel and Gyromitra species of mushroom. DMH induced colon tumor in rats mimics human colon tumor in morphological and histological aspects. Thus, DMH model is well established and correlated with human problems.The effects of dietary fenugreek seeds on induced colon tumor and oxidative stress was investigated in male Wistar rats. Rats administered with a weekly subcutaneous injection of DMH (20 mg/kg body weight) for 15 weeks developed colon tumor with 100% incidence, and showed a significant, i) decrease in lipid peroxidation (LPO) measured interms of thiobarbituric acid reactive substances (TBARS), ii) decrease in phospholipid, a major substrate for LPO and iii) increase in glutathione dependent enzymes- glutathione peroxidase (GPx) and glutathione S-transferase (GST), when compared to control rats. However, supplementation of dietary fenugreek to DMH treated rats significantly decreased the tumor incidence to 16.66%, increased the TBARS and phospholipid content and decreased the GPx and GST activities when compared to DMH treated rats. It is suggested that fenugreek act as antitumorigenic agent by influencing DMH induced colon tumor incidence and oxidative stress through its constituents flavonoids, saponin, protease inhibitors and dietary fibre.

Key words : Fenugreek, Colon cancer, Phospholipid, Lipid peroxidation, Glutathione, Mushroom.

INTRODUCTION

1,2-dimethylhydrazine (DMH) is a toxic environmental pollutant. Humans are exposed to DMH through rocket fuel and Gyromitra species of mushroom. DMH induced colon tumor in rats mimics human colon tumor in morphological and histological aspects. Thus, DMH model is well established and correlated with human problems. Several biomarkers have been suggested for biomonitoring the action of antitumorigenic agents of which oxidative stress is well established. This is because oxidative stress is an important aspect in contributing to the cancer development (Bobek *et al.*, 2000). 1,2-Dimethylhydrazine (DMH) a potent colon carcinogen has been reported to elicit oxidative stress associated with decreased lipid peroxidation and increased antioxidant enzymes during experimental colon tumorigenesis (Manoj *et al.*, 1999).

Dietary intervention is one of the most promising approaches in the suppression of colon cancer (Garay and Engstrom, 1999; Tiwari, 2001). Several natural foods act as antitumorigenic agent by ameliorating the oxidative stress during experimental tumorigenesis. Phytochemicals such as flavonoids, saponins and protease inhibitors from plant foods ameliorate oxidative stress (Hayatsu *et al.*, 1998; Richter *et al.*, 1999) and prevent tumor development (Lipdkin *et al.*, 1985; Mure and Rossman, 2001)

From this aspect it is logical to assume that plants rich in phytochemicals could be used for the amelioration of oxidative stress and prevention of tumor incidence. Seeds of fenugreek (*Trigonella foenum graecum*) is consumed as a spice and food additive in many parts of country. It is a good source of phytochemicals (Jain and Aggarwal, 1990)

Recently we have reported that fenugreek seeds prevent colon tumorigenesis by influencing the metabolism of bile acid, phospholipid and microfloral enzymes (Devasena *et al.*, 2003a; Devasena *et al.*, 2003b). It was also demonstrated that fenugreek modulates hepatic and colonic oxidative stress during its antitumorigenic action (Devasena and Menon, 2002. Devasena *et al.*, 2005).

This part of our study was carried out to investigate whether fenugreek influences colonic oxidative stress during DMH induced colon tumorigenesis. We have measured colonic i) phospholipid (a major substrate for lipid peroxidation - LPO), ii) thiobarbituric acid reactive substances – TBARS (an index of lipid peroxidation) and iii) activities of glutathione dependent enzymes as markers of oxidative stress.

MATERIALS AND METHODS

Animals:

Male albino rats of Wistar strain weighing between 100 and 120 g were obtained from the Central Animal House, Department of Experimental Medicine, Annamalai University. Animals were housed in polypropylene cages. Commercial pellet feed containing 5% fat (obtained from Hindustan Lever Limited, Mumbai, India) was powdered and mixed with 15% peanut oil making a total of 20% fat in the diet. The feed and water were given ad libitum

(Manoj *et al.,* 1999, Devasena *et al.,* 2003).

Preparation of fenugreek seed powder:

Fenugreek seeds were purchased from the local market of Annamalai Nagar, Tamil Nadu, India. The seed was identified by a Botanist in the Department of Botany, Annamalai University. Seeds were cleaned of extraneous matter, dried in shade and finely powdered in a mechanical mixer. Fenugreek powder was weighted every day and given in the diet at a dose of 2 g/kg body weight.

Experimental design:

Animals were randomly divided into 4 groups of 6 animals each.

Group 1: Control rats.
Group 2: Rats administered DMH (20 mg/kg body weight) once in a week for 15 weeks.
Group 3: Rats given fenugreek powder in the diet (2 g/kg body weight) daily for 30 weeks.
Group 4: Rats administered DMH as in group 2 as well as fed with fenugreek powder as in group 3.

The experiment was terminated at the end of 32nd week (including 2 weeks of acclimatization). All animals were sacrificed by cervical decapitation after an overnight fast. Colon was slit open longitudinally and the number of gross tumors/polyps were counted visually. The colon was then cut and immediately transferred to ice cold saline for further investigation.

Biochemical estimations:

Phospholipid was estimated by the method of Zilversmit (Zilversmit and Davis, 1950).

TBARS was estimated by the method of Yagi (Yagi, 1978) and reduced glutathione by the method of Ellman (Ellman, 1959). The activities of glutathione peroxidase (GPx) and glutathione S-transferase (GST) were estimated by the method of (Rotruck *et al.,* 1973; Habig *et al.,* 1974), respectively.

Statistical analysis

The data presented as mean ± SD, were analysed using analysis of variance (ANOVA) and the group means were compared by Duncan's Multiple Range Test (DMRT). The results were considered statistically significant when $P \leq 0.05$.

Results and Discussion

Macroscopic observations:

Effect of fenugreek on percentage incidence, multiplicity (average number of gross tumors per rat) and size of colonic tumors in DMH treated rats are summarized in Table 1. There was no tumor formation in untreated control rats (Group 1). In the case of animals given DMH (Group 2), the tumor incidence was 100% and the average size of the tumor was 2 cm. Dietary supplementation of fenugreek to DMH treated rats (Group 4) decreased the tumor incidence (16.66%) and also reduced the tumor multiplicity and tumor size (< 0.5 cm). No tumor was observed in fenugreek treated control rats (Group 3).

Data on the effects of dietary fenugreek on colonic phospholipid content and TBARS in the control and experimental rats are given in Table 2. Phospholipid and TBARS in DMH-induced colon tumor bearing rats (Group 2) was lowest among the groups. However, supplementation of fenugreek seed powder to DMH administered rats (Group 4) increased the phospholipid content and TBARS towards near normal values (Group 1). Supplementation of fenugreek to control rats (Group 3) did not significantly affect the phospholipid content and TBARS.

GSH content and the activities of GSH dependent enzymes are also given in Table 2. The levels of GSH as well as the activities of GPx and GST were significantly increased in DMH treated rats when compared to untreated control (Group 1), while all these parameters were significantly decreased in DMH + fenugreek treated rats compared to DMH treated rats.

After 32 weeks of experimental period, the present study investigated the (1) tumor incidence, (2) phospholipid content, (3) LPO and (4) the activities of GPx, GST in the colon.

Present results show that a marked colonic oxidative stress has been elicited (decreased LPO, increased GPx and GST; Table 2) with simultaneous development of colonic tumors (Table 1) in response to the DMH treatment.

Reduced incidence of colonic neoplasms (16.6%) in DMH + fenugreek treated rats when compared to DMH treated rats (100%) and absence of tumor in fenugreek treated control rats were noticed. These macroscopic observations together with our previous report or microscopic histopathologic studies of DMH and DMH + fenugreek treated rats (Devasena *et al.,* 2003) suggest that fenugreek exhibit chemopreventive activity and that fenugreek is non toxic and lack side effects.

DMH is a procarcinogen which induces tumor formation after undergoing various metabolic changes in colon as shown in Fig. 1 (Fiala, 1977). Blocking agents are chemopreventive agents that prevents tumor formation

Table 1 : Incidence of colonic neoplasms

Group	Treatments	Number of rats examined	Number of rats with tumor	Tumor incidence (%)	Number of tumors per rat (average)	Tumor size (cm) (average)
1.	Control	6	0	0	0	-
2.	DMH	6	6	100	6	≈ 2
3.	Fenugreek	6	0	0	0	-
4.	DMH + Fenugreek	6	1	16.66	2	< 0.5

Table 2 : Colonic phospholipid, TBARS, GSH and activities of GPx and GST in control and experimental rats

Group	Phospholipid (mg/g tissue)	TBARS (mg/g tissue)	GSH (mg/g tissue)	Gpx (units[1])	GST (units[2])
Control	7.55 ± 2.18^{abc}	4.06 ± 0.16^{abc}	1.35 ± 0.12^{dbc}	5.65 ± 1.22^{dcb}	601.12 ± 22.84^{cdb}
DMH	4.13 ± 0.22^{d}	1.23 ± 0.08^{d}	3.02 ± 0.14^{a}	12.51 ± 1.23^{a}	766.15 ± 24.44^{a}
Fenugreek	7.42 ± 1.11^{b}	3.87 ± 0.12^{c}	1.53 ± 0.10^{b}	5.92 ± 1.92^{c}	598.98 ± 21.26^{d}
DMH + Fenugreek	6.98 ± 1.23^{c}	3.95 ± 0.11^{b}	1.51 ± 0.09^{c}	6.64 ± 0.96^{b}	611.18 ± 21.49^{b}

Values are mean ± SD from 6 rats in each group
[a-d] values not sharing a common superscript differ significantly at $P < 0.05$
Unit[1] -µg of GSH utilised/min/mg protein
Unit[2] -µM of CDNB-GSH conjugate formed/min/mg protein.

by blocking carcinogen-DNA adduct formation (Fig. 1). Flavonoids and saponins are good examples for blocking agents (Wattenberg, 1985). Fenugreek seeds are rich in flavonoids (quercetin, kaempferol, luteolin and vitexin) and saponins (Jain and Aggarwal, 1990). Suppressing agents are chemopreventive agents that prevents cancer by suppressing the neoplastic manifestation. Protease inhibitors are reported to be cancer suppressing agents (Wattenberg, 1985) and widely distributed in fenugreek seeds (Jain and Aggarwal, 1990). Thus, it is suggested that the colon tumor inhibitory activity of fenugreek seeds in DMH treated rats (Table 1; Group 4) could be due to its constituent flavonoids, saponins and protease inhibitors which could act as blocking agents and suppressing agents, respectively.

Colon of DMH challenged rats were less susceptible to LPO, as evidenced by the low levels of TBARS. DMH is reported to enhance *in vivo* bile acid concentration (Devasena *et al.*, 2003). Bile acids stimulate phospholipase activity which inturn hydrolyses

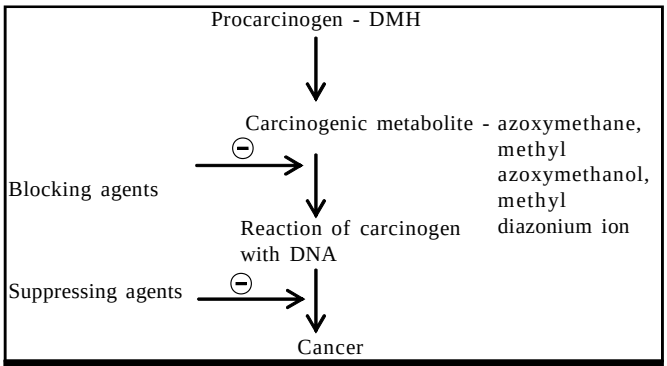

Fig. 1: Mechanism of Cancer Prevention

phospholipid into arachidonic acid. Finally arachidonic acid is converted into Prostaglandin (PG), which promotes growth and proliferation of colon cells to form tumor (Longo *et al.*,1999).

These findings related to bile acid phospholipid and tumor incidence have already been published (Devasena *et al.*, 2003(a); Devasena *et al.*, 2003(b) as well as by other workers (Nalini *et al.*, 1997). Further, Cheesman *et al.* (1986) have reported low LPO in proliferating tumor cells when compared to normal counterparts. Tanaka *et al.* (1998) and Barrera *et al.* (1991) have also highlighted an inverse relationship between LPO and proliferation. These reports support present findings. Thus, it is suggested that, in DMH treated rats decreased LPO is due to a decrease in phospholipid (a major substrate for LPO) and increased tumor incidence is due to PG induced proliferation. Thus, LPO and tumor incidence are inversely related.

Fenugreek seeds are good source of dietary fibre and saponin (Jain and Aggarwal, 1990), both of which binds to bile acid and prevent its stimulative action on phospholipases (Stark and Madar, 1993). This may consequently lead to prevention of 1) phospholipid hydrolysis, 2) arachidonic acid formation and 3) prostaglandin generation, finally leading to inhibition of proliferation and tumor formation. Thus we could suggest that fenugreek through its fibre and saponin content not only increases LPO by increasing the availability of the substrate phospholipid but also evokes antiproliferative response by preventing prostaglandin generation.

Elevated levels of colonic GSH and GSH dependent

enzymes (GST and GPx) in DMH administered animals may be associated with the observed decrease in the LPO. Exposure to carcinogens that cause oxidative stress, results in elevation of tissue GSH levels in rodents (Borroz *et al.,* 1994; Forman *et al.,* 1999). Moghadasian *et al.* (1996) used DMH-induced colon cancer model to show that GSH utilising enzymes are induced in cancer tissues. Elevation of GSH and GST dependent enzymes were considered as the adaptation of the tissues against toxic challenge (Singh *et al.,* 2000). Thus, the increased colonic GSH, GPx and GST may be due to the toxicity produced by the DMH.

Decrease in colonic GSH, GPx and GST in DMH + fenugreek treated rats when compared to DMH treated rats suggest that fenugreek act as antitoxic agent and protects colon from DMH induced toxicity by influencing GSH dependent enzymes.

Conclusion:

It could be concluded that fenugreek blocks colon tumorigenesis by ameliorating DMH induced oxidative stress and colonic toxicity. Antitumorigenic action of fenugreek may be due to its constituent flavonoids, saponin, protease inhibitors and dietary fibre. However, identification of the active antitumorigenic constituent needs further study.

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