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Anticancer Activity of Acetone Extract of Quercus infectoria Olivier Fagaceae in 1,2 Dimethyl Hydrazine Induced Colon Cancer

Review Article

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Abstract

Quercus infectoria Olivier (*Fagaceae*) which contains abundant amount of hydrolysable tannins and traces of gallic acid, ellagic acid and sitosterol is reported to be effective in inflammatory bowel disease. In the present study, chemopreventive potential of acetone extract of *Q. infectoria* (AEQI) 450 mg/kg was assessed in 1,2-dimethylhydrazine (DMH) (20 mg/kg) induced colon cancer. DMH produced significant development of aberrant crypts (AC) and aberrant crypt foci (ACF) associated with loss of body weight and high mortality. Treatment of rats with AEQI not olnly prevented development of AC and ACF but also reduced mortality and loss of body weight. There was a significant increase in oxidative stress (increase in MDA, MPO, NO and decrease in SOD) as well as TNF α, TGF β and VEGF. Treatment with AEQI decreased not only oxidative stress but also oxidative stress and inflammatory factors. Our data suggest that *Q. infectoria* possesses potential anti carcinogenic activity against colon cancer. Decrease in growth factors and anti oxidant activity may be responsible for this anti carcinogenic effect.

Key Words: *Quercus infectoria*; TNF α; TGF β; VEGF; Aberrant Crypt Foci.

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Introduction

Adenoma of colon and rectum (colorectal cancer) is one of the commonly occurring cancers reported to cause 655,000 deaths worldwide. It is fourth most common form of cancer in United States and third leading cause of cancer-related deaths in the Western world (WHO 2006, NCI 2009). In India also the incidence of colon cancer is very large. During a 32-year period (1941–1972), 555 cases of colorectal cancer were recorded at the Tata Memorial Hospital, Mumbai[1]. In contrast a total of 560 cases of colorectal cancer were treated at the same institution in 2006 alone. Colon cancer as compared to rectal cancer occurs more frequently as a result of mutations in the signaling pathway that artificially increases signaling activity. The mutations can be inherited or acquired and occur in the intestinal crypt stem cell [2].

Aberrant crypt foci (ACF) are putative preneoplastic lesions of colonic neoplasia in rodents and humans. During the process of colon carcinogenesis, ACF appears in the early stages and subsequently develop into polyps, adenomas and eventually carcinomas [3]. Hence ACF development is commonly used as a parameter for assessment of colorectal cancer even in animals.

Current medical treatment in colon cancer consists of chemotherapy, hormonal treatment and targeted therapy. They are sophisticated, expensive and not widely available. Therefore, a search for novel anticancer agents from natural products may provide an alternative and cost-effective treatment modality [4]. The herbal drug *Quercus infectoria* is reputed plant in Ayurvedic system of medicine and commonly known as "*Majuphul*" in Ayurvedic literature. Tannins(gallotannic acid) is one of the main constituent found in the galls of *Quercus infectoria* in 50%-70%. Gallotannic acid a tannic acid is known for its anti-mutagenic, anticancer and antioxidant properties [5]. In present investigation we have studied the anticancer potential of *Q. infectoria* using 1,2-dimethylhydrazine induced colon cancer model in rats.

Materials and Methods

Materials

1,2-dimethylhydrazine (DMH) was purchased from Sigma Chemical Company. ELISA kit for TNF- alpha, TGF β and VEGF from Nucleus inc. Ahmedabad. All other chemicals including solvents were of high purity analytical grade marketed by Dutt enterprise chemicals, Gujarat, India.

Identification, Collection and preparation of Acetone extract of *Quercus infectoria*

Dried galls of *Q.infectoria* were obtained from a commercial supplier in Ahmedabad. It was authenticated by Dr. Hitesh A. Solanki, Reader Department of Botany, Gujarat University, Ahmedabad. Acetone extract of *Q.infectoria* was prepared. The yield of extract was 46.6%. It was standardized by HPTLC taking gallotannic acid as marker.

Induction of colon cancer

Male albino Wistar rats weighing 150-200 gms obtained from Zydus research center Moraiya. Animals were cared for in compliance with the principles and guidelines of Council for the purpose of control and supervision of experimental animals (CPCSEA), ministry of environment and animal welfare, Govt. of India and approved by institutional animal ethical committee, in accordance with the Indian National Law on Animal Care and Use (Registration number: 1338/C/10/CPCSEA). The animals were housed 4 per polypropylene cage with a wire-mesh top and a hygenic bed of husk in a specific pathogen-free animal room under controlled conditions of a 12 h light/12 h dark cycle with temperature of 24 ± 2°C and relative humidity of 50 ± 5% until the end of the experimental period. The rats were held in quarantine for 1 week and had access to food and tap water *ad libitum*. Commercial pellet diet containing 4.2% fat was powdered and mixed with 15.8% peanut oil, making a total of 20% fat. This modified diet was fed to all rats throughout the 16 week experimental period.

Administration of carcinogen

Colon cancer was induced by subcutaneous injections of 1,2 dimethylhydrazine (DMH) at 20 mg/kg once a week for the first 4 consecutive weeks. DMH was dissolved in 1 mM EDTA; the pH was adjusted to 6.5 with 1 mM NaOH.

Treatment with acetone extract of *Q. infectoria* (AEQI) to animals

After 1 week of acclimatization, animals were randomly divided into six groups each containing ten rats.
Group I: Control
Group II: Rats treated with DMH 20 mg/kg sc weekly for four weeks.
Group III: Rats treated with DMH and AEQI 450 mg/kg p.o for 16 weeks.
Throught the period body weight of animals and mortality were recorded. At the end of sixteen weeks blood samples were collected and then rats were sacrificed.

Assessment of anti cancer activity

Animals were sacrificed by ether anesthesia at the end of fourth month from start of the experiment. The colons were removed, cleaned with saline solution (0.9%) and slit open longitudinally from cecum to anus. Each colon was cut into proximal, middle and distal portion of equal length, and fixed flat between two pieces of filter paper in 10% neutral-buffered formalin for at least 24 h. Later, they were stained with 5% methylene blue for 5 to 10min, and they were then placed on a microscopic slide; the mucosal side was observed through light microscope at 40x magnifications. ACF were counted by the method of Bird (1987) [6]. Crypts or distinct foci of crypts were counted as an ACF if they displayed at least two of the following characteristics [7]: (i) occupy a greater area than surrounding crypts; (ii) have a thickened epithelial lining; (iii) have elongated or altered shape of luminal opening; and (iv) have an increased pericryptal zone separating the crypt or foci from surrounding crypts.

Estimation of Oxidative stress

Part of colon isolated after 16 weeks were homogenized (50gm/L) in 50mM/L ice-cold potassium phosphate buffer (pH 6.0) containing 0.5% of hexadecyltrimethylammonium bromide. The homogenate was frozen and thawed thrice, then centrifuged at 4000 rpm for 20 min at 4°C.for the measurement of myeloperoxidase activity. Supernatant was collected and analyzed spectrophotometrically for malondialdehyde (MDA)[8], nitric oxide (NO) [9], myeloperoxidase (MPO)[10] and superoxide dismutase (SOD) [11].

Detection of tissue vascular endothelial growth factor

Yet another part of colon tissue was homogenized in 50 mM/l Tris buffer (pH 8.0) and centrifuged at 10000 RPM for 10 min. 50 µL supernatant were used for VEGF measurement. VEGF concentration were assayed by ELISA. Total protein was measured by Lowry's method[12].

Detection of serum TNF α and TGF β

At the end of 16 weeks before the animals were sacrificed blood samples were collected from retro-orbital plexus of the eye from 12 h fasted rats into vacutainer clotted tubes, where sera were separated by centrifugation within 30 min at 4000 rpm at 4°C for 10 min using centrifuge, then divided into aliquots and kept at -80°C for further assay of TNF-a and TGF β immediately. Serum TNF-α and TGF β were estimated by enzyme-linked immunosorbent assay (ELISA) [13].

Statistical Analysis

Results have been expressed as mean ± standard error of the mean (SEM). Data were analyzed as a completely randomized design using One Way Analysis of Variance (ANOVA) followed by Dunnett's Post Hoc Test. The statistical significance was set at $P < 0.05$.

Result

Body weight and mortality

Body weight of animals treated with DMH were found to be significantly reduced after 4 weeks and 16 weeks study period. There was also high mortality found in these animals. Treatment with AEQI significantly prevented not only the loss of body weight (Table 1) but also mortality in these animals (Table 1).

Effect of AEQI on development of ACF induced by DMH

There was development of colon ACF and AC in rats treated with DMH (Figure 1). AEQI treatment for 16 weeks significantly reduced the number of AC, ACF and crypt multiplicity as compared to DMH group (Table 2).

Oxidative stress parameters

Treatment with DMH produced significant increase in MDA,

MPO, NO levels associated with significant decrease in colonic SOD as compared to control animals. Treatment with AEQI significantly prevented DMH induced increase in MDA, MPO and NO levels further it also produce increase in SOD levels (Figure 2).

Serum TNF α, TGF β and tissue VEGF

Serum TNF α, TGF β and tissue VEGF levels were increased with DMH administration to rats. This up regulation was prevented by treatment with AEQI. DMH caused elevation in TNF α, TGF β and tissue VEGF levels. Oxidative stress could be the additive factor which was induced by administration of DMH (Figure 3).

Discussion

In the present study rats treated with DMH were found to show presence of ACF and severity of dysplasia which indicates development of carcinomatous changes. Our findings are in consonance with those reported earlier.

ACF are indeed the earliest manifestations of colon cancer, and then ACF could provide insight into the earliest events of colon tumorogenesis.

The genesis of colon tumor is a multistep process, and ACF have been proposed to precede the earliest adenoma [6,14,15].

DMH is used to produce Colon cancer, which is metabolized into the carcinogenic metabolite without previous metabolism by other tissues or colon bacteria in rat [16,17]. The ultimate carcinogenic metabolite of DMH is responsible for methylation of the DNA bases of various organs, including epithelial cells in the proliferative compartment of the crypts, which results in a great loss of colonic cells by apoptosis, an increase in proliferation, and an apparent increase in mutations of colonic epithelial cells[18]. All these alterations are likely to produce increase in inflammatory growth factors such as TNF α, TGF β and VEGF as well as oxidative stress. There was also an increase in mortality and decrease in body weight of the animals treated with DMH, which may be due to induction of cancer by DMH.

Weight loss is an important prognostic factor in cancer; the higher the extent of weight loss, the shorter the survival time. The prognostic effect of weight loss is greatest in patients with a more favorable prognosis [19]. Treatment with AEQI reduces weight loss and DMH induced mortality in animals showed good prognosis in colon cancers.

Table 1. Effect Of Aeqi On Body Weight And Mortality Of Animals.

Group/treatment	% mortality	Body weight		
		1st wk	4th wk	16th wk
Group I	0	248±7.5	257±4.216	250±3.416
Group II(DMH)	50a	247±7.6	222±7.92a	168±3.651a
Group III (DMH+ AEQI)	10b	250±7.7	238±6.541b	225±8.466b

Each value is mean ± SEM for 6 replicates in each group. [a] $P < 0.05$ significant different from control and [b] $P < 0.05$ significant different from DMH treated control.

Table 2. Effect of Acetone extract of Q.infectoria on ACF formation in DMH treated rats.

Group/treatment	ACF formation in rat colon			
	Total AC	Total ACF	Crypt multiplicity AC/ACF	% of ACF inhibition
Group I	0	0	0	--
Group II(DMH)	128±11.2	47±5.1	2.7±0.3	--
Group III (DMH+ AEQI)	46±9.7a	22±2.1a	2.1±0.1a	53.2

Figure 1. Histopathology of isolated rat colon staining with Hematoxylin and Eosine.

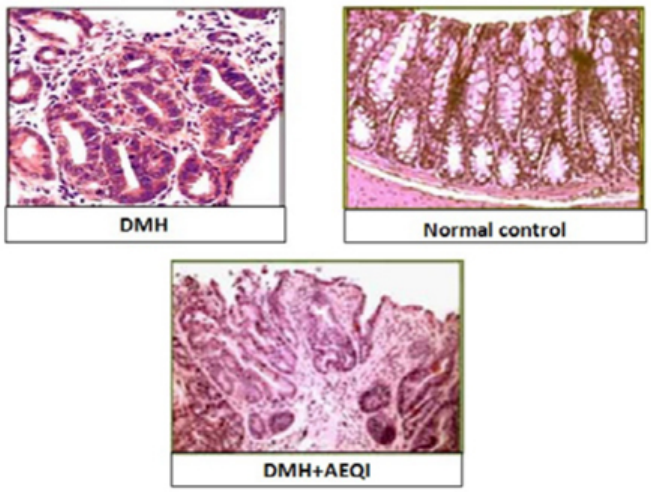


Figure 2: Effect of AEQI on MDA, MPO, NO and SOD.

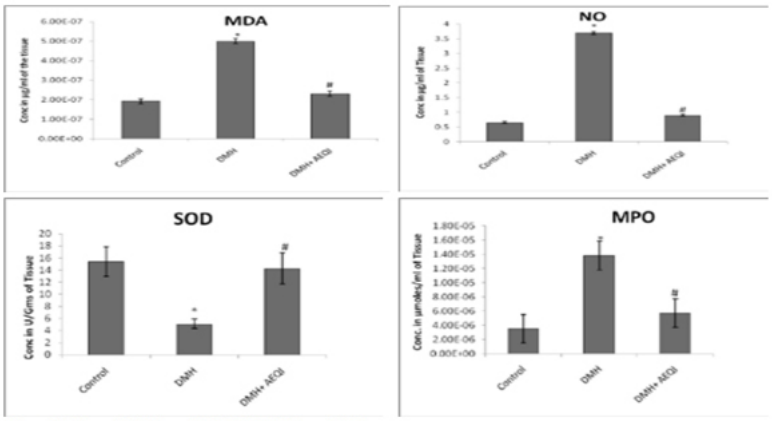
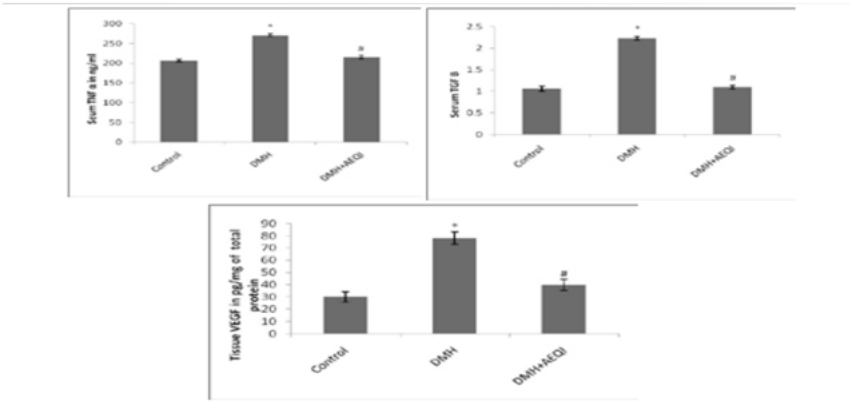


Figure 2: Effect of AEQI on MDA, MPO, NO and SOD
Each value is mean ± SEM for 6 replicates in each group. * P <0 .05 significant different from control and # P <0 .05

Each value is mean ± SEM for 6 replicates in each group. * *P* <0 .05 significant different from control and # *P* <0 .05 significant different from DMH treated control.

Figure 3: Effect of AEQI on VEGF, TNF α and TGF β Level.



Each value is mean ± SEM for 6 replicates in each group. * P <0 .05 significant different from control and # P <0 .05 significant different from DMH treated control.

ACF and AC were found to significantly reduced in colon of rats treated with both DMH as well as AEQI, thus suggesting that AEQI possesses activity against DMH induced colon cancer. This is further strengthened by observation that there was significant reduction in mortality of rats treated with AEQI and significant prevention of the loss of body weight in rats treated with AEQI in addition to DMH.

The phytochemical work carried by Umachigi et al., (2008) revealed that tannin is one of the active compounds which may be responsible for the antiproliferative activity [20]. Srivastava 2000 reported that gallotannic acid is antimutagenic in nature [5]. Several antioxidants in plants have been suggested to contribute to the anti carcinogenic effects and other such as flavanols have been able to inhibit cancer cell proliferation in vitro [21]. Inhibit ACF and AC generation by AEQI in colon may be due to presence of tannic acid.

Important to carcinogenesis, the unregulated or prolonged production of cellular oxidants has been linked to mutation (induced by oxidant-induced DNA damage), as well as modification of gene expression. In particular, signal transduction pathways, including AP-1 and NFκB, are known to be activated by reactive oxygen species, and they lead to the transcription of genes involved in cell growth regulatory pathways [22].

In addition to these many other chemical mediators are involved in carcinogenesis such as TNF- α, which is an acute phase reactive protein which is responsible for many inflammatory diseases including caner of colorectal metastases confined to the liver [23] Further in colon cancers, loss of TGF- β sensitivity is frequently accounted for by loss or mutation of known components of the TGF- β signaling pathway, notably TβRII and Smad4. Moreover VEGF expression was closely related with biological behavior of colon cancer and significantly associated with high intratumoral microvessel density (MVD) [24].

As mentioned above increased luminal activities of NO might be the aiding factor in tumor development. Taylor et al (1996) reported that iNOS gene expression is mediated by NFκB [25]. Malondialdehyde is final product of oxidative stress and is good indicator for extent of oxidative stress [26]. Myeloperoxidase catalyses the conversion of proportionally more stable hydrogen peroxide to unstable hydrochlorus acid. This in turn promotes oxidative stress and additionally it induces neutrophil infiltration on mucosal area causing further damage to the tissue [27]. Preventive anti-oxidant, such as superoxide dismutase (SOD) enzyme is the first line of defense against reactive oxygen species [28]. Superoxide dismutase (SOD) is widely distributed in cells with high oxidative metabolism and has been proposed to protect such cells against the deleterious effect of superoxide anion[29].

In our studies we found anti oxidant and anti inflammatory po-

tential of *Q. infectoria.* Kaur et al., (2003) showed anti oxidant and anti inflammatory potential of *Q. infectoria* which supports our finding [30]. Thus our data suggest that antioxidant effect of AEQI might be due to presence of tannic acid.

TNF is a member of a family of cytokines that are important for immune function and tissue homeostasis. Over expression of these ligands could contribute to tumor development by providing a defense that protects tumor cells by killing host immune cells. Similarly TGF β induces angiogenesis and metastasis at late stage of tumor growth [31]. Guetz et al., 2006 said that overexpression of VEGF in colon cancer tissue indicated poor prognosis [24]. All these are important proinflammatory cytokines and their expression is under the transcriptional regulation NFκB. In our studies we found that there was significant increase in TNF α, TGF β and VEGF. These fairly support the contention that TNF α, TGF β and VEGF are involved in colon cancer.

Conclusion

Data from our study suggest that *Q. infectoria* exerts a chemopreventive effect in DMH-induced experimental carcinogenesis in rats indicating its potential as an anticancer drug for the treatment of colon cancer. The anticarcinogenic property of *Q. infectoria* in DMH induced colon carcinogenesis may be due to antioxidant property and reduction in the level of TNF α, TGF β and VEGF.

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