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Original Article

Influence of myrtenal on the levels of surface glycoproteins, nucleic acids, and lysosomal enzymes against 1, 2-dimethyl hydrazine-induced experimental colon carcinoma

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ABSTRACT

Myrtenal, a monoterpenoid present in essential oil act as a therapeutic substance against cancer growth. Its specific isoprene residues may reduce the risk of developing cancer by modulating the factors responsible for carcinogenesis. The major carcinogen-metabolizing enzyme activity results in apoptosis and post-translational isoprenylation of cell growth-regulating proteins. The present study was carried out to evaluate the efficacy of myrtenal against 1, 2-dimethylhydrazine (DMH)-induced experimental colon cancer with reference to surface glycoproteins, bodyweight determination, nucleic acids, lysosomal enzymes, and histopathological examinations. Wistar Albino rats were subcutaneously injected with 20 mg/kg body weight (b.wt.) of DMH for 15 weeks to induce colon cancer and were treated with myrtenal (230 mg/kg/b.wt.) for 30 weeks by intragastric administration. The results revealed that there was a significant elevation in the levels of glycoproteins, nucleic acids, lysosomal enzymes, and also significant alterations in tissues of cancer-bearing animals with histological findings. Interestingly, the altered levels of these parameters were remarkably reverted back to near-normal in myrtenal treatment. It is proposed that the effect of myrtenal during DMH-induced colon cancer could be due to the intervention strategies of myrtenal in the protein, nucleic acid biosynthesis, membrane stabilizing potentials on lysosomal compartment, and inhibitory effect on cell surface glycoproteins and biofuel such as lipids.

Keywords: 1, 2-dimethylhydrazine, colon cancer, glycoproteins, lysosomal enzymes, myrtenal, nucleic acids

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INTRODUCTION

Epidemiological studies have diagnosed the incidence of colorectal cancer cases to about 40% annually and it is the third most leading cause of deaths among cancer patients worldwide.[1] Incidence of colorectal cancer is often associated with imbalance diet, lack of physical exercise, and high-fat consumption.[2] In addition, environmental chemical carcinogens induce free radicals, which alter the cell membrane potential, and produce an oxidative stress-related malignancy.[3] In response to the intra- and extra-cellular environmental conditions signaling pathways on the cell surface macromolecules are distorted.[4] Signaling molecules such as cell surface glycans are involved in many physiologically important functions that include normal embryonic development, differentiation, growth, contact inhibition, cell–cell recognition, host–pathogen interaction during infection, host immune response, disease development, metastasis, intracellular trafficking and localization, rate of degradation, and membrane rigidity.[5,6] Alterations on the surface glycans of the malignant cells undergo transformation to from glycolconjugates with cancer cells resulting in excessive replication potential. Energy created by this configuration is released through reactions with adjacent molecules, such as inorganic or organic chemical – proteins, lipids, carbohydrates – particularly with key molecules in membranes and nucleic acids.[7] Experimental colon cancer induced with 1, 2-dimethylhydrazine (DMH) produce free radical-mediated cancer cell glycolconjugates with increased byproducts

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leading to non-specific markers in cancer conditions.[8] A correlation between increased oxidative stress and improved glucose homeostasis states the combat of self-antioxidant defense system to eliminate the free radicals.[9] It is also well established that free radicals are known to react with all components of deoxyribonucleic acid (DNA), thus damaging its bases and the deoxyribose backbone causing mutations in crucial genes, which ultimately may lead to cancer.[10] Elevated levels of glycoconjugates express a direct relationship between glycoproteins and tumorogenesis.[11] Thereby, the excess glycoprotein level triggers the production of primary lysosomal enzymes beyond the control leading to degradation of the cells and ultimately causes cancer.[12] Studies have focused on the marker lysosomal enzymes, acid phosphatases, and many cathepsins, DNAases, ribonucleases, sulfatases, and glucuronidases are other important cellular lysosomal enzymes. Oxidative stress-related biochemical reactions have been implicated in cancer pathology, especially in colon cancer conditions were diet is predominantly considered to inhibit lysosomal enzymes.[13] Animal experimental studies have implicated that a large number of dietary phytochemicals may have anti-carcinogenic properties such as anti-oxidative action, induction of detoxification enzymes, and signal transduction.[14] Many dietary anti-carcinogens exhibit anti-oxidant properties. The compounds function by directly scavenging free radicals, altering the activity of antioxidant defense enzymes, or by affecting other biochemical processes involved in redox homeostasis. Of which monoterpenes are dietary components found in the essential oils of citrus fruits and other plants. A number of these dietary monoterpenes have antitumor activity. For example, d-limonene, which comprises >90% of orange peel oil, has chemopreventive activity against rodent mammary, skin, liver, lung, and fore-stomach cancers. Similarly, other dietary monoterpenes have chemopreventive activity against rat mammary, lung, and fore-stomach cancers when fed during the initiation phase.[15] The monoterpenes have several cellular and molecular activities that could potentially underlie their positive therapeutic index. Myrtenal a compound of monoterpenes was shown to have excellent pharmacological activities against many diseases among which cancer is imperative. Even though, it has been reported to have various biological activities, there is still a paucity of information, especially against the experimental colon cancer. Therefore, in light of the aforementioned reasons, this investigation was undertaken to evaluate the chemotherapeutic potential of myrtenal against DMH-induced colon carcinogenesis in Wistar Albino rats with reference to enzymes of carbohydrate metabolism, lysosomal enzymes, and nucleic acids.

MATERIALS AND METHODS

Reagents

DMH, myrtenal was purchased from Sigma Chemical Company, St. Louis, MO, USA. All the other chemicals used in this study were of analytical grade available commercially.

Experimental Animals

Experiments were carried out with 5-week-old male Wistar rats procured from central animal house facility, Dr. A. L. M. Postgraduate Institute of Basic Medical Sciences, University of Madras, Taramani, Chennai - 600 113. They were maintained in the controlled environmental conditions of temperature and humidity on alternative 12 h light/dark cycle, noise level maintained below 85 db and had unrestricted access to standard diet consisting of 24% protein, 4.5% fat and 4% fiber. The experiment was sanctioned and approved by the Institutional Animal Ethical Committee (IAEC No. 01/13/2013).

Experimental Design

The experimental animals were divided into four groups, each group comprising six animals.

- Group 1: Control animals fed with standard diet and pure drinking water
- Group 2: Animals were administered with 20 mg/kg body weight (b.wt.) of DMH, in 1 mM ethylenediaminetetraacetic acid, pH · adjusted to 6.5 with 1 mM NaOH and subcutaneously injected once in a week for 15 weeks
- Group 3: Animals were treated with myrtenal (230 mg/kg b.wt.) with corn oil as vehicle for 30 weeks by intragastric administration. Myrtenal treatment was started 1 week before the first dose of 20 mg/kg b.wt. of DMH (as in Group 2)
- Group 4: Animals were treated with myrtenal (230 mg/kg b.wt.) for 30 weeks by intragastric administration to assess the cytotoxicity if any, induced by myrtenal, and rats were referred as drug control.

After the end of the experimental period, the rats were fasted overnight and anesthetized using diethyl ether and sacrificed by cervical decapitation. A portion of colon was used for histopathological studies and remaining tissue was homogenized in 0.1 M Tris–HCl buffer pH 7.4 and centrifuged, the supernatant was used for biochemical studies.

Colon Analysis

Colon was excised from experimental rats, and were blotted dry and opened longitudinally, with the inner surface examined for visible macroscopic lesions. Tumor incidence (percentage of animals with tumors) and multiplicity (mean counted tumors per animals) were determined for the colons. Immediately following sacrifice, colons were removed and washed with ice-cold saline, and colon homogenates (10%) were prepared in ice-cold Tris-buffer saline (Tris 50 mM and NaCl 150 mM; pH 7.2) then centrifuged at 10,000 g for 10 min at 4°C and were stored as aliquots at or below −20°C for subsequent assays.

Biochemical Analysis

The glycoproteins such as hexose, hexosamines, and sialic acid were estimated according to the methods of Niebes[16] and Warren,[17] respectively. The absorbance was recorded at

540 nm and their levels were expressed as mg/dL for serum and mg/g for wet tissue. The nucleic acids were extracted by the method of Schneider[18] and the DNA was estimated by the method of Burton[19] and RNA was estimated by the method of Rawal *et al*.[20] The lysosomal enzymes such as the activities of β-D-galactosidase, β-D-glucuronidase, β-N-acetyl-D-glucosaminidase, and cathepsin-D were estimated according to the methods of Kawai and Anno,[21] Delvin and Gianetto,[22] Maruhn[23] and Sapolsky *et al*.,[24] respectively.

Histopathology - Periodic Acid and Schiff's (PAS) Staining

Histochemical staining of glycoconjugates was carried out as per the method of Kierman (1990), using 2% PAS's reagent in dark for 20 min. Photomicrographs were obtained using a Nikon Camera (Japan) to measure the relative intensity of PAS staining with the aid of a ×40 magnification lens in control and experimental groups.

Statistical Analysis

Values are expressed as mean ± standard deviation. The results were statistically evaluated using one-way analysis of variance by SPSS 10.0 student version followed by Tukey's multiple comparison method to compare means of different groups. The mean difference is significant at the 0.05 levels.

RESULTS

Glycoprotein Contents in Colon Control and Experimental Animals

Effect of Myrtenal on the levels of hexose, hexosamines, and sialic acid in colon of control and cancer-bearing animals is shown in Figure 1. The levels of glycoproteins were significantly increased ($P < 0.05$) in Group II cancer-bearing animals, when compared with group I control animals. On the other hand, a considerable decrease in the glycoproteins levels were observed in Group III myrtenal treated animals when compared with Group II cancer-bearing animals. Whereas, no significant changes were noticed in Group IV myrtenal alone treated animals, when compared to Group I control animals.

Nucleic Acid Content in Colon of Control and Experimental Animals

The effect of myrtenal on the levels of nucleic acids (DNA and RNA) in colon of control and experimental animals are shown in Figure 2. In Group II cancer-bearing animals, the levels of nucleic acids were found to be significantly elevated ($P < 0.05$) in colon tissues, when compared to Group I control animals. Conversely, these elevated levels were significantly decreased in myrtenal treated Group III animals ($P < 0.05$) when compared to Group II cancer-bearing animals. However, no significant changes were observed in Group IV myrtenal alone treated animals when compared to the Group I control animals.

Levels of Lysosomal Enzymes in Colon of Control and Experimental Animals

The effect of myrtenal on lysosomal enzyme activities in colon tissues of control and experimental animals are presented in the Figure 3. The activities of lysosomal enzymes were significantly increased in Group II cancer-bearing animals ($P < 0.05$) when compared to Group I control animals. On the contrary, the lysosomal enzyme levels were significantly decreased toward normal range ($P < 0.05$) in Group III myrtenal treated animals when compared with Group II cancer-bearing animals. Whereas, no significant alterations were observed in Group IV myrtenal alone treated animals when compared to Group I control animals.

PAS's Staining Findings

The effect of myrtenal in colon tissues of control and experimental animals are presented in the Figure 4. With effect of the PAS staining on the glycoprotein the intensity of mucin content are depicted. The normal architecture was

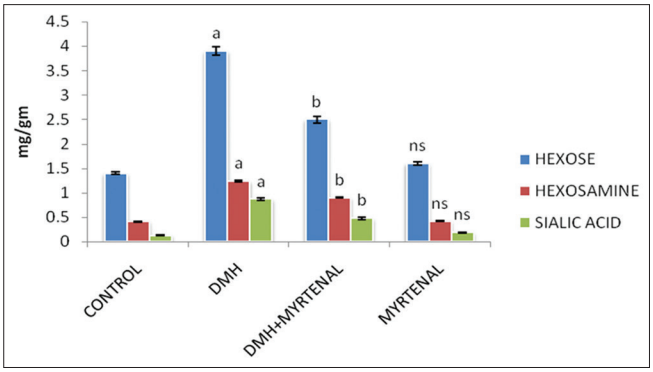

Figure 1: Effect of myrtenal on levels of glycoproteins in colon of control and experimental animals. Values are expressed as mean ± standard deviation of six animals. Statistical significance $P < 0.05$. Control with 1, 2-dimethylhydrazine (DMH), DMH with DMH + myrtenal. Units: mg/g tissue

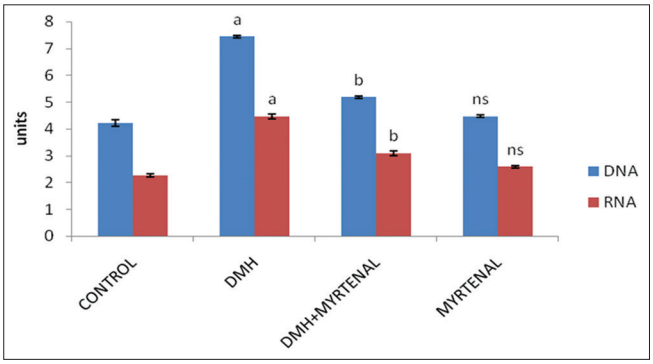

Figure 2: Effect of myrtenal on the levels of nucleic acids in colon of control and experimental animals. Values are expressed as mean ± standard deviation of six animals. Statistical significance $P < 0.05$. Control with 1, 2-dimethylhydrazine (DMH), DMH with DMH + myrtenal. Units: mg/g wet tissue

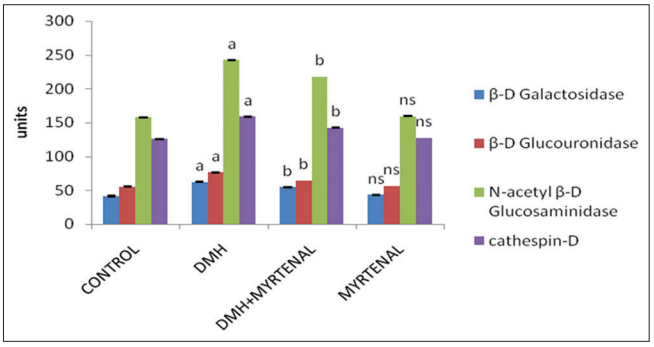

Figure 3: Effect of myrtenal on the levels of lysosomal membrane in colon of control and experimental animals. Values are expressed as mean ± standard deviation of six animals. Statistical significance $P < 0.05$. Control with 1, 2-dimethylhydrazine (DMH), DMH with DMH + myrtenal. Units: β-D galactosidase (p-nitrophenol liberated/mg protein/h), β-D glucouronidase (p-nitrophenol liberated/mg protein/h), β-D glucosaminidase (p-nitrophenol liberated/mg protein/h), cathespin (p-nitrophenol n moles of tyrosine/mg protein/h)

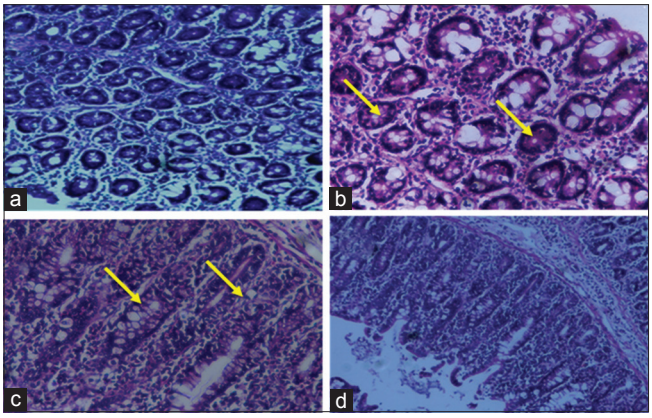

Figure 4: Histochemical analysis by periodic acid Schiff's staining in control and experimental animals. (a) Control, (b) 1, 2-dimethylhydrazine (DMH) induced, (c) DMH + myrtenal, (d) myrtenal. Arrows depicts the expression of glycoconjugates

seen profound in Figure 4a control animals. The neutral mucin intensity clearly expresses the increased levels of glycoproteins in Figure 4b DMH-induced Group II colon cancer animals compared to Group I control animals. Group III myrtenal treated animals shows active morphology in Figure 4c compared to Group II animals. Whereas, no significant alterations were observed in Group IV myrtenal alone treated animals in Figure 4d when compared to Group I control animals.

DISCUSSION

Glycoproteins are integral membrane proteins which play a major role in cell differentiation and absorption of macromolecules.[25] The increased levels of glycoproteins associated with cancer produces changes in macromolecular structure affecting cell–cell and cell–matrix interactions associated with decreased elasticity and increased fluid filtration.[26] In our study, the increased levels of hexose, hexosamines, and sialic acid in Group II animals are well in accordance with the excess free radical generated by DMH in the liver leading to oxidative stress-related inflammatory damage to cellular membranes.[27] Elevated levels of glycoproteins arise as a result of chemical glycosylation with hydroxyl groups by the free radicals on lipid membrane. The levels were seen decreased in myrtenal-treated animals providing effective free radical scavenging activity and thereby stabilizing the cell membrane integrity in glycosylated moieties.[28] Lysosomes are components of the endocytic pathway responsible for storage and processing of digestive enzymes for terminal degradation of cellular material delivered to autophagosomes during cell renewal and cell death.[29,30] Lysosomes play a critical role in protein, lipid, and carbohydrate catabolism; plasma membrane recycling; lipid and sterol trafficking; antigen processing; and autophagy. Disruption of lysosomal membrane integrity, and release of lysosomal enzymes into the cytosol, can have grave cytotoxic consequences resulting in apoptotic or necrotic death.[31,32] Increased levels of lysosomal enzymes in the cellular environment are as a result of free radical-mediated enzyme leakage of lysosomal sacs observed with the biomarkers such as β-D-galactosidase, β-D-glucuronidase, N-acetyl-B-glucosaminidase, and cathespin-D in colon cancer-induced animals.[33] Comparatively myrtenal supplementation reduced the lysosomal enzyme activity, which may be due to the enzyme stability of the lysosomal sacs with raise to membrane potency.

Cells become cancer cells because of DNA damage. In a normal cell, when DNA is damaged the cell either repairs the damage or dies. In cancer cells, the damaged DNA is not repaired but proceeds with abnormalities in their DNA content leading to malignancy.[34] Abnormal cells grow out of control as a result of DNA damage in the cellular environment delivering them as a potential biological target for initiation of many carcinogenesis thereby leading to sensitive indicators.[35] Altered physiological behavior triggers the risk of more exposure to chemical carcinogens and reduce the repair leading to DNA adduct formation.[36] These findings clearly suggest the uncontrolled accumulation of DNA damage and lack of repair mechanism could proceed to colon cancer as initiated by DMH. The chemical adduct between DMH-DNA thus indicate the important role of DNA in carcinogenesis.[37] RNA levels were also found to be increased in cancer condition as DNA and RNA are directly related. The abnormal increased level of DNA may lead to an increased transcription thereby increasing the RNA levels in tumor cells.[38] Thus, upon myrtenal treatment the levels were reverted back to near-normal which supports the anti-tumor activity by inhibiting the progression of tumor growth with tumor DNA content in malignant conditions.

Histochemical studies with PAS staining shows the neutral mucin content in colon cancer-induced animals stating the increased levels of mucin in the mucous epithelial cells of colon compared to myrtenal treated animals. In conclusion, our study revealed that monoterpenes and its derivatives mainly myrtenal have possessed significant anti-carcinogenic and anti-oxidative property against DMH-induced colon carcinogenesis in Wistar Albino rats.

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Original Article

In vitro cytotoxicity of ethanol extract of *Gmelina arborea* and *Grewia umbellifera* in HepG2 and Vero cell lines

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ABSTRACT

Medicinal plants are plants containing inherent active ingredients used to cure diseases. The use of traditional medicines and medicinal plants in most developing countries as therapeutic agents for the maintenance of good health has been widely observed. *Grewia umbellifera* (GU) and *Gmelina arborea* (GA) leaves are reported to possess a multifunctional pharmacological property. The result of our study revealed that ethanol extract of GA and GU has a cytotoxic effect on Vero cell line in a concentration-dependent manner. The extract showed moderate therapeutic values on Vero cell line with inhibitory concentration 50% values 541.42 and 651.95, respectively.

Keywords: 3-(4,5-dimethylthiazol-2-yl)-5-diphenyltetrazolium bromide, cytotoxic, HepG2, plant herbs, Vero

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INTRODUCTION

Herbal drugs have gained importance and popularity in recent years because of their safety, efficacy and cost-effectiveness. The association of medical plants with other plants in their habitat also influences their medicinal values in some cases.[1] One of the important and well-documented uses of plant products is their use as hepatoprotective agents. Hence, there is an ever increasing need for safe hepatoprotective agent.[2] A number of herbs are traditionally used in different countries during drug or toxin-induced in hepatic, renal and cardiac disorders. *Grewia umbellifera* (GU) and *Gmelina arborea* (GA) leaves are commonly known as Phalasa and Kumizh is reported in Ayurvedic Pharmacopoeia. Traditionally, GU and GA are used for its cardioprotective, cardiotonic, diuretic, and aphrodisiac activities, and as an antidote to certain poisons.[3] Modern pharmacological studies have shown that leaves of GU and GA possess various positive effects in preventing various conditions including pain and inflammation, has diuretic activities, and is also effective in hyperlipidemic condition, cardiotoxicity, and stress. GU and GA are traditionally used for its cardioprotective, cardiotonic, general tonic, diuretic, aphrodisiac, antidote to certain poisons and scorpion stings, alternative purgative, and cooling effects.[4] It cures pain, ulcer, and fever and is used for pectoral-cough, asthma, and other bronchial disorders.[5] Hence, the present investigation was undertaken to evaluate the *in vitro* cytotoxicity of ethanol extract of GA and GU in HepG2 and Vero cell lines.

MATERIALS AND METHODS

Chemicals

3-(4,5-dimethylthiazol-2-yl)-5-diphenyltetrazolium bromide (MTT), fetal bovine serum (FBS), phosphate buffered saline (PBS), Dulbecco's modified Eagle's medium (DMEM), and trypsin were obtained from Sigma-Aldrich Co., St. Louis, USA. Ethylenediaminetetraacetic acid (EDTA), glucose and antibiotics from Hi-Media Laboratories Ltd., Mumbai, dimethyl sulfoxide (DMSO) and propanol from E. Merck Ltd., Mumbai, India.

Cell Lines and Culture Medium

Human, liver hepatocellular cells (HepG2) cell line and Vero cell lines were procured from National Centre for Cell Sciences, Pune, India. Stock cells were cultured in

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DMEM supplemented with 10% inactivated FBS, penicillin (100 IU/ml), streptomycin (100 µg/ml), and amphotericin B (5 µg/ml) in an humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with trypsin phosphate versene glucose solution (0.2% trypsin, 0.02% EDTA, and 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks, and all experiments were carried out in 96 microtiter plates (Tarsons India Pvt. Ltd., Kolkata, India).

Collection and Identification of Plant Material

The barks of GA were collected from south India, Kanyakumari district during the month of January and February. The plant was identified by S. Balasubramanium, ABS Botanical Garden, Salem.

Extract Preparation

The freshly collected barks were dried in the shade, then coarsely powdered. For extraction of crude phytochemical, 25 g of powdered bark material was kept in a closed conical flask with 20 mL various solvents such as petroleum ether, benzene, chloroform, ethanol, acetone, ethyl acetate, and distilled water in a shaker at room temperature for 24 h. After incubation, the extracts were filtered, and the extracts were collected and stored in the refrigerator at 4°C for further studies.

Preparation of Test Solutions

For cytotoxicity studies, each weighed test drugs were separately dissolved in distilled DMSO and volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serial dilutions were prepared from this for carrying out cytotoxic studies.

Cytotoxicity Assay

The MTT assay was performed as described by Cardile *et al*.[6] The viability of the cell was assessed by MTT assay, which is based on the reduction of MTT by the mitochondrial dehydrogenase of intact cells to a purple formazan product. Cells were seeded in 96-well microplates (1×10^4 cells/well in 180 µl medium) and routinely cultured in a humidified incubator at 37°C in 5% CO_2 for 24 h. The extracts were added in serial concentrations such as 125, 250, 500, and 1000 µg/ml and reincubated for 24 h. Then, the medium was discarded, and 30 µl of MTT dye solution (5 mg/ml in PBS) was added to every well and reincubated for 4 h. After removing un-transformed MTT reagent, 100 µl of DMSO was added to dissolve the formed formazan crystals. Amount of formazan was determined by measuring the absorbance at 540 nm using an enzyme-linked immunosorbent assay plate reader.

Light Microscopic Studies

Light microscopic examination of the cells was performed to observe the morphological changes after the treatment with ethanolic extracts of GA and GU for 24 h. HepG2 and Vero cells were grown in 35 mm sterile Petri plates and treated with ethanolic extracts of GA and GU at the concentration of 125, 250, 500, and 1000 µg/ml for 24 h. The cells were then fixed for 5 min with 10% methanol, PBS. The morphological changes were observed under inverted microscope (Nikon, Japan).

Statistical Analysis

Values are expressed as mean ± standard deviation. The results were statistically evaluated using independent sample–*t*-test using SPSS 10.0 student version. $P < 0.05$ was considered statistical significant.

RESULTS

Effect of Ethanolic Extracts of GA and GU on the Cytotoxicity of HepG2 and Vero Cells

The cytotoxic effects of ethanolic extracts of GA and GU on HepG2 and Vero cells were performed by the MTT method, which is reliable to detect proliferation of cells. The results of the MTT assay are shown in Tables 1 and 2. The results clearly confirm that the exposure of ethanolic extracts of GA and GU at different concentrations such as 125, 250, 500, and 1000 µg/ml for 24 h resulted in decrease of cell proliferation in a dose-dependent manner. The percentage of inhibitory concentration 50% (IC_{50}) inhibition of cell proliferation was found to be initiated at the concentration of 412.88 µg/ml of ethanolic extracts of GA and GU in HepG2 cells and 438.61 µg/ml in Vero cells, respectively.

Effect of Ethanolic Extracts of GA and GU on the Apoptosis and Morphology of HepG2 and Vero Cells

In this study, the light microscopic analysis was studied to determine the presence of apoptosis in ethanolic extracts of GA and GU treated HepG2 and Vero cells. The morphological changes of control and ethanolic extracts of GA and GU treated HepG2 and Vero cells at the concentration of 125, 250, 500, and 1000 µg/ml after 24 h of exposure are shown in Figures 1 and 2. The HepG2 and Vero cells treated with dose-dependent concentrations of ethanolic extracts of GA and GU significantly changed the structural alterations and reduction cells populations.

DISCUSSION

The biological activity of any phytocompounds depends on the type of chemical composition and the concentration of active constituents as well as types and developmental stages of the cancer.[7] The screening of plants for their anticancer properties used cell-based assays and established cell lines, in which the cytotoxic effects of plant extracts could be measured. MTT assay is a nonradioactive, fast and economical assay widely

Table 1: Cytotoxic properties of test drugs against HepG2 cell line of GA and GU

Name of the plant	Concentration (µg/ml)	Inhibition (%)±SD	IC_{50} (µg/ml)
Ethanol extract of GA	1000	75.69±1.4	412.88
	500	55.12±1.6	
	250	51.72±1.5	
	125	29.14±2.5	
Ethanol extract of GU	1000	67.16±1.4	438.61
	500	55.21±1.7	
	250	57.18±0.2	
	125	25.14±1.8	

GA: *Gmelina arborea,* GU: *Grewia umbellifera*, IC_{50}: Inhibitory concentration 50%, SD: Standard deviation

Table 2: Cytotoxic properties of test drugs against Vero cell line of GA and GU

Name of the plant	Concentration (µg/ml)	Inhibition (%)±SD	IC_{50} (µg/ml)
Ethanol extract of GA	1000	64.11±1.4	541.42
	500	51.11±1.4	
	250	45.41±1.2	
	125	29.14±1.8	
Ethanol extract of GU	1000	62.12±1.2	651.95
	500	49.23±1.4	
	250	35.11±1.4	
	125	24.12±1.6	

GA: *Gmelina arborea*, GU: *Grewia umbellifera*, IC_{50}: Inhibitory concentration 50%, SD: Standard deviation

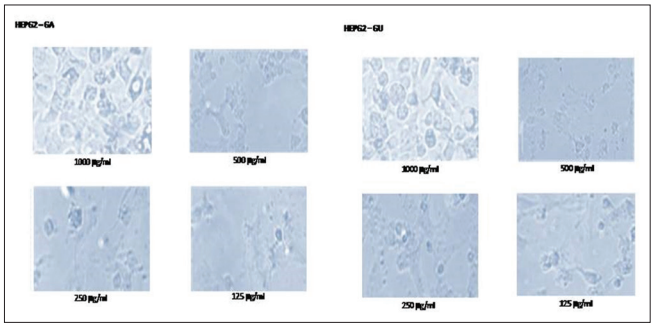

Figure 1: Effect of ethanolic extracts of *Gmelina arborea* and *Grewia umbellifera* on the apoptosis and morphology of HepG2 cells

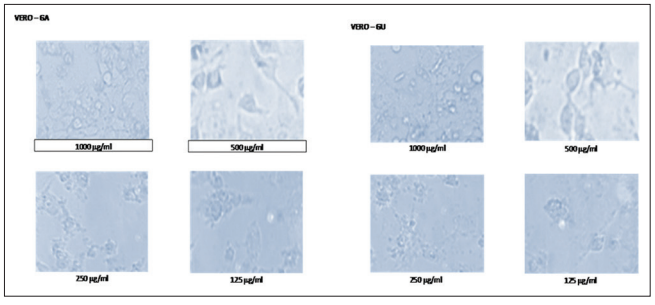

Figure 2: Effect of ethanolic extracts of *Gmelina arborea* and *Grewia umbellifera* on the apoptosis and morphology of vero cells

used to quantify cell viability and proliferation. MTT is a yellow water-soluble tetrazolium salt. Metabolically active cells are able to convert the dye to water-insoluble dark blue formazan by reductive cleavage of the tetrazolium ring.[8] The result of our study revealed that ethanol extract of GA and GU has a cytotoxic effect on human liver hepatocellular cells (HepG2) cell line in a concentration-dependent manner. The extract showed moderate therapeutic values HepG2 cell line with IC_{50} values 412.88 and 438.61, respectively. Morphological studies also confirmed that the ethanol extract of GA and GU has got the potential cytotoxic effect. The result of our study revealed that ethanol extract of GA and GU has a cytotoxic effect on Vero cell line in a concentration-dependent manner. The extract showed moderate therapeutic values on Vero cell line with IC_{50} values 541.42 and 651.95, respectively. Morphological studies also confirmed that the ethanol extract of GA and GU has got the potential cytotoxic effect.

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Original Article

Anti-inflammatory effect of myrtenal against cytokines in experimental colon cancer

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ABSTRACT

Colon cancer incidence advances and metastasis of cancer cells have been widely targeted in treatment regime. Inflammation-related colon cancer treatment is being widely evaluated, since free radical-mediated inflammation leads to lipid peroxidation and excess free radical formations. In this connection, erythrocyte membranes degrade in the mechanism which ultimately leads to altered levels of Na^+/K^+, Ca^{2+}, Mg^{2+} adenosine triphosphatase. Major ion gradients face a deficit in cellular mechanism and normal biochemical functions. Decreased phosphorylation of these key enzymes favors further inflammation of the cell membranes. Inflammatory cytokines have been studied to determine the prognostic approach toward cancer treatment. Various interleukins and tumor marker enzymes were analyzed and this in turn can be used to determine the dosage of chemo-preventive drugs. In the present study, myrtenal was used against 1, 2-dimethylhydrazine-induced colon cancer in experimental rats, and their inflammatory markers were determined. Myrtenal an essential oil of monoterpene family was studied for its various activities. Hence, the present study focused on the anti-inflammatory property of myrtenal and its therapeutic efficacy against colon cancer in the developmental stage and also in regulating further prognosis.

Keywords: 1, 2-dimethylhydrazine, adenosine triphosphatase, cytokines, inflammation, myrtenal

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INTRODUCTION

Colon cancer remains a serious health problem around the world despite its advances in diagnosis and treatment.[1] It ranks third in overall cancer incidence and is the second most common cause of cancer-related mortality in the United States.[2] The prognosis is especially poor for metastatic colorectal cancer (CRC), with overall 5-year survival rates of 5-8%.[3] The incidence rate continues to rise as people change their lifestyles and food habits.[4] Diets high in red meat and processed food show established risk factors for CRC.[5] In this connection, various enzymes have been reported in the correlation between colon cancer incidence and their alterations in the biochemical levels. Na^+/K^+, Ca^{2+}, Mg^{2+} adenosine triphosphatase (ATPase) is a membrane bound enzyme involved in the transport of various cations and the steroidal glycoside. Inhibition of this enzyme results in a depletion of intracellular Mg^{2+} and Ca^{2+} that leads to the pathogenesis of the various disorders. The transport takes place against ion gradient and the energy required for the process is provided by ATP, which is hydrolyzed to ADP and Pi during these transport phenomena.

In cancer condition, the levels of Na^+/K^+, Ca^{2+}, Mg^{2+} ATPase were seen decreased and this may be due to the inhibition of the phosphorylation of ATPase by the chemical carcinogens. Experimental colon cancer induced by 1, 2-dimethylhydrazine (DMH) in rats a potent carcinogen that acts as a DNA methylating agent-inducing colon tumors in experimental animals. It is further transported to colon through bile or blood to generate its ultimate carcinogenic

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metabolite, diazonium ion which elicits an oxidative stress by methylating biomolecules of colonic epithelial cells, and leads to promutagenic events as a result of inflammation and tumor promotion. The inflammatory pathway between liver and liver-mediated DMH induction in colon, serves a channel, and knocks off the immune system during its metastatic approach. Tumor markers comprise predominately the substances that are produced by malignant cells or the substances that are produced by other cells under the influence of malignant cells that are determined in body fluids.[6] Tumor markers can be either newly synthesized substances or the substances that can be found in normal organisms in much lower concentrations.[7] The determination of tumor markers in cancer condition reveals the extent of tumor pathogenesis and its metastatic approach to distinct organs. In the present study, the levels of tumor markers such as carcinoembryonic antigen (CEA) and CA 19-9 were found increased and this may be due to the cytotoxic degradation of normal cells and its malignant transformation tendency to release excess levels of the marker enzymes from the tumor cells.

Cytokines are small glycoproteins produced by a number of cell types predominantly leukocytes, which regulate immunity, inflammation, and hematopoiesis.[8] Hence, recent studies have focused on the regulation of inflammation and biochemical enzyme alterations in cancer studies. Natural chemo-preventive drugs have been analyzed in respect to its less side effects and anti-inflammatory properties. Myrtenal and essential oil of monoterpene family present in cumin, pepper, mint, eucalyptus, etc., was found to suppress the proliferation of murine B16 melanoma, human HL-60 leukemia cells, and other carcinogenic process. In this connection, various studies have postulated its biological activities such as anti-malarial, anti-plasmodial, anti-radicular, hypocholesterolemic, gonadotrophic, cyclooxygenase-inhibitor, and immunostimulant effects.[9] A variety of studies have been followed, but there is a paucity of information regarding its anti-inflammatory property; hence, the present study determines the anti-inflammatory property of myrtenal in DMH-induced colon cancer in experimental rats.

MATERIALS AND METHODS

Reagents

DMH, myrtenal was purchased from Sigma Chemical Company, St. Louis, MO, USA. All the other chemicals used in this study were of analytical grade available Commercially.

Experimental Animals

Experiments were carried out with 5-week-old male Wistar rats procured from the Central Animal House Facility, Dr. A. L. M. Postgraduate Institute of Basic Medical Sciences, University of Madras, Taramani, Chennai - 600 113. They were maintained in the controlled environment conditions of temperature and humidity on alternative 12 h light/dark cycle, noise level maintained below 85 dB and had unrestricted access to standard diet consisting of 24% protein, 4.5% fat, and 4% fiber. The experiment was sanctioned and approved by the Institutional Animal Ethical Committee (IAEC No. 01/13/2013).

Experimental Design

The experimental animals were divided into four groups, each group comprising six animals.

- Group I: Control animals fed with standard diet and pure drinking water
- Group II: Animals were administered with 20 mg/kg body weight (b.wt.) of DMH, in 1 mM ethylenediaminetetraacetic acid (EDTA), pH adjusted to 6.5 with 1 mM NaOH and subcutaneously injected once in a week for 15 weeks
- Group III: Animals were treated with myrtenal (230 mg/kg b.wt.) with corn oil as vehicle for 30 weeks by intragastric administration. Myrtenal treatment was started 1 week prior to the first dose of 20 mg/kg b.wt. of DMH (as in Group II)and continued till end of the experimental period
- Group IV: Animals were treated with myrtenal (230 mg/kg b.wt.) for 30 weeks by intragastric administration to assess the cytotoxicity if any, induced by myrtenal, and rats were referred as drug control.

After the end of the experimental period, the rats were fasted overnight and anesthetized using diethyl ether and sacrificed. A portion of the colon was used for histopathological studies and remaining tissue was homogenized in 0.1 M Tris-HCl buffer pH 7.4 and centrifuged, the supernatant was used for biochemical studies.

Colon Analysis

Colons were excised from experimental rats, and were blotted dry and opened longitudinally, with the inner surface examined for visible macroscopic lesions. Tumor weight was determined for the colons. Immediately following sacrifice, colons were removed and washed with ice-cold saline, and colon homogenates (10%) were prepared in ice-cold Tris-buffered saline (Tris 50 mM and NaCl 150 mM; pH 7.2) then centrifuged at 10,000 g for 10 min at 4°C and were stored as aliquots at or below −20°C for subsequent assays.

Estimation of Membrane Bound ATPases

ATPases catalyzes the conversion of ATP into adenosine diphosphate. During the conversion, one molecule of inorganic phosphate is liberated. The inorganic phosphate is estimated according to the method of Fiske and Subbarow.[10] The proteins were precipitated with trichloroacetic acid. The free filtrate reacts with acid molybdate to form phosphor-molybdic acid which is reduced by the addition of 1-amino 2-naphthol-4-sulphonic acid to produce blue color. The intensity of the color

is proportional to the amount of inorganic phosphate present in the sample.

Preparation of Hemolysate and Isolation of Erythrocyte Membrane

Blood collected with EDTA was centrifuged at 2000 rpm for 20 min at 4°C. The packed cells were washed with isotonic saline to remove the buffy coat. An aliquot of packed cells were then washed three times with isotonic Tris-HCl (0.3 M, pH 7.4) buffer. An aliquot of 1.0 ml washed cells were lysed using 9.0 ml of hypotonic Tris-HCl buffer (0.015 M, pH 7.2). The lysed cells were centrifuged for 30 min at 15,000 rpm. The pellet was repeatedly washed with hypotonic Tris-HCl buffer until a clear pale pink or colorless supernatant was obtained. The resulting erythrocyte membrane pellet was suspended in 0.01 M Tris-HCl buffer, pH 7.4 for subsequent analysis.

Na+ K+ - ATPase was assayed according to the method of Israel *et al*.[11] Ca^{2+} - ATPase was estimated as described by the method of Hjertén and Pan.[12] Mg^{2+} - ATPase was assayed by the method of Ohnishi.[13]

Tumor Markers

Estimation of CEA

The UBI MAGIWEL CEA Quantitative CM-201 is a solid phase enzyme-linked immunsorbent assay kit. This test provides quantitative measurement of CEA in serum.

Estimation of CA 19-9

The UBI MAGIWEL CA 19-9 Quantitative CM-701 is a solid phase enzyme-linked immunsorbent assay kit. This test provides quantitative measurement of in serum CA 19-9.

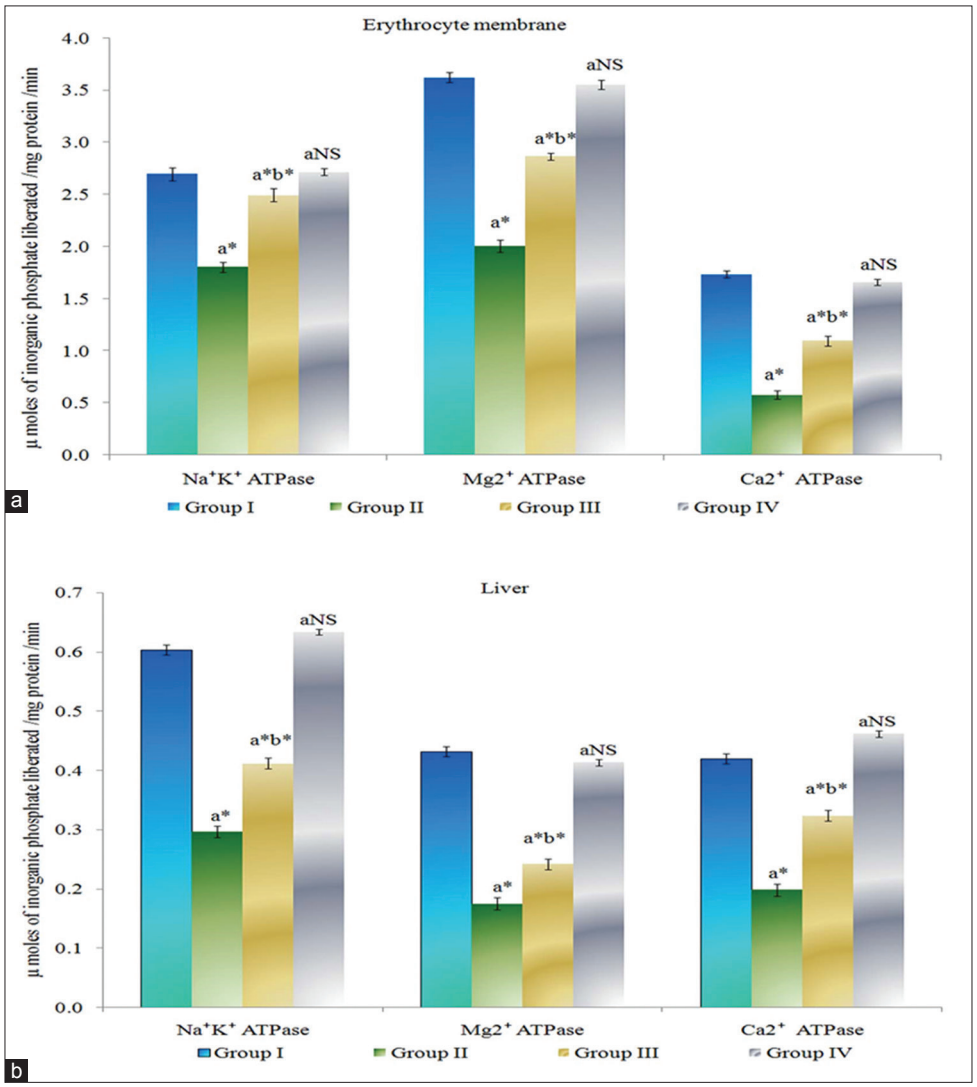

Figure 1: (a and b) Effect of myrtenal on adenosine triphosphatase levels in erythrocyte membrane and liver tissue of control and experimental animals. Results are expressed as mean ± standard deviation for six animals, (a) Group II, III, and IV compared with Group I, (b) Group III compared with Group II. NS – Nonsignificant, **P* < 0.05

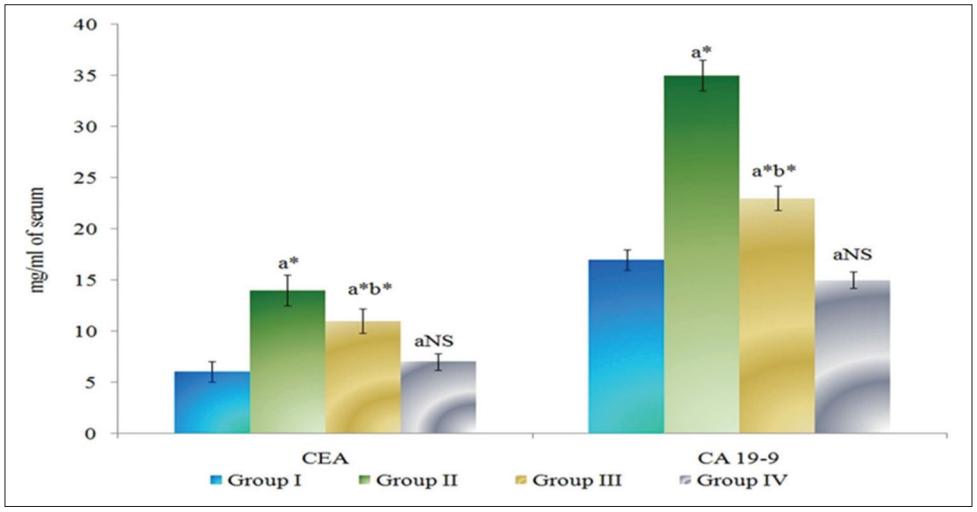

Figure 2: Effect of myrtenal on levels of tumor markers in serum of control and experimental animals. Results are expressed as mean ± standard deviation for six animals, (a) Group II, III, and IV compared with Group I, (b) Group III compared with Group II. NS – Nonsignificant, **P* < 0.05

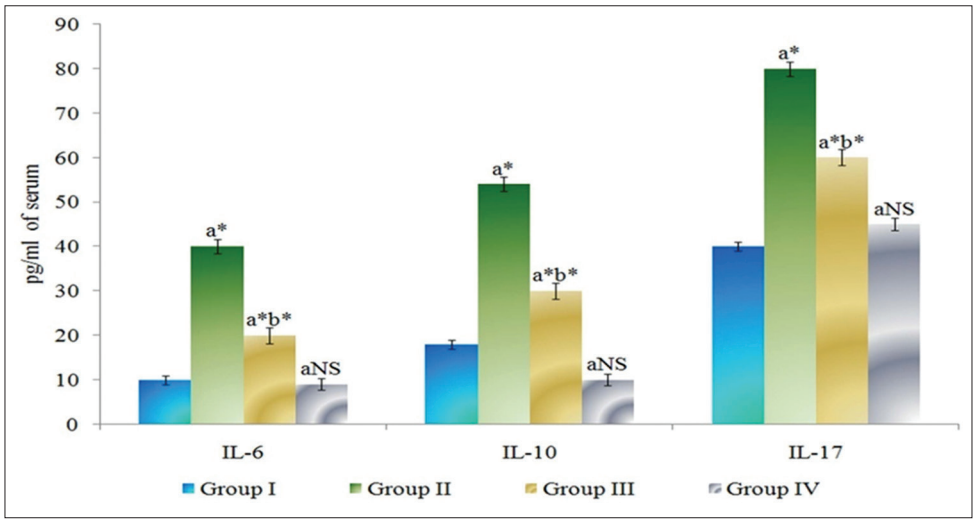

Figure 3: Effect of myrtenal on levels of cytokines in serum of control and experimental animals. Results are expressed as mean ± standard deviation for six animals, (a) Group II, III, and IV compared with Group I, (b) Group III compared with Group II. NS – Nonsignificant, **P* < 0.05

Inflammatory Markers

Determination of cytokines

The levels of cytokines such as interleukins (IL-6; IL-10; IL-17) in serum were determined using specific enzyme-linked immunosorbent assay kits (Biosource, California, US). The analyses were performed according to instructions of the manufacturer's. Standard plots were constructed using standard cytokines and the concentrations for unknown samples were calculated from the standard plot.

RESULTS

Effect of Myrtenal on Membrane Bound ATPase

The activities of Na^+/K^+, Ca^{2+}, Mg^{2+} ATPase in erythrocyte membrane and liver of control and experimental animals are provided in Figure 1. The membrane bound ATPase were found to be significantly decreased ($P < 0.05$) in erythrocyte membrane and liver of Group II colon cancer-bearing animals compared to Group I control animals. Myrtenal-treated Group III animals show a significant increase ($P < 0.05$) in the levels of membrane bound ATPase in erythrocyte membrane and liver compared to Group II cancer bearing animals. Group IV myrtenal alone treated animals show no significant change compared to Group I control animals.

Effect of Myrtenal on Levels of Tumor Markers

The levels of tumor markers CEA and CA 19-9 in serum of control and experimental animals are presented in Figure 2. Tumor marker enzymes levels were found to be significantly increased ($P < 0.05$) in DMH-induced Group II colon

cancer-bearing animals compared to Group I control animals. The Group III myrtenal-treated animals show a significant decrease ($P < 0.05$) in the levels of tumor markers compared to Group II animals. No significant changes were observed in Group IV myrtenal alone treated animals compared to Group I control animals.

Effect of Myrtenal on Levels of Cytokines

The levels of cytokines in serum of control and experimental animals are presented in Figure 3. IL-6, IL-10, and IL-17 were found to be significantly increased ($P < 0.05$) in DMH-induced Group II cancer-bearing animals compared to Group I control animals. The Group III myrtenal-treated animals show a significant decrease ($P < 0.05$) in the levels of cytokines compared to group II animals. No significant changes were observed in Group IV myrtenal alone treated animals compared to Group I control animals.

DISCUSSION

The present study provides evidence for a synergy between inflammation and colon cancer prognosis. Chemical-induced experimental animals have expressed increased levels of tumor markers and inflammatory marker.[14] In this connection, the increase in the levels of membrane bound ATPase delivers effective cellular and electrogenic pump in normal cells.[15] In colon cancer, condition induced by DMH the anaerobic pathways of tumor cells express decreased ATP levels as this in turn reflect the production of membrane ATPases. Activation of vesicles in the cytoplasm is favored by Ca^{2+} ATPase, and thus, in cancer condition the altered levels of calcium ATPases results in inactivation of major cellular components. Calcium-modulated protein is a calcium-binding messenger protein that mediates many crucial processes such as inflammation, metabolism, apoptosis, smooth muscle contraction, intracellular movement, short-term and long-term memory, and the immune response.[16] Thereby, alterations in the levels of calcium ATPases regulate the normal cellular functions. Mg^{2+}ATPase functions as a key factor in the activation of cofactor to regulate kinase enzymes.[17] In colon cancer, condition the levels of Mg^{2+} ATPase inhibit as a cofactor activator. Myrtenal-treated animals expressed altered levels of membrane bound ATPase to near normal and this might be due its specific activation of cell membranes by preventing lipid peroxidation reaction.

Colon cancer cases are mostly proved by increased CEA levels in the experimental models.[18] In addition to the levels of tumor markers biopsy and other diagnosis are essential to confirm the extent of tumorogenesis. Tumor markers are also essential in the diagnosis of cancer treatment as they respond to the chemo-preventive drugs. In the present scenario, myrtenal-treated animals expressed very little tumor marker enzymes compared to colon cancer bearing animals and this might be due to the anti-proliferative effect of myrtenal against DMH-induced animals. In addition, cytokines have a much larger distribution of sources for their production, with nearly all cells that have a nucleus capable of producing IL-1, IL-6, and tumor necrosis factor alpha, particularly endothelial cells, epithelial cells, and resident macrophages.[19] The levels of cytokines such as IL-6, IL-10, and IL-17 were seen increased in DMH-induced animals and this might be due to severe inflammation on normal cells and their profound inflammatory action during cancer conversion. Myrtenal treatment reduced the levels of cytokines and their activity in the cellular environment and this might be due to the immunomodulating property of myrtenal that modulate the immune response across the whole body against chemical carcinogens and its pathological effect. In addition, regulated cytokine levels help in combat against various diseases including cancer. Hence, myrtenal supplementation might have triggered the beneficial cytokine modulation against cancer cells. Thereby, the present study provides an insight on the effect of myrtenal on inflammatory cytokines and their regulation which can regulate colon cancer proliferation.

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Original Article

Methicillin-resistant *Staphylococcus aureus*; identification from clinical isolates and analysis of antibiotic resistance pattern

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a leading cause of hospital-associated infections. *S. aureus* develops resistance to commonly used antibiotics which results in therapeutic failure. We aimed to study the rate of MRSA and their antibiogram in a tertiary care hospital in South India. 94 isolates were collected from minor wounds to surgical site infections of patients of all ages and both sexes. Antibiotic resistance was studied by Kirby-Bauer disc diffusion method. Methicillin resistance was genotypically identified by specific amplification of the mecA gene by conventional polymerase chain reaction (PCR). A prevalence of 59.6% MRSA was observed through the study. MRSA strains were also resistant to other beta-lactams (penicillin and oxacillin) and other commonly used antibiotics, namely, gentamicin - 38% and cephalosporin −44%. No strains were resistant against, vancomycin, teicoplanin, and linezolid. The prevalence of MRSA was found to be higher than upper limits. Both PCR and disc diffusion methods were accurate in the detection of hospital-associated MRSA. Vancomycin, teicoplanin, and linezolid were found to be effective against MRSA. Physicians should be aware of the prevalence and pattern of antibiotic sensitivity of hospital-acquired MRSA and manage therapy accordingly.

Keywords: Beta lactams, mecA, methicillin-resistant *Staphylococcus aureus*, penicillin binding protein

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INTRODUCTION

Evolution of methicillin-resistant *Staphylococcus aureus* (MRSA) against beta-lactam antibiotics is a challenging issue for clinicians in the management of infections.[1] *S. aureus* is one of the major pathogens causes wide range of minor and major infections leading to morbidity and mortality. Penicillin invention reduced the mortality rates of infectious diseases including *S. aureus* infection.[2] Widespread use of antibiotics results in the evolution of more resistant strains. An organism can become resistant to a particular antibiotic through repeated exposure. The resistance is a result of genetic alterations or transfer of resistant gene.[3] *S. aureus* acquired resistance to beta-lactam antibiotics by harboring a gene called mecA. This restricts the use of beta-lactam antibiotics against MRSA infections.[4]

mecA is the gene responsible for resistance against beta-lactam antibiotics in *S. aureus*. mecA encodes a unique (altered) penicillin-binding protein (PBP) called PBP2 or MRSA-PBP, which executes low affinity to almost all beta-lactam antibiotics.[5]

A 21-60 kb DNA segment called chromosome cassette mec CCMec harbors the mecA gene along with other genes encoding resistance to non-beta-lactam antibiotics. mecA is a 2010 bp gene flanking from 47917 to 49926 in the genomic DNA of MRSA, which ensures resistance to beta-lactams. The gene product is a 78kDa protein PBP2 which is a modified form of native PBP. Methicillin-sensitive *S. aureus* (MSSA) encodes the native PBP and beta-lactams bind and disrupt the peptidoglycan layer synthesis. In MRSA, beta-lactam antibiotics cannot bind to the modified PBP (PBP2).

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Hence cell wall synthesis continues even in the presence of antibiotic.[4,6] Through horizontal gene transfer, mecA could be transferred from MRSA to MSSA by bacterial conjucation. This mechanism increases the prevalence of beta-lactam resistant strains among nosocomial infections which results in the failure of antibiotic therapy.[7,8] Identifying methicillin resistance in *S. aureus* infections is essential to choose right antibiotics for treatment.

Exposure of MRSA to beta-lactam antibiotics results in the production of PBPs excessively. It has been found that the induction of mecA and penicillinase production occurs simultaneously in the presence of beta-lactam antibiotics. This explains the fact that mechanism behind the stimulation and overexpression of PBP and penicillinase would be the same.[9]

MATERIALS AND METHODS

Sampling

94 strains of *S. aureus* isolated from minor wounds and postoperative wounds of patients, attending a rural tertiary care hospital were included in the study.

Wound swabs were collected and 5% blood agar and MacConkey quadrant streaking was for the isolation of bacteria following standard operating procedures. *S. aureus* was identified by slide and tube coagulase tests.

Antibiotic Sensitivity Assay

All isolates were subjected to antibiotic sensitivity test by modified Kirby-Bauer technique based on NCCLS regulations. The antibiotic panel includes, methicillin (M), penicillin (P), oxacillin (OX), amikacin (AK), Co-trimoxazole (COT), gentamicin (G/GEN), vancomycin (VA), clindamycin (CD), erythromycin (E), teicoplanin (TEI/TE), ciprofloxacin (CIP), cefazolin (CZ), cefoxitin (CX), and linezolid (LZ) and the isolates were observed as sensitive or resistant based on the measurement of zone of inhibition (Table 1).

DNA Isolation and mecA Gene Amplification

DNA was isolated by rapid heat and cool method. A loopful of bacterial culture was transferred to 100 µl of 50 mM NaoH and incubated at 95°C for 5 min. The sample was immediately transferred to 4°C. It was followed by the addition of 20 µl of 1Mtris HCL and centrifugation at 8000RPM for 3 min. The supernatant containing DNA was transferred to a fresh tube.

Amplification of mecA gene was done using primers described previously, F: 5'-TGCTATCCACCCTCAAACAGG-3', R: 5'-AACGTTGTAACCACCCCAAGA-3' (Kondo *et al*., 2007). The 286 bp sequence was amplified with 500 µm of each forward and reverse primer, 5 µl of template DNA were used in a 50 µl of 1X master mix (Takara- EmraldAmp). Amplification was done in BIO-RAD T100 thermal cycler with 35 cycles of initial denaturation at 93°C for 5 min, cycle denaturation at 93°C for 1 min, annealing at 55°C for 1 min, cycle extension at 72°C for 1 min, final extension at 72°C for 5 min and hold at 4°C for 5 min. The resulting product was viewed in 1% agarose gel with 100bp DNA marker.

Table 1: Antibiotic resistance pattern of *S. aureus* isolates

Antibiotic	MRSA (56) (%)	MSSA (38) (%)
Beta-lactams		
Methicillin (M)	56 (59.6)	0 (0)
Penicilin (P)	56 (59.6)	20 (21.2)
Oxacillin (OX)	56 (59.6)	18 (19)
Aminoglycosides		
Amikacin (AK)	8 (8.5)	2 (2.1)
Gentamicin (G/GEN)	38 (40.4)	11 (11.7)
Glycopeptides		
Vancomycin (VA)	0 (0)	0 (0)
Teicoplanin (TEI/TE)	0 (0)	0 (0)
Cotrimoxazole (COT) (sulfonamide)	16 (17)	11 (11.7)
Clindamycin (CD) (licosamide)	6 (6.4)	2 (2.1)
Erythromycin (E) (macrolid)	10 (10.6)	8 (8.5)
Ciprofloxacin (CIP) (quinolone)	44 (46.8)	16 (17)
Linezolid (LZ) (oxazolidinone)	0 (0)	0 (0)

MRSA: Methicillin resistant *S. aureus,* MSSA: Methicillin sensitive *S. aureus, S. aureus: Staphylococcus aureus*

RESULTS

S. aureus infection was predominant in males (62) than females (32). The study population was aged from a minimum of 1 to a maximum of 74 years with an average of 39 years. More number of isolates were obtained from older age groups of 50+ years (Table 2). Polymerase chain reaction (PCR) amplified a 286 bp sequence of the mecA gene in 56 of the 94 isolates (59.6%), which was confirmed in 1% agarose gel electrophoresis (Figure 1). All MRSA identified by PCR were resistant to methicillin with varying inhibitory concentrations by disc diffusion.

The resistance rate of both MRSA and MSSA were more against penicillin and oxacillin followed by ciprofloxacin and gentamicin. Linezolid, vancomycin and teicoplanin were equally effective against both MRSA and MSSA. None of

Table 2: Age and sex distribution of *Staphylococcal* infection

Age	Male	Female
1-10	4	4
11-20	6	0
21-30	8	8
31-40	8	6
41-50	6	6
51-60	16	6
60+	14	2

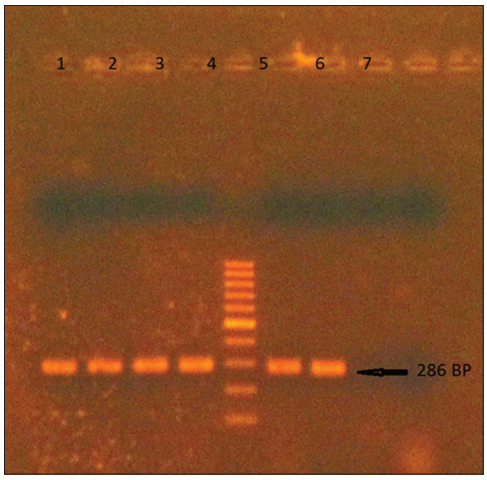

Figure 1: Amplified product of mecA gene in 1% agarose gel. Lane 5: 100bp DNA marker

the isolates were resistant against linezolid, vancomycin and teicoplanin (Table 1).

DISCUSSION

The prevalence of hospital-acquired MRSA is more than the previous reports available in the literature across the country.[10,11] This shows that the prevalence rate is increasing gradually over time. Global prevalence rates vary among countries from a minimum of <1% to higher rates of more than 50%.[12] The infection rate of our study was found to be more in males than in females like the other community-based studies.[6,13,14] This study indicates MRSA is more common in wound infections and open wounds are more susceptible to acquire hospital based transmission of MRSA. Thereby supports the report of Kini *et al*. 2013, who have observed the prevalence of MRSA in joint wounds of children in India.[15]

The PCR protocol amplified the mecA gene in all methicillin-resistant strains identified through disc diffusion analysis. Both PCR and disc diffusion tests are equally efficient, and PCR is more rapid than the culture protocols, by which results can be obtained within few hours. The antibiotic resistance pattern shows that all mecA positive strains are phenotypically resistant to penicillin and oxacillin. Some mecA negative isolates were also observed to be resistant to penicillin and oxacillin. This could be explained by the fact that production of beta-lactamase but not the novel PBP, would contribute to the resistance in these strains.[16]

Glycopeptides; both vancomycin and teicoplanin exhibited 100% activity against MRSA. Vancomycin has been the drug of choice for MRSA infections, but vancomycin-resistant *S. aures* (VRSA) also emerged as a new challenge in infection management.[17] However, we have not found any VRSA strains among the isolates included in the study.

S. aureus more quickly develops resistance to most of the commonly used antibiotics. This creates a necessity to use more recent antibiotics like linezolid. Linezolid-resistant *S. aureus* (LRSA) has been found to be <1%. However higher prevalence of around 40% LRSA was reported from North America and Europe. The existance of resistance to linezolid in LRSA is a mutation in the 23srRNA gene.[18,19]

CONCLUSION

Prevalence of hospital-associated MRSA is increasing drastically in the past few decades. More precautions and aseptic procedures should be followed in hospitals to minimize the spread of MRSA. Both phenotypic and genotypic determination of MRSA is recommended, and mecA PCR is found to be a rapid and reliable method. The presence of mecA gene in all MRSA strains indicates mecA is responsible for methicillin resistance. According to the present study, MRSA was found to be resistant to all beta-lactams. Vancomycin, teicoplanin and linezolid were 100% effective against all the hospital isolated strains and recommended as drugs of choice for MRSA.

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Original Article

Antidepressant-like effects of *Ficus deltoidea* aqueous extract on chronic unpredictable mild stress-treated rats

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ABSTRACT

Depression is a common and debilitating mental disorder. The present study was undertaken to investigate the antidepressant-like effects of the aqueous extract of *Ficus deltoidea* (FD) leaves. Sprague-Dawley rats treated with chronic unpredictable mild stress (CUMS) for 4 weeks were used as depression models. The body weight, sucrose preference test, open field test, and forced swimming test were measured to assess depressant-like symtoms. Hematoxylin-eosin staining was performed to evaluate neuronal morphology of hippocampal CA1 region and the toxicity of FD on liver and kidney. The administration of FD showed no obvious damage to the liver and kidney. It increased the body weight, improved depressive behaviors, and decreased the amounts of pyknotic and dark stained neurons in hippocampus in CUMS-treated rats. FD aqueous extract possessed antidepressant-like effects on CUMS-treated rats.

Keywords: Behavioral test, chronic unpredictable mild stress, depression, *Ficus deltoidea*, neural damage

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INTRODUCTION

Depression is a common comprehensive mental disorder. The core symptoms of depression are characterized by weight loss, low morale, anhedonia, and disrupted sleep.[1] There are more than 300 million people living with depression now, an increase of more than 18% between 2005 and 2015.[2] The curative effects are far away from satisfactory, which primarily reflected in slow work (weeks to months), low efficiency (only one-third of patients achieve full remission of their depressive symptoms and gain functional recovery) and many side effects (insomnia, headache, and anxiety).[3] Therefore, it has become a research hotspot to explore other mechanisms of depression and develop new natural antidepressants with high efficiency and low toxicity.

In Malaysia, *Ficus deltoidea* (FD) is commonly known as Mas cotek. It is an epiphytic shrub which has been traditionally used to treat sores, wounds, pain, diabetes, disorders of the menstrual cycle, and rheumatism.[4] It was reported that FD possessed antioxidant activity *in vitro* models[5,6] and anti-inflammatory activity.[7,8] Oxidative stress and inflammation play a key role in the pathophysiology of depression.[9,10] We speculate FD extract possesses antidepressant-like effect due to its traditional efficacy and chemical composition. In this study, chronic unpredictable mild stress (CUMS)-treated rats were used to investigate the antidepressant-like effect of FD treatment.

MATERIALS AND METHODS

Animals

Forty-two healthy adult male Sprague-Dawley rats (200-220 g, 6-8 weeks) were supplied by TAKRIF BISTARI ENTERPRISE (Malaysia) and housed with 12 h illumination, 25 ± 1°C room temperature and free access to water and food. After 7-day

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acclimatization to their new environmental conditions, the rats were randomly divided into control, CUMS, FD alone (800 mg/kg), different doses (50, 200, and 800 mg/kg) of FD, and fluoxetine (10 mg/kg, positive control) groups. There were six rats in every group. There was no stress in control and FD alone group. The rats in other groups were subjected to the following stressors once a day for 28 days: Inversion of the light/dark cycle, wet bedding for 24 h, nip tail for 1 min, shaking for 10 min, water deprivation for 24 h, fasting for 24 h, swimming in 45°C hot water for 5 min, swimming in 4°C cold water for 5 min, and cage tilting for 24 h.[11,12] A stressor was performed randomly every day and the same stressor could not appear continuously. The animals were given oral administration once a day for 28 days. Rats in control and CUMS group were given normal saline with equal volume (Figure 1).

Drug Administration

The leaves of FD were collected from a farm in Malacca. After being air-dried, the leaves were coarsely powdered, and then, extracted with boiling water for 1 h. The infusion was filtered, and the filtrate was spray-dried to form a powder. FD and fluoxetine (Zhongxi Pharmaceutical Co., Ltd. Shanghai, China) was diluted in sterile water to suitable concentration. All treatments were administered 30 min before stress exposure.

Detection of Body Weight

Body weight of rats in every group was monitored on 0 day, 7 days, 14 days, 21 days, and 28 days of the experiment.

Sucrose Preference Test (SPT)

All rats were applied adaptation to sucrose solution 2 days before SPT. Two bottles were put in every cage at the same time. One was 1% sucrose solution, the other was water. To avoid the preference of the position where the rats drank, the position of the two bottles was exchanged every 12 h. After rats were deprived of water for 24 h, the volumes of consumed water and sucrose solution were recorded in 2 h. The sucrose preference (%) = sucrose consumption/(water consumption + sucrose consumption).

Open Field Test (OFT)

After 1 h of habituation to the test room, the rats were placed in a black square open box (100 × 100 × 40 cm) one by one, of which the base consisted of 25 same squares. When the rat was put at the center of the box, the locomotor activities (defined as locomotion on at least three paws) and rearing activities (defined as an upright posture sustained on hind paws) of the rat in 5 min were recorded with a video-computerized tracking system.

Forced Swimming Test (FST)

Rats were subjected to an open cylindrical container (height 45 cm, diameter 18 cm) filled with water (depth 35 cm) at 25 ± 2°C. The activities of rats in 6 min were recorded with a video-computerized tracking system. After each test, rats were dried by towels and returned into their cage under a heater. The immobility time was defined when no obvious activity was observed except essential activity to keep the head out of water.

Morphology of Hippocampus, Liver, and Kidney with hematoxylin-eosin (HE) Staining

After behavioral tests, 10% chloral hydrate (0.3 mL/100 g, i.p.) was used to anesthetize the animals. Hippocampus was rapidly separated from the skull. Kidneys and livers were also

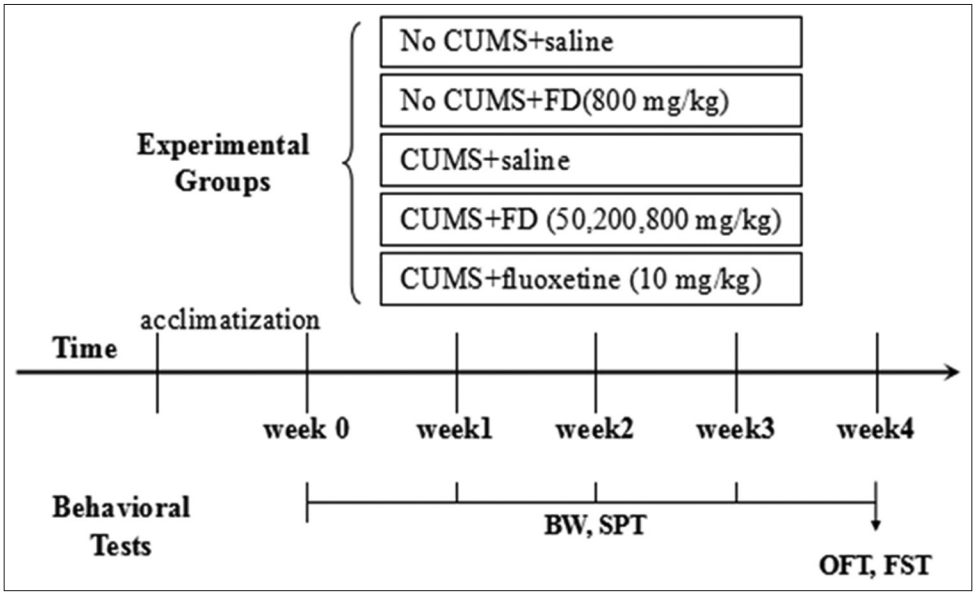

Figure 1: Schematic representation of the chronic unpredictable mild stress (CUMS) experimental procedure and behavioral tests. After 4 weeks of CUMS procedure or control condition, rats were performed behavioral tests before being sacrificed. The brains, kidneys, and livers were then collected for morphology

removed from peritoneal cavity. Then, the tissues were fixed in 4% paraformaldehyde, manufactured into paraffin specimen, cut into 6 μm thick sections, and stained with HE. Light microscopy (Olympus, U-25ND6) was used to observe the morphology of liver, kidney, and hippocampal neurons in CA1 region in each group. Pyknotic hippocampal neurons (%) = the number of pyknotic neurons/the number of total neurons in the same field of microscope.

Statistical Analysis

Data were presented as mean ± standard deviation and analyzed with the GraphPad Prism 5 software. One-way ANOVA followed by the Tukey *post-hoc* test was used for statistical analysis of the results as appropriate. Significance level was set at $P < 0.05$.

RESULTS

The Effects of FD on Body Weight of CUMS-treated Rats

As shown in Table 1, there was no significant difference in each group for body weight at the beginning of the experiment ($P > 0.05$). Compared with the control group, CUMS treatment resulted in a significant reduction in body weight increase from the 3rd week ($P < 0.05$). At the 4th week, the average body weight of rats in CUMS group was reduced by 21.30%, being 255.83 ± 5.78 g for the CUMS group and 324.50 ± 9.99 g for the control group. The decrease of body weight induced by CUMS was ameliorated by the pretreatment of FD (800 mg/kg) or fluoxetine (10 mg/kg) ($P < 0.01$).

The Effects of FD on SPT of CUMS-treated Rats

There was no significant difference in each group for sucrose solution intake at the beginning of the experiment ($P > 0.05$). The sucrose preference of rats in CUMS group tended to decrease over time and was notably reduced compared with that of scheme group from the 3rd week ($P < 0.001$). This indicated anhedonia and decreased response capability to happiness events. The sucrose solution intake of rats in FD200 ($P < 0.05$), FD800 ($P < 0.01$), and flu ($P < 0.01$) group increased considerably at the 4th week compared with CUMS group (Table 2).

The Effects of FD on OFT of CUMS-treated Rats

Compared with the control group, the locomotor and rearing activities number of rats in CUMS group decreased significantly ($P < 0.01$). Decreased locomotor and rearing activities indicated that animals showed depression, loss of interest, and lack of pleasure. The locomotor and rearing activities number of rats in FD (200, 800 mg/kg) and flu group were significantly increased ($P < 0.01$, $P < 0.001$, respectively) compared with CUMS group (Figure 2).

Table 1: Effect of FD on body weight of CUMS-treated rats (g) (*n*=6)

Groups	0	1	2	3	4 (week)
Control	194.67±5.31	226.67±7.52	260.33±9.75	291.67±11.99*	324.50±9.99**
FD	195.17±5.09	227.33±6.92	261.67±9.28	292.17±7.71*	324.33±9.02**
CUMS	196.17±5.25	226.17±6.89	253.00±7.39	256.83±6.34$^{\#}$	255.83±5.78$^{\#\#}$
FD50	193.00±3.41	222.00±4.63	248.50±5.84	251.67±5.10$^{\#}$	256.17±5.53$^{\#\#}$
FD200	191.33±2.93	222.00±4.00	249.50±3.92	258.67±4.43	280.50±4.02$^{\#\#}$
FD800	201.17±5.49	234.00±7.41	263.33±9.59	278.67±9.99	298.67±10.64**
Flu	195.83±4.98	228.17±5.26	258.00±5.26	275.67±5.55	296.00±5.60**

FD pretreatment significantly suppressed the decrease of body weight induced by CUMS. FD: *Ficus deltoidea*, CUMS: Chronic unpredictable mild stress. $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{*}P<0.001$ versus control group, $^{**}P<0.01$ versus CUMS group

Table 2: Effect of FD on SPT of CUMS-treated rats (%) (*n*=6)

Groups	0	1	2	3	4 (week)
Control	88.17±2.90	91.67±3.08	91.33±2.40	92.50±3.72	90.50±3.16
FD	87.83±2.97	89.00±3.89	90.00±2.38	92.00±2.88	89.83±3.01
CUMS	87.67±3.08	90.83±4.55	71.83±9.78	50.50±4.92$^{\#\#\#}$	44.33±6.34$^{\#\#\#}$
FD50	87.17±3.16	91.33±3.96	76.17±7.70	56.00±3.45$^{\#\#\#}$	50.17±4.22$^{\#\#\#}$
FD200	90.50±3.50	88.83±2.88	81.17±5.53	71.50±2.31$^{\#}$*	69.67±6.77*
FD800	86.83±3.25	89.83±2.63	90.67±2.09	81.67±5.16***	78.17±3.06**
Flu	89.33±2.72	93.67±3.00	89.00±4.34	75.50±6.72**	74.17±6.58**

FD pretreatment significantly improved the decrease of sucrose solution intake induced by CUMS. FD: *Ficus deltoidea*, SPT: Sucrose preference test, CUMS: Chronic unpredictable mild stress. $^{\#}P<0.05$, $^{\#\#\#}P<0.001$ versus control group, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ versus CUMS group

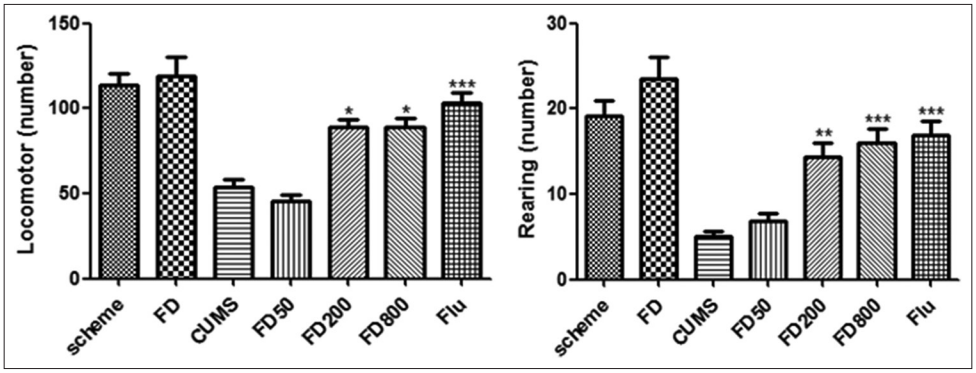

Figure 2: Effect of FD on OFT of CUMS-treated rats. FD pretreatment significantly increased the number of locomotors and rearing activities compared with CUMS group. FD: *Ficus deltoidea*, OFT: Open field test, CUMS: Chronic unpredictable mild stress. $^{**}P < 0.01$, $^{***}P < 0.001$ versus CUMS group

The Effects of FD on FST of CUMS-treated Rats

Compared with the control group, the immobility time of rats in CUMS groups was significantly longer during the 6-min test period ($P < 0.001$). The rats in FD (200, 800 mg/kg) or flu (10 mg/kg) group spent less time immobile compared with CUMS group ($P < 0.01$, $P < 0.001$, respectively) (Figure 3).

Neuronal Morphology in the CA1 Region of Hippocampus

Neurons in control group were normal, with a clearly round appearance, intact membranes, a clear cytoplasm, a clear nucleus, and distinct nucleoli. Compared with the control group, the density of dark stained and pyknotic hippocampal neurons in CA1 region was significantly increased in CUMS group. In hippocampal CA1 region of rats in CUMS group, we also observed nucleus shrinkage or disappearance and neuron loss. However, the amount of pyknotic cells was significantly decreased in FD group, especially 800 mg/mL, compared with CUMS group (Figure 4).

Morphology of Liver and Kidney

As shown in Figure 5, glomerular with normal volume formed regularly. Renal corpuscles were clear. There was no abnormality of the number of mesangial cells, endothelial cells, and epithelial cells. The apical end of each proximal tubule cell has a brush border of microvilli. The boundaries between adjacent proximal tubule cells are inconspicuous. Most of round nucleus located at the base. There was a usual appearance of a larger, clearer lumen in each distal tubule which had a lesser degree of acidophilia. The central vein located at the center of the lobule. Portal triads situated at the periphery of the lobule, which were composed of one or more small branches of the portal vein, a branch of the hepatic artery, and a small bile duct. Hepatocytes usually had one, sometimes two, large round to slightly oval nuclei, which were distinctly round, with one or two prominent nucleoli. There was no obvious difference of renal and liver histology between control and FD group.

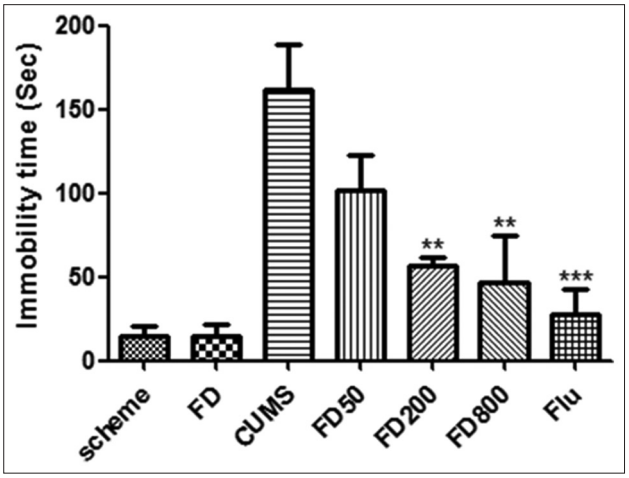

Figure 3: Effect of FD on FST of CUMS-treated rats. A significant decrease in floating behavior was observed in rats pretreated with FD (200, 800 mg/kg) and flu compared with CUMS group. FD: *Ficus deltoidea*, FST: Forced swimming test, CUMS: Chronic unpredictable mild stress. $^{**}P < 0.01$, $^{***}P < 0.001$ versus CUMS group

DISCUSSION

Animal model of depression are often used to study the pathophysiology and symptomatology of depression, screen novel antidepressants, and identify the mechanism of antidepressants.[13] CUMS is one of widely used rodent depressed models because the behaviors of CUMS-treated animals approximate the symptoms of patients with depression such as appetite loss, weight changes, decreased exploratory activities, diminished cognitive functioning, anhedonia, and hopelessness.[11,12]

Rodents prefer sweet solutions or foods from birth. SPT can be used to evaluate the motivation and affective state of subject rats. Decreased preference for sweet solution in SPT means anhedonia.[14] OFT is an experiment used to assay anxiety-related and exploratory behavior of rodents.[15] During the

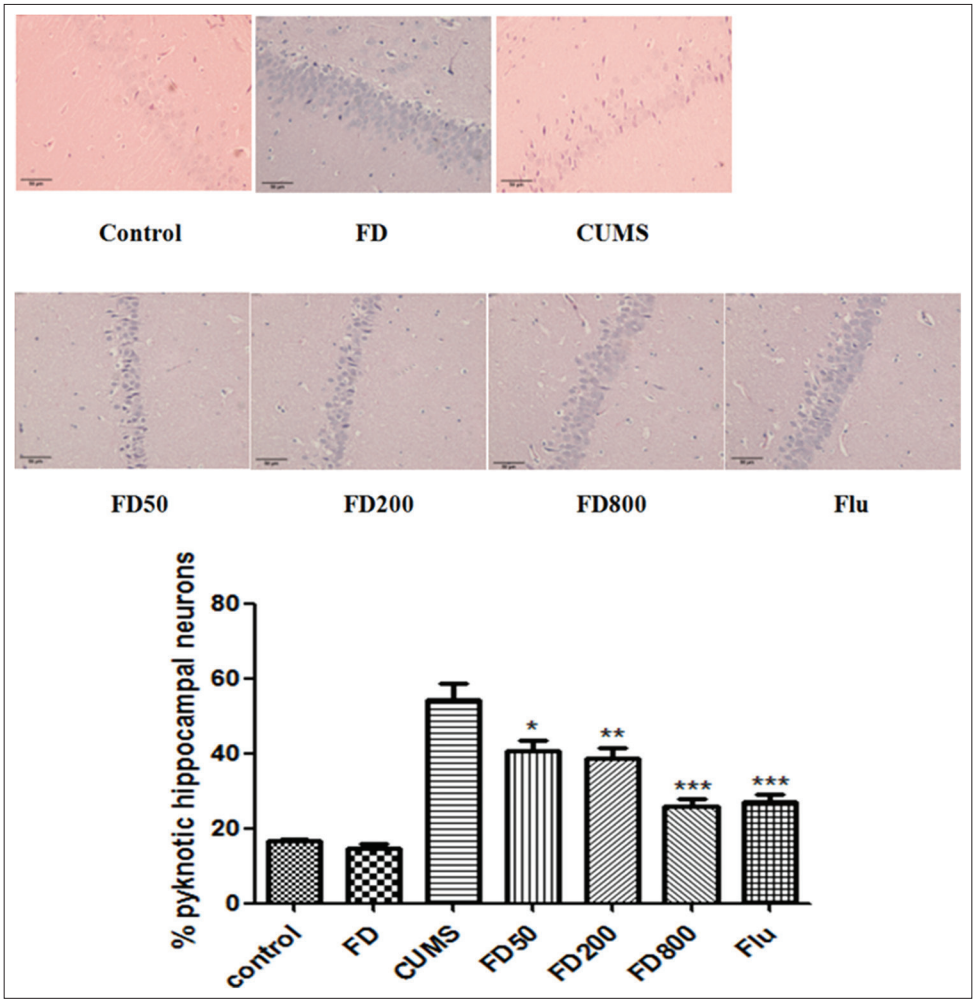

Figure 4: Effects of FD on neuronal cell survival in CA1 region in hippocampus of CUMS-treated rats. FD pretreatment significantly decreased the number of dark stained and pyknotic hippocampal neurons in CA1 region compared with CUMS group. Representative photographs of CA1 region of hippocampus stained by HE in each group (original magnification ×200). The bar graphs reflected the rate of pyknotic and dark-stained neurons in CA1 region in each group. Data were presented as mean ± SEM. FD: *Ficus deltoidea*, CUMS: Chronic unpredictable mild stress, SEM: Standard error of the mean. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ versus CUMS group

test, rats were put in a bright and novel environment. The parameters, such as motion distance and the number of rearing, are often used to evaluate anxiety of the animals. FST is an experiment widely used to measure susceptibility to negative mood in depression research.[16] At first, rats kept on swimming and leaping to escape from the water-filled container. When they gave up, they developed an immobile posture. Immobility is thought as depression-like behavior or negative stress-coping strategy. In the present study, we adopted CUMS depression model to observe the effects of FD extract on depression-like behaviors of rats by detecting body weight, SPT, OFT, and FST. The results showed that rats exhibited many depressive-like symptoms after being exposed to CUMS for 4 weeks. The treatment of FD (200, 800 mg/kg) extract attenuated or reversed these changes including decreased body weight, sucrose intake and autonomous activities, and increased immobility time. These results suggested the rat model of depression was successful and FD extract possessed an anti-depressive effect on CUMS-treated rats.

It has been reported that CUMS treatment could induce neuronal degeneration in hippocampus which plays a key role in many respects of depression.[17,18] The present study exhibited similar findings after exposure to CUMS for 4 weeks. Treatment with FD (200, 800 mg/mL) decreased the number of dark stained and pyknotic hippocampal neurons in CA1 region, suggesting its potential neuroprotective effect against CUMS exposure.

Increased inflammation and oxidative stress are involved in the pathogenesis of depression[9] including the metabolism of neurotransmitters,[19-21] neuroendocrine function,[22] and synaptic plasticity.[17] Phytochemical studies confirmed FD contained numerous constituents' classes, such as phenol,[23]

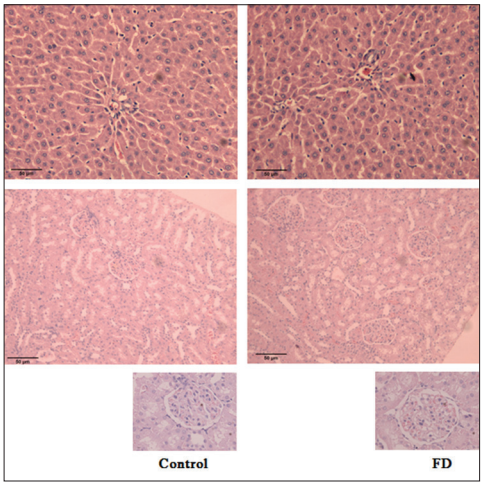

Figure 5: Effects of FD on histology of liver and kidney. FD pretreatment had no obvious toxicity to liver and kidney. Representative photographs of liver and kidney stained by HE in control and FD group (original magnification ×100 and ×200). G: Glomerular, PT: Portal triads, FD: *Ficus deltoidea*, HE: Hematoxylin-eosin

terpenes, steroids, tannins, saponins, and flavonoids.[24] It has been reported that several tannins and flavonoids isolated from FD had been identified for their strong anti-inflammatory activity[25] and anti-oxidant activity.[23] The aqueous extract of FD leaves possess anti-inflammatory effects against various inflammatory responses.[25] Abdullah *et al*.[5] used three *in vitro* assays to examine the anti-inflammatory activity of standard extracts of different varieties of FD. Therefore, it is possible that the antidepressant-like effect of FD observed in our study may be due to its phenolics and flavonoids components. Moreover, the preliminary acute oral toxicity test showed that there is no observed adverse effect even at the dose of FD 2500 mg/kg.[26,27] This indicates that FD may have a considerable safety range to acute toxicity. In our study, different doses of FD showed no observable damage to kidney and liver. It is beneficial to the wide application of this plant in various disease conditions.

CONCLUSIONS

The present study indicated that CUMS caused depression-like behaviors of rats and increased neuronal degeneration in hippocampus. However, the aqueous extract of FD attenuates or reverses these changes. The results suggest the active compounds in FD and the mechanisms of its anti-depressant effects may be worth further investigating and elucidating.

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Review Article

Salivary antioxidants and dental *problems* - A review literature

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ABSTRACT

Antioxidants deficiency is indirectly related with a group of diseases worldwide. Dental studies related with free radicals and their toxic effects in dentistry have been focused recently. Major inflammation in periodontics and gingivitis is treated with antioxidants by its free radical scavenging activity. Recent studies with oral cancer incidence and its adjuvant treatment with antioxidants sustain the preventive effect of various antioxidants. Reactive oxygen species and other hydroxyl radicals tend to degrade the oral maxillary and induce carcinoma in a step-wise manner which ultimately leads to death. Data correlating the anticarcinogenic properties of antioxidants provide major insight in the mechanism of action.

Keywords: Antioxidants, oral cancer, salivary antioxidants

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INTRODUCTION

Antioxidants are substances that inhibit oxidation in the cellular environment and maintain homeostasis.[1] They are natural or man-made substances that can neutralize free radicals and eliminate them. Free radicals are chemical species having a single unpaired electron in its outer orbit, which create an imbalance in the microenvironment of the cells and reconstitute a pathological effect.[2] Free radicals such as reactive oxygen species (ROS) are formed within the mitochondrial inner membrane and induce lesions in the nucleoproteins which serve as a binding protein during carcinogenic process.[3] DNA damage can result in dysfunction, mutation, or oxidation of protein. Antioxidants can be enzymatic or non-enzymatic which donate an electron to the free radical and make them stable.[4] Polyphenol antioxidant is a type of antioxidant derived from fruits and vegetables and is considered as a major therapeutic drug in treating dental pathologies.[5] It contains a polyphenolic substructure with the presence of catechol group in the polyphenol which acts as an electron acceptor.[6] Polyphenol antioxidants are mainly obtained from diet rich in honey, legumes, apple, blackberry, blueberry, grapes, cherries, pomegranate, strawberry, and vegetables such as broccoli, cabbage, onion, and parsley.[7]

SALIVARY ANTIOXIDANTS

Saliva is rich in antioxidants and acts a first-level defense along with immune response during oral inflammation.[8] Dental studies reveal that primary inflammation in the mouth cavity if left untreated with adequate consumption of dietary antioxidant might lead to chronic conditions.[9] Thus, salivary antioxidants such as uric acid, albumin, ascorbic acid, and glutathione act as a medium of natural defense in maintaining oral health and can be measured by total antioxidant capacity (TAC).[10] It has been reported that periodontal diseases are high in patients with significant decrease in salivary antioxidants. In periodontal diseases such as gingivitis, there is an increased amount of free radicals released due to inflammation.[11] Bacteria such as *Porphyromonas gingivalis* and *Entamoeba gingivalis* are causative agents of *Gingivalis*, and hence, enhanced salivary antioxidants are known to reduce inflammation by quenching the free radicals produced by pathogens.[12] In response to the bacteria which acts as a pathogen, our body produces a number of inflammatory mediators such as cytokines, prostaglandins, and certain enzymes. Neutrophils appear due to the inflammatory responses which phagocytose the bacteria.[13] This process results in the formation of large quantities of ROS.

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DENTAL CARIES

It is a common oral disease which is caused by the interaction of bacteria and dietary carbohydrates, leading to the destruction of tooth structure.[14] It is an infectious transmissible disease having a multifactorial etiology which depends on the interaction of three main group factors that must exist simultaneously for sufficient time including host, microbial, and substrate factors.[15] Saliva plays an important role in caries prevention. Saliva has an antibacterial action due to its complex structure of organic and inorganic molecules, such as electrolytes, mucins, antiseptic substances, immunoglobulin, proteins, and various enzymes.[16] Saliva peroxidase controls oral bacteria which lead to dental caries and periodontal diseases. Statistical comparisons have correlated the levels of TAC in saliva where significantly increased in caries patient in comparison to non-caries subjects.[17]

ORAL CANCER

Oral cancer is a class of disease characterized by uncontrolled cell growth to form masses of tissue called tumors. Tumor development might occur due to imbalanced cell proliferation and cell death which can further progress into cancer.[18] In addition, genetic and epigenetic alterations drive cellular transformation with multiple signals delivered within the tumor microenvironment by modifier genes[19] with an accumulation of mutations in oncogenes, tumor suppressor genes, and genes that maintain the genomic integrity of the cell.[20] Cancer development is characterized by cumulative action of multiple events occurring in single cell and can be described by three stages: Initiation, promotion, and progression.[21] ROS is involved in all these stages. The effect of oxidative stress at a certain stage of carcinogenesis is directly proportionate to the type and the reactivity of radicals involved.[22] During tumor development, tumor cells release nucleic acids into the blood circulation, and this process occurs by apoptotic and necrotic cell deaths along with active cell secretion. Increased ROS levels report the imbalance between the generation and elimination, leading to oxidative stress-related malignancy characterized by functional decline of the cellular antioxidants during the primary defense.[23] Antioxidants act as reducing agents to get oxidized and prevent excess free radical production, but under malignant condition, the levels of antioxidants provided are insufficient to overplay the emerging free radicals all over. Hence, treatment with antioxidants in the initial stages might reduce the effect of cancer metastasis.[24]

MECHANISM OF ACTION

Enzymic and non-enzymic antioxidants deliver a wide variety of action against dental and other disease conditions. Hence, a detailed mechanism of action is studied and followed with yet future research. Superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase are considered as the primary antioxidant enzymes since they are involved in the direct elimination of ROS.[25] They catalyze the dismutation of the superoxide (O_2^-) radical into either ordinary molecular oxygen (O_2) or hydrogen peroxide (H_2O_2), and the regulation of hydrogen peroxide provides a key in the downregulation of cellular damage. Salivary antioxidants enhance a wide variety of action as the majority of saliva is watery, and hence, function in the transport and lubrication in the body.[26]

CONCLUSION

Antioxidant review helps us to understand the potent activity in scavenging the free radicals and maintaining homeostasis. Salivary antioxidant present naturally may be enriched with proper hygiene and a dietary habit as per dental research explains. Various researches on natural products reveal the antioxidant and anticarcinogenic effects which emphasize the intake of natural food. Recent research has focused mainly in antioxidant study and its action mechanism for various diseases. Hence, in the future, antioxidant-based artificial drugs will be prescribed and followed in curing ailments for the betterment of humanity.

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