Phytochemical Protection against Diethylnitrosoamine Induced Hepatocarcinogenesis by *Trigonella foenum graecum* in Female Rats

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**ABSTRACT**

*Trigonella foenum graecum* (fenugreek) is traditionally used to treat. Recent studies suggest that fenugreek and its active constituents may possess anticarcinogenic potential. The preventive efficacy of dietary fenugreek seed 2% and 4% (w/w feed) on diethylnitrosamine-induced rat liver carcinogenesis were evaluated. Rats were sacrificed when rats became very weak with unstable shelf live were chosen as an end point. Egypt is an Islamic country alcoholic prohibition so the possible use of ethanol extraction may be accepted with high restriction towards its applications in human, that it may affect the active constituents of fenugreek seeds. Whereby, fenugreek seeds contain >8% oil, many valuable phenolic compounds, protein and amino acids, etc...with different concentrations according to the extraction method. So, the present study was carried out to evaluate the effect of fenugreek powder on adult female rats fed experimental diets containing 2% or 4% (w/w) fenugreek seed powder (FSP) for 2 weeks and have phenobarbital in a dose of 200mg/L *ad lib. before and after a single injection with diethylnitrosamine* (200 mg/kg body weight). Rats were sacrificed 20 weeks after intra-peritoneal (*i.p*) diethylnitrosamine injection and their livers, spleens, kidneys and lungs were excised washed well with cold saline, weighted and processed through paraffin wax preparation, staining and pathological changes were examined. It was found that, by comparison with control, continuous feeding of FSP 2% and 4% suppressed hepatocarcinogenesis up to 2% and 50%, respectively. In addition, on the basis of these findings, the invaluable and precious fenugreek constituents are diosgenin [(25R)-5-spirosten-3h-ol] and [5,7-dihydroxy-2-(4-hydroxyphenyl)-6-(3,4,5-trihyd-roxy-6(hydroxymethyl) tetra- hydro-2H-pyran-2-yl)chroman-4-one] besides, others. Finally, fenugreek seeds seem to have potential role as a novel cancer preventive agent and that needs further investigations.

**Key words:** Fenugreek seeds / Liver / Toxin / DietylNitrosamine / HCC / Thyroid Hormones / Female Rats.

**INTRODUCTION**

Phytochemicals are defined as bioactive non-nutrient plant compounds in fruits, vegetables, grains, and other plant foods that have been linked to reducing the risk of major chronic diseases. The word ‘phyto-’ is derived from the Greek phyto which means - plant. The presence of these bioactive components are said to confer them with resistance against bacterial, fungal and pesticidal pathogens. These bioactive components are said to be responsible for the antimicrobial effects of plant extracts in *vitro*.

Fenugreek (*Trigonella foenum graecum*) is an annual herb that belongs to the family Leguminosea. It has a long history as both a culinary and medicinal herb. The seeds of fenugreek are commonly used as a spice in food preparations due to the strong flavour and aroma. The seeds are reported to have restorative and nutritive properties. Fenugreek seeds have antioxidant activity and have been shown to produce beneficial effects such as neutralization of free radicals and enhancement of antioxidant apparatus.
The protective effect of a polyphenolic extract of fenugreek seeds (FPEt) against ethanol (EtOH)-induced toxicity was investigated in human Chang liver cells. EtOH treatment suppressed the growth of Chang liver cells and induced cytotoxicity, oxygen radical formation and mitochondrial dysfunction. Incubation of FPEt along with EtOH significantly increased cell viability in a dose-dependent manner, caused a reduction in lactate dehydrogenase leakage and normalized GSH/GSSG ratio. The findings suggest that the polyphenolic compounds of fenugreek seeds can be considered cytoprotective during EtOH-induced liver damage\(^6\). Fenugreek seed polyphenol extract (FPEt) administration had a positive influence on both lipid profile and on the quantitative and qualitative properties of collagen in alcoholic liver disease and the protective effect is presumably due to the bioactive phytochemicals in fenugreek seeds\(^7\).

Fenugreek seeds contain approximately 40 percent of mucilage fibers. Fenugreek has been studied for its support of gastrointestinal health\(^8,9\). Fenugreek contains fatty oils, 20-30 % mucilage, trigonelline, bitter substances, and saponins and has a softening effect\(^10\). Steroid saponins are found in oats, capsicum peppers, aubergine, tomato seed, alliums, asparagus, yam, fenugreek, yucca and ginseng\(^11\). Dixit et al.\(^12\) reveals that germinated fenugreek seeds have significant antioxidant activity in which may be due partly to the presence of flavonoids and polyphenols. Raju et al.\(^13\) found that the fenugreek constituent diosgenin seems to have potential as a novel colon cancer preventive agent.

Epidemiological studies implicate apoptosis as a mechanism that might mediate the Fenugreek's anti-breast cancer protective effects suggests significant chemopreventive effects of Fenugreek seeds against breast cancer\(^14\). Extracts of fenugreek seeds and some of their saponin constituents have been found to have anticarcinogenic potency in different settings \(^15,16\). Saponins also show wide-ranging cytostatic effects against cancer cells\(^11\).

Fenugreek seed extract has been evaluated in the Ehrlich ascites carcinoma model in BALB/c mice, where it effected 70% inhibition of tumor cell growth compared with controls\(^15\). The findings of Hibasami et al.\(^16\) suggest that growth inhibition of human leukemia HL-60 cells by protodioscin, isolated from fenugreek seeds, results from the induction of apoptosis. Diosgenin [(25R)-5-spirosten-3h-ol], a steroid sapogenin constituent of fenugreek seeds, is a precursor of steroid hormones, such as progesterone, and anti-inflammatory steroids, such as cortisone\(^17\). Moalic et al.\(^18\) reported that diosgenin inhibits cell proliferation in the human osteosarcoma 1547 cell line by induction of apoptosis and G1 phase cell cycle arrest. Furthermore, in the osteosarcoma 1547 cell line, it was showed that diosgenin caused cell cycle arrest and apoptosis principally by increasing the expression of the tumor suppressor oncoprotein p53\(^19\). On the basis of the information described above, diosgenin and other fenugreek seed constituents possess anticarcinogenic properties, suggesting their potential role as suitable phytochemicals for liver cancer prevention.

The main objective of this study was to evaluate the potential efficacy of fenugreek seed, a commonly used herb in preventing liver carcinogenesis.
MATERIALS AND METHODS

Preparation of fenugreek seed powder (FSP)

Dried, viable and fresh batches of fenugreek seeds were obtained from a commercial source (Fenugreek seeds were purchased from the Egyptian local market, identities was confirmed by Botanist). Seeds were washed in distilled water, and dried in an oven at 60 °C. Dried and clean seeds were ground to a fine powder mechanically in a mixer twice a week, packed in a well closed small sacs and ready for use daily during the further experiments.

Phytochemical analysis

In a previous study the authors illustrates fatty acids compositions of fenugreek seed oil (FSO) contain 20.68% of the medium fatty acids (C12-16), while its content of the long chain fatty acids (>C16) represents 79.32%. The percentage of palmetic and palmetto in the fenugreek seed oil reached 13.3% and 4.06%, respectively, while its content of stearic, olic, lenolic and lenolenic fatty acids amounted to 4.45% 13.48%, 33.48% and 26.25%, respectively, besides, its containing of arackedonic acid represents 1.66%, crude protein, 25.18%, ether extract 8.2%, nitrogen free extract 54% (20). Amino acids contents, alanine 1.3%, arginine 2.38%, aspartic acid 2.39%, cysteine+systine 0.33%, glutamic acid 4.32%, glycine 1.73%, histadine 0.76%, Lucine+isoleucine 3.4%8, lysine 0.86%, methionine 0.72%, phynylalanine 2.54%. Proline 0.29%, serine 1.73%, threonine 1.27%, tyrosine 1.27%, valine 0.81% and traces of tryptophane (Essential amino acids 9.97% whereby, nonessential aminoacids 16.41% and total aminoacids 26.38%), there was difference in using different extracts compared with seed flour protein, and hence amino acids, as follow: Seed flour crude protein% (on dry weight basis) 28.18% where, in ethyl alcohol, petroleum ether, ether, acetone, germinated seeds and boiled seeds extracts was 26.82, 26.65, 26.09, 25.7, 24.29 and 23.23, respectively (21).

Among bioactive compounds isolated from fenugreek seeds are protodioscin, trigoneoside, diosgenin, yamogenin, and others (22,23). The past phytochemical investigations on the seeds reveals the presence of Diosgenin, Trigonelline, Gitogenin, Vicenins 1 and 2, Vitexin, Quercetin, Luteolin, Kaempferol, Sitosterol etc., moreover the endosperm of the seeds is rich in galactomannan (24).

Quantitative phytochemical analysis of the ethanolic extract of the plant indicated the presence of flavonoids, alkaloids, steroids, proteins, saponins, glycosides, gum, mucilages and some sugars (25). Egypt is an Islamic country alcoholic prohibition, so the possible use of ethanol extraction may be accepted with high restriction towards its applications in human because it may affect the efficacy of fenugreek constituents. Finally, Mishra (26) reported that, the major constituents in fenugreek are diosgenin [(25R)-5-spirosten-3h-ol] and {5,7-dihydroxy-2-(4-hydroxyphenyl)-6-(3, 4, 5-trihydroxy-6(hydroxyl methyl) tetrahdro- 2H -pyran -2-yl) choman-4-one}.

Experimental animals

In all experiments adult female rats (190±10 g) were used and maintained in a temperature controlled room (25°C±2°C), under 12 hours light/dark cycle. Rats were fed with a standard laboratory diet containing 19% crude proteins, 3.8% fiber and 4400 Kcal of energy, prepared by the Animal Nutrition Unit, Biological Applications Department, Nuclear Research Center, Egyptian Atomic Energy Authority and water ad libitum.
Chemicals

Diethylnitrosamine (DEN) was purchased from Sigma Diagnostics Inc, USA.

Dosage and administration of FSP

FSP was administered to rats. The effect of two doses of the FSP was studied. Dose 1 was 2% (w/w). This dose corresponded to normal dose administered to rats in a previous study. Dose 2 provides a higher dose of 4% (w/w) which gives worth results in the study mentioned before (20), liver tumors were induced in groups 4, 5 and 6 by two steps model according to Yoshiji et al. (27) & Thriunavukkarasu and Saktishekaran (28) with little modifications. Briefly, phenobarbital (Pb) (200mg/L) was administered two weeks for preinitiation before initiation by a single intraperitonal injection of DEN at a dose of 200 mg/Kg-body weight in saline and two weeks later, promotion by Pb add lib. till the end of the experiment, rats were categorized as follow:-

Group 1: Control (injected with normal saline only).
Group 2: Administered with FSP 2% (FSP 2% + i.p. injected with normal saline only).
Group 3: Administered with FSP 4% (FSP 4% + i.p. injected with normal saline only).
Group 4: Administered with FSP 2% and Pb in drinking water before 2 weeks and after i.p. injected with 200mg/kg DEN till the end of the experiment (FSP 2% + Pb + DEN).
Group 5: Administered with FSP 4% and Pb in drinking water before 2 weeks and after i.p. injected with 200mg/kg DEN till the end of the experiment (FSP 4% + Pb + DEN).
Group 6: Administered with Pb in drinking water before 2 weeks and after i.p. injected with 200mg/kg DEN (DEN + Pb only).

Experimental Design

Sixty female rats were randomly divided into 6 groups of 10 animals in each group (groups 1–6). Groups 4 and 5 served as the test groups to which the two doses of FSP under investigation were administered. Animals in these two groups were injected with a single dose of DEN dissolved in normal saline (200 mg/Kg-body weight) to initiate hepatocarcinogenesis. By the end of the experiment at 20 weeks, rats in each group were sacrificed for examination. Weights of rats in each group were recorded at the beginning of the experiment and at the end of every week which was varied.

Laboratory investigations

Tissue processing

At autopsy, livers were excised and slices of 2–3 mm thick (six slices of liver, two each from the right posterior, right anterior and caudate lobes) were cut with a surgical blade, fixed in 10% phosphate buffered formalin and embedded in paraffin. They were used for pathological examination.

Blood sample collection

At the end of experiment, rats were fasted overnight and then blood samples were drawn by cardiac puncture using stainless steel needle a part was used for determination of complete blood picture, another part of blood samples were collected without anticoagulant; serum was separated by centrifugation at 3000 rpm for 15 minutes. Sera were used for the determination of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (AIP) activity and albumin, total protein, glucose, urea, creatinine (Cr) concentration by colorimetric technique, while T3 and T4 levels were assayed by radioimmunoassay technique. The effects of FSP on diethylnitrosamine (DEN) induced hepatocarcinogenesis were examined in adult female rats. Rats were randomly divided into 6 groups of 10 each.
Statistical Analysis

The data are expressed as mean (±SE) statistical comparisons were made using Student's two-tailed unpaired t-test and P-values <0.05 were considered significant.

RESULT AND DISCUSSIONS

DEN was used as a carcinogen to initiate hepatocarcinogenesis because it is a proven and specific carcinogen for hepatocarcinogenesis (29,30).

General Observations: To ascertain that dietary fenugreek seed 2% and 4% had either negative effect on body weight gain or eating habit, all rats were monitored on a routine basis. The initial body weight (mean ±SE) before dietary interventions with fenugreek seed and DEN injection was 190±10g. At the time of termination, there was significant difference in body weights of control and treated rats. The food intake of animals in the experimental groups varies (Fig. 1-a&b).

Fenugreek seed at dose 2% was well tolerated than at dose 4% and caused slight adverse effects in rats, where continuous feeding of FSP 2% and 4% suppressed hepatocarcinogenicity (2/10 and 5/10 rats) up to 20% and 50%, respectively, compared with control. Fig (2-a) shows control (H&E 200X) with normal tissues, fig. (2-b) shows HCC nodule (H&E 400X) at the end of the experiment, where, fig. (2-c) shows sever cells degradation (H&E 400X).
- Effect of FSP at different doses during late stage hepatocarcinogenesis chemically induced in rats by a single dose i.p DEN and phenobarbital ad lib.

In the present study, a single dose of 200mg/kg DEN i.p injection after initiation with phenobarbital was used to induce rat liver carcinogenesis model to determine the efficacy of fenugreek seed at two different doses 2% and 4% as a phytochemo-protective herb.

- Effect of FSP on hematological parameters:

Hematological parameters were deteriorated with FSP administration and DEN injection plus phenobarbital ad lib as follow:

a) CBC

Fig (3) illustrates that there is insignificant difference (P>0.05) of mean WBCs in comparing all groups with each other or in between; there is also, insignificant changes (P>0.05) of mean lymphocytes % in comparing 2% group with 4% group, and 4%+DEN with 2%+DEN whereby, there is a significant difference between the other compared groups (P<0.001 and P<0.0001).

Where mean platelets volume shows insignificant difference in comparing control group with 2% and 4% groups; 2% group with 4% group; DEN group with 4%+DEN group (Fig. 3).

In the same time the mean platelets count was significantly increased (P<0.05) in 2% group compared with 2%+DEN group; 4%+DEN group with 2% and 4% groups (P<0.05); DEN group significantly decreased (P<0.0001) with other groups (Fig. 3).

Fig (3): Mean (±SE) of WBCs, platelets, platelets volume and lymphocytes values of adult female rats fed balanced diet with or without FSP and DEN.

Fig (4) shows significance difference (P<0.0001) of mean RBCs in comparing DEN group with control, 2%, 4%, 2%+DEN and 4%± DEN groups; also, there is a significant
increase \((P<0.05)\) in 4%+DEN group compared with 2% and 4% groups, and the mean red blood distribution width illustrates only insignificant changes in 2% group compared with 4% and 2%+DEN groups; 4% group with 2%+DEN group; 4%+DEN with DEN group. Where mean HB% shows significant changes \((P<0.05, P<0.01\) and \(P<0.0001)\) in 2%+DEN group compared with control, 2% and 4% groups; DEN group compared with 2% and 4% and 2%+DEN groups.

![Fig (4): Mean (±SE) of RBCs, RDW and hemoglobin values of adult female rats fed balanced diet with or without FSP and DEN.](image)

In Fig (5); mean HCT% shows significant changes \((P<0.001)\) in DEN group compared with control, 2%, 4%, 2%+DEN and 4%+DEN groups; control group with 2%, 4% and 2%+DEN groups.

Mean MCV values shows significant changes \((P<0.05\) and \(P<0.001)\) in control group compared with 2%+DEN and 4%+DEN groups; 2% group and 4%+DEN group \((P<0.001)\); DEN group with 2% group \((P<0.05)\), 4% \((P<0.001)\), 2%+DEN and 4%+DEN groups \((P<0.001)\) (Fig. 5).

MCH shows significant changes \((P<0.001)\) in 2%+DEN group compared with control, 2%, 4%, 4%+DEN and DEN groups; 4%+DEN groups with DEN group (Fig. 5).

Mean MCHC shows significant difference in 2%+DEN group compared with control, 2%, 4% and DEN group \((P<0.001)\) and 4%+DEN group \((P<0.01)\) (Fig. 5).

![Fig (5): Mean (±SE) of Haematocrite %, MCV, MCH and MCHC values of adult female rats fed balanced diet with or without FSP and DEN.](image)

b) Liver and renal functions

In Fig (6): Mean serum AST and ALT activity was significantly changes \((P<0.05, <0.01\) and \(<0.001)\) in control group with 2%+DEN , 4%+DEN and DEN groups; 4%+DEN group with 2%, 4%, 2%+DEN and DEN groups \((P<0.001, P<0.001, P<0.05, P<0.001)\).
Mean serum AST/ALT ratio shows insignificant difference (P>0.05) in comparing all groups with each other and in between (Fig. 6).

Mean serum Alp activity indicated that there is significant difference in compared control group with 2%+DEN, 4%+DEN and DEN groups (P<0.05, <0.0001 and <0.0001); 2% group with 4%+DEN and DEN groups (P<0.01 and P<0.001); 2%+DEN group with DEN group (P<0.01) (Fig. 6).

Mean serum glucose level shows significant decrease in comparing 4%, 4%+DEN, and DEN group with control group (P<0.01, <0.05 and P<0.001), respectively; 2% group compared with 4%+DEN group (P<0.05) (Fig. 6).

Mean serum urea level illustrate significant difference in 2% group compared with 2% group (P<0.05); DEN group with control, 2%, 4%, 2%+DEN and 4%+DEN groups (P<0.001), respectively (Fig. 6).

In fig (7) mean serum albumin level was significantly decreased in 4%+DEN group in compared with control, 2%, 4% and 2%+DEN groups (P<0.001); DEN group with other groups (P<0.001). Where total protein level was also significantly decreased in 2%+DEN compared with DEN (P<0.0001). Also, mean serum globulin was significantly changes only in 2%+DEN group compared with 2% and 4% groups (P<0.05).

Mean A/G ratio illustrated significant changes in control group with 2%, 4% groups (P<0.001); 2% group compared with control, 2%+DEN and 4%+DEN groups (P<0.0001); DEN group compared with 2%, 4% and 2%+DEN groups (P<0.01 and P<0.001) (Fig 7).

Mean serum creatinine levels shows significant increase in 2%+DEN, 4%+DEN and DEN groups compare with control group(P<0.05, P<0.05 and P<0.0001), respectively; DEN group with 2%, 4%, 2%+DEN and 4%+DEN groups (P<0.001) (Fig 7).
In Fig (8): Illustrates that mean total bilirubin shows significant changes in control group compared with other groups (P<0.01 and P<0.001); DEN group with 2%, 4%, 2%+DEN and 4%+DEN groups (P<0.001).

c) Thyroid Hormones

In Fig (8): Mean serum T3 levels illustrates significant increase (P<0.05 and P<0.001) in 2% and 4% groups compared with control group; 4% group with DEN and 4%+DEN groups (P<0.05 and P<0.001); 2% group compared with DEN and 4%+DEN groups (P<0.05 and P<0.001). Where mean serum T4 level shows significant changes (P<0.001) between all groups and within each other, except for the comparison between 4% group with DEN group (P>0.05).

![Graph showing mean ±SE of serum thyroid hormones and total bilirubin levels of adult female rats fed balanced diet with or without FSP and DEN.](image)

Fig (8): Mean (±SE) of serum thyroid hormones and total bilirubin levels of adult female rats fed balanced diet with or without FSP and DEN.

In this regard, plant-derived diets containing phytochemicals could be used in preventive strategies. The antioxidant property of fenugreek seeds might be contributing to modulatory action resulting in its protective effect in immunosuppressed mice by deltamethrin\(^{31}\). Administration of Fenugreek extract to STZ-diabetic rats (Streptozotocin-Induced Diabetes) reduced blood glucose level by 58%, restored liver glycogen content. These findings suggest that the hypoglycemic effect of Fenugreek and Balanites is mediated through insulinomimetic effect as well as inhibition of intestinal K-amylase activity\(^{32}\).

In this regard, plant-derived diets containing phytochemicals could be used in preventive strategies to reduce the risk and inhibit or retard the development of liver cancer. The present study demonstrating that fenugreek seed have the potential to prevent liver cancer. Using the diethylnitrosamine induced rat liver carcinogenesis model, dietary fenugreek seed reduce or retard the appearance of cancers when given during the initiation/post-initiation stages. Polyphenols may inhibit carcinogenesis by affecting the molecular events in the initiation, promotion and progression stages. Epidemiological studies concerning polyphenol consumption and human cancer risk suggest the protective effects of certain food items and polyphenols\(^{33}\), but more studies are needed for clear-cut conclusions. Perspectives on the application of dietary polyphenols for the prevention of human cancer and possible concerns on the consumption of excessive amounts of polyphenols are discussed by Yang et al.\(^{33}\).

Fenugreek seeds and their active constituents have been reported to be excellent antidiabetic agent based on several in vivo studies, including human intervention studies\(^8\), and their possible mechanisms of action as antidiabetic have been described\(^{34,35}\).
Al-Habori et al.\(^{(35)}\) revealed that the level of diosgenin in fenugreek seeds ranges from 0.42% to 0.75% depending on the cultivars and seed quality. Moreover, Mason\(^{(36)}\) revealed that no acute toxic effects were reported when rats were given dietary diosgenin at 1%, 0.2%, or 0.05% doses. Diosgenin, also downregulated the expression of various STAT3-regulated gene products, inhibited proliferation and potentiated the apoptotic effects of paclitaxel and doxorubicin. Overall, these results suggest that diosgenin is a novel blocker of the STAT3 activation pathway, with a potential role in the treatment of HCC and other cancers\(^{(37)}\). Because the whole seed was used in the present study, active seed constitutes other than diosgenin might influence the transformation of normal hepatocytes into a preneoplastic state and progresses toward advanced neoplasia. As in a previous study, 2% and 4% (w/w) fenugreek seed was used in the bioassay with rats, the chronic administration for up to 30 weeks (~210 days) gives disaster results with 4% more than dose 2%\(^{(20)}\) whereby in a 90-day subchronic study, rats fed fenugreek seeds, at doses between 1% and 10% in pure diet, had no toxic effects\(^{(38)}\). Mason\(^{(36)}\) indicates, no acute or chronic distress was observed in 1% fenugreek seed treated animals.

Different mechanisms of action of phytochemicals have been suggested. They either act as antioxidants, or may modulate gene expression and signal transduction pathways\(^{(39-41)}\). They may either be used as chemotherapeutic or chemopreventive agents with chemoprevention referring to the use of agents to inhibit, reverse, or retard tumorogenesis. In this sense, chemopreventive phytochemicals are applicable to cancer therapy, since molecular mechanisms may be common to both chemoprevention and cancer therapy\(^{(42,43)}\).

Molecular mechanisms of herb–drug interaction have been investigated. The most notable involves the ATP-binding cassette drug transporters such as P-glycoprotein\(^{(44)}\) and the drug metabolizing enzymes (known as phase I and phase II enzymes), especially cytochrome P450 3A4 (CYP3A4)\(^{(45,46)}\). Multiple molecular targets of dietary phytochemicals have been identified, from pro- and anti-apoptotic proteins, cell cycle proteins, cell adhesion molecules, protein kinases, transcription factors to metastasis and cell growth pathways\(^{(47-49)}\). Polyphenols particularly are among the diverse phytochemicals that have the potential to inhibit carcinogenesis\(^{(1)}\). The polyphenolic phytochemicals are virtually ubiquitous in plant materials and may occur at very high levels. Phenolics in plants are mostly synthesized from phenylalanine via the action of phenylalanine ammonia lyase (PAL). They are very important to plants and have multiple functions. The most important role of plant phenolics may be in plant defense against pathogens and herbivore predators, and thus are applied in the control of human pathogenic infections\(^{(50)}\). With the discovery of health benefits of plant polyphenols, it has been proposed to optimize the phenolic content of the diet so as to obtain favorable consequences for general health of the population\(^{(51)}\).

Phytochemicals including plant polyphenols that show health benefits may act via similar or different mechanisms in humans as those functional in plants. This mechanism may be novel to those of synthetic antibiotics for the control antibiotic resistant pathogenic strains. Phytochemicals may also modulate transcription factors\(^{(52)}\), redox-sensitive transcription factors, redox signaling, and inflammation\(^{(41)}\). As an example, nitric oxide (NO), a signaling molecule of importance in inflammation, is modulated by plant polyphenols and other
Many phytochemicals have been classified as phytoestrogens, with health-promoting effects resulting in the phytochemicals to be marketed as nutraceuticals. The mechanism by which the FSP mediates its anticarcinogenic effects is not clear. Antitumor effect of FSP may be mediated by one or more of the following mechanisms: (a) detoxification of the carcinogen by inducing detoxification enzymes such as GSTs. (b) antioxidant activity. (c) immuno modulatory action. (d) cytotoxicity (cytotoxic to several cancer cell lines). Diosgenin induced apoptosis in HT-29 cells; this is at least in part mediated by the suppression of bcl-2 and the induction of caspase-3 proteins. As mentioned earlier, colonic tumors potentiate their growth and survival by suppressing apoptosis. Other mechanism(s) of diosgenin that could possibly be involved in the inhibition of HT-29 cells could be those relating to modulation of cyclooxygenase-2 and the activation of nuclear factor-κB, p53, or p21 expression as shown earlier in the inhibition of osteosarcoma cells. Panda, et al. stated that administration of fenugreek (methi) seed extract to both mice and rats significantly decreases serum triiodothyronine (T3) concentration and T3/T4 ratio, but increases thyroxine (T4) levels and body weight. These findings suggest that fenugreek seed extract induced inhibition in T4 to T3 conversion is not peroxidation-mediated and the inhibition in SOD activity could be the result of a decrease in the protein anabolic hormone, T3, however in the present study, FSP increased T4 to T3 conversion in most studied groups. Decreased body weight has also been reported and may be attributed to decreases in T3 and decreases in the serum level of T3 and in the T3/T4 ratio, as well as an increase in the serum level of T4, have been observed in mice and rats given fenugreek. Fenugreek is also purported to contain an estrogenic constituent which previously, Shimizu et al. suggested that exogenous and endogenous oestradiol can suppress chemical hepatocarcinogenesis in male and female Fischer rats, and oestrogen receptors may be involved in the inhibition of malignant transformation or preneoplastic liver cells, while androgen and androgen receptors are involved in hepatocarcinogenesis and as a result of that fenugreek seeds may have protective effect against chemical hepatocarcinogenesis.

In summary, the present results show for the first time that (a) fenugreek seed inhibit early events of phenobarbital / diethylnitrosamine / phenobarbital model-induced liver cancer when given during initiation/post-initiation or promotion stage. (b) Phytochemicals or food-based compounds hold promise for novel strategies in cancer chemoprevention and control. Whereas the clinical potential of fenugreek seeds and their active constituents in the control of hypercholesterolemia or diabetes has been documented, the findings of this study and those of earlier ones demonstrating the anticancer properties of fenugreek constituents may have potential clinical relevance for cancer prevention and control. Thus, the role of fenugreek seed and its main active constituents as new supplements in diet-based preventive/therapeutic strategies to potentially alleviate human liver cancer remains an important field of study for future investigations.
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