Phytochemical and pharmacological activity of Aegle marmelos as a potential medicinal plant: An overview

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Citation

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Abstract

Aegle marmelos (Linn) family rutaceae is highly reputed ayurvedic medicinal tree commonly known as the bale fruit tree, is medium sized tress growing throughout the deciduas forest of India of altitude 1200 meter. It is found whole over India, from sub-Himalayan forest, Bengal, central and south India .All the parts of the tree viz, root, leaf, trunk, fruit, are used in traditional system of medicine: root parts are used in dysentery (pravahika), dyspepsia (agnimandya), chronic diarrhea with mal absorption (graham roga). The dried roots are used in the disorder of nervous system (vatavadhjy), oedema (uotha), vomiting (chardi), and rheumatism (amavata). Various phytochemical and biological evaluations have been reported in this literature for the importance of the Aegle marmelos.

INTRODUCTION

A medium sized armed deciduas tree upto 8.0 m high with straight sharp auxiliary thorns and yellowish brown shallowly furrowed corky bark ([1,2]). Older branch neither are straight sharp single nor paired, 2.5 cm long. Young branches are green slightly zigzag and compressed. Leaves are alternate, attenuate trifoliate, occasionally digitately 5-foliate; petiole 2.5 to 6.3 cm long. Leaflets ([5,6,7,8,9,10]) are ovate or ovate- lanceolate, margins crenate, apex acuminate, glabrous and densely minutely glandular-punctuate on both surfaces; lateral leaflets to 7 cm long and 4.2 cm wide, petiolules 0-3mm long. Flowers are large, bisexual, greenish-white, sweet scented, in short axillary panicles 4 to 5 cm long. Calyx flat, pubescent, 4-lobed, lobes rounded sometimes obscure. Petals 4, spreading, oblong, thick, gland-dotted, much exceeding the sepals, imbricate. Stamens numerous; another elongate, apiculate; filaments free or fascicled, inserted round an inconspicuous disk. Ovary ovoid, cells 10-20; style terminal, short, deciduous; stigma capitate; ovules numerous, 2-seriate. Fruits are globose, grey, or yellowish, upto 20 cm in diameter, with woody rind; seeds numerous, oblong, compressed, embedded in sacs covered with thick, orange coloured sweet pulp root bark is 3 to 5 cm thick covered, with creamy yellowish surface. The surface is rough, irregular and shallow with ridges along the line of development of lenticels cream colored flaring edges. It has a firm leathery texture, a sweet taste and fracture is fibrous. Stream bark is extremely gray and internally cream in colour. The outer surface is rough warty due to a number of lenticels, ridges and furrows. It is 4-8 mm thick, film in texture and occurs as flat or channeled pieces. The fracture is tough and gritty in outer region and fibrous in the inner. The taste is sweet and there is no characteristic odour ([3,4]).

PHYTOCHEMICAL EVALUATION

Riyanto et al, isolated few compound from A. marmelos bark from petroleum ether extract. The crude extract was subjected to column chromatography. The isolated compounds were identified on the basis various spectral data (UV, IR, Mass and NMR). Two triterpenes, lupenone and lupeol were obtained in the form of white crystal ([5]).

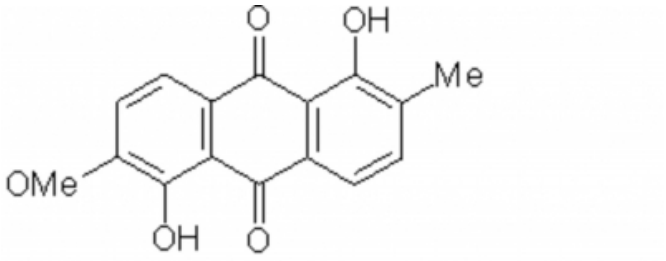
Khangan et al, studied the binding of copper ions with modified Aegle marmelos bark substrate. The effect of pH, contact time, temperature, anion, light metal ion, concentration and effect of amount of substrate on the uptake of Cu^{2+} were studied. Substrate indicated that Cu was removed to <0.02 mg/L from solutions. The capacity has been found to be 0.9756 meq of Cu^{2+} per g of substrate. Cu sorbed on the substrate was removed by simple leaching dil HNO_3 and the substrate was used for a fresh treatment cycle. Studied on the sorption of different heavy metal ions ON packed column of Aegle marmelos stem bark substrate indicated that Pb and Cu are strongly sorbed followed by Cd, Zn, Ni, Co and Mn ([6]).

Ohashi et al isolated four isomeric lignan-glucosides from the bark of Aegle marmelos .Two new lignan – glucoside, (-) – lyoniresinol 2-O--D glucopyranoside and (-) 4 - epi-lyoniresinol, 3 -O - -D-glucopyranoside, have been isolated together with two known lignan - glucosides, (+)-lyoniresinol. 3-O--D-glucopyranoside and (-)-lyoniresinol 3-O--D-D-glucopyranoside ([7]).

Ohashi et al isolated two new 7-geranyloxycoumarins from the bark of the Aegle marmelos. Two new 7-gerayloxycoumarins and aeglin, were isolated from the bark of Aegle marmelos, and there structures were assigned on the basis of the NMR data .The absolute configuration was confirmed by chemical synthesis ([8]).

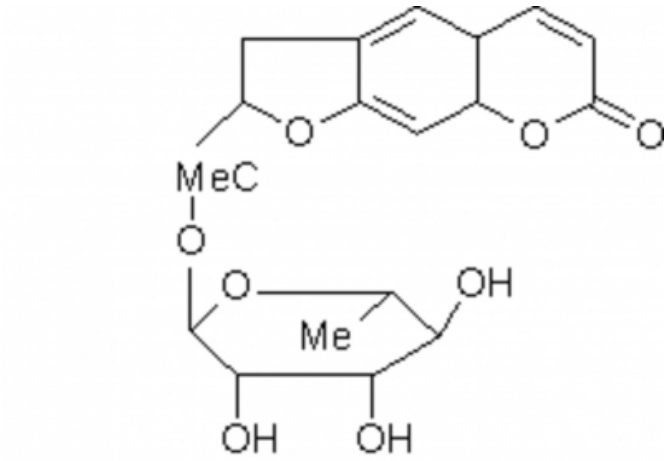
Nema et al isolated new pigment from stem bark of the Aegle marmelos. The isolation and structure elucidation of new compound is marmesin – 1”- -L - rhamnopyranoside and 1,5 -dihydroxy - 6 - methoxy -2 -methyl anthraquinone, which occur together with lupeol and -sitosterol in the stem bark of Aegle marmelos were describes ([9]).

Figure 1



1,5-dihydroxy-6-methoxy-2-methylanthraquinone

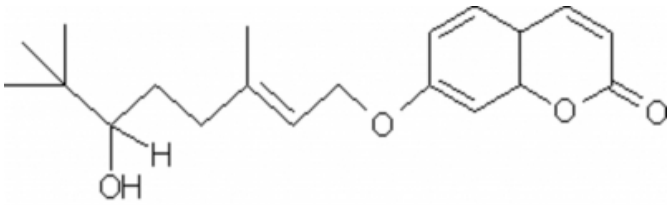
Figure 2



Marmesin–1[1]-α–L–rhamnopyranoside

V.K. Gupta et al studied the sample coumarin compound R-(+) – marmin from the trunk bark of the Aegle marmelos by methanol extract. Then extracted compound concentrated and chromatographed over the silica gel, and the chemical structure were assigned on the basis of the H^+ NMR and mass spectra ([10]).

Figure 3

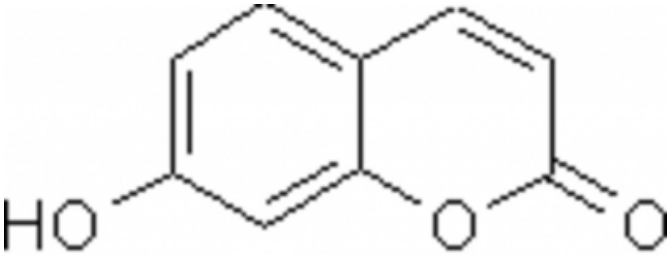


R-(+) – marmin

Chatterjee et al studied the isolation and constitution of marmin, a new coumarin from Aegle marmelos umbelliferone (i), skimmianine (ii) and a sitosterol (iv), were isolated from the immature bark of Aegle marmelos. The constitution of (iii) was established as 7- (3, 7 dihydroxy-3, 7- dimethyloctyloxy) coumarin ([11]).

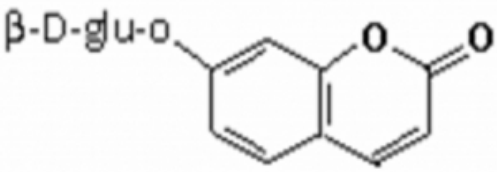
Samarasekera et al isolated various coumarin present in the various part of the Aegle marmelos. These are Umbeliferone, Skimmin, Impertonin. The structures of these coumarins are given below ([12]).

Figure 4



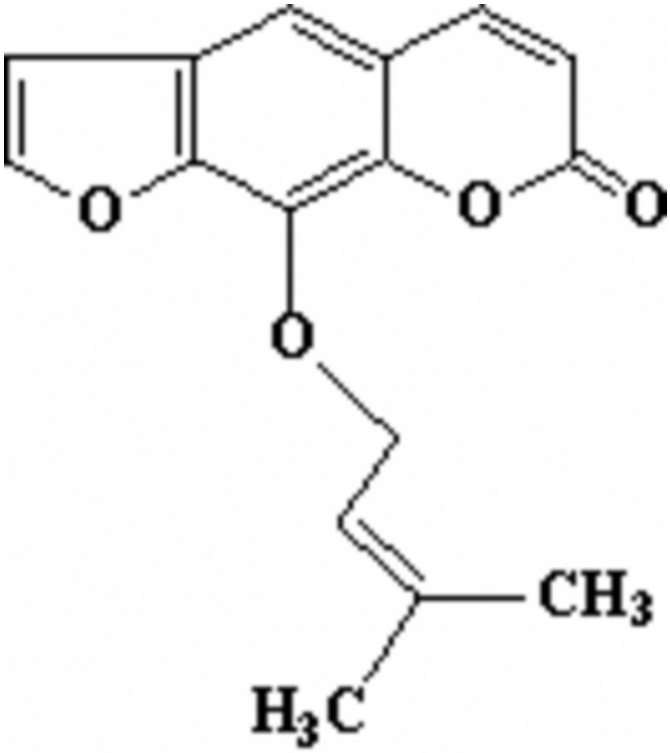
Umbeliferone

Figure 5



Skimmin

Figure 6



Impertonin

PHARMACOLOGICAL EVALUATION

Leticia veras et al, evaluated the anticancer potential used in Bangladeshi folk medicine. The extracts of Aegle marmelos were tested for cytotoxicity using brine shrimp lethality assay; sea urchin eggs assay, and MTT assay using tumor cell lines. The extract of Aegle marmelos exhibited toxicity on all used assays ([13]).

Rana et al evaluated the anti fungal activity of essential oil isolated from leaves of the Aegle marmelos using spore germination assay. The oil established variable efficacy against different fungal isolation and 100% spore germination off all the fungi tested and observed 500 PPM. However the most resistant fungus, Fusarium udum was inhibited 80% at 400 PPM ([14]).

P.S. Marinzene et al determines the effect of an aqueous extract of Aegle marmelos fruits on serum and tissue lipids in experimental diabetes. Albino Wistar rats were rendered diabetic by intraperitoneal administration of streptozotocin

(45 mg kg^{-1}). Serum and tissue lipids such as total cholesterol, triglycerides, free fatty acids and phospholipids were elevated in diabetic rats. Oral administration of A. marmelos fruit extract at doses of 125 and 250 mg kg^{-1} to diabetic rats twice daily for 1 month led to a significant lowering of these lipids in diabetic rats. The effect exerted by the fruit extract at a dose of 250 mg kg^{-1} was greater than that of the dose of 125 mg kg^{-1} or of glibenclamide (300 µg kg^{-1}). The results of this study demonstrate that an aqueous Aegle marmelos fruit extract exhibits an antihyperlipidaemic effect in streptozotocin-induced diabetic rats ([15]).

S. Miyazaki et al designed to elucidate the toxicity of the widely used plant A. marmelos in rats. They used total alcoholic, total aqueous, whole aqueous and methanolic extracts isolated from the leaves of Aegle marmelos and studied their toxic effects. Acute, sub-acute and LD_{50} values were determined in experimental rats. The dead animals were obtained from primary screening studies, LD_{50} value determination experiments and acute studies subjected to postmortem studies. The external appearance of the dead animals, the appearance of the viscera, heart, lungs, stomach, intestine, liver, kidney, spleen and brain were carefully noted and any apparent and significant features or differences from the norm were recorded. Following the chronic administration of A. marmelos for 14 days, the vital organs such as heart, liver, kidney, testis, spleen and brain were carefully evaluated by histopathological studies and any apparent and significant changes or differences from the norm were studied. From the acute administration of A. marmelos, the LD_{50} values were determined using graphical method. The hearts stopped in systolic stand-still in the acute experiments. There were no remarkable changes noticed in the histopathological studies after 50-mg/kg body wt of the extracts of A. marmelos when administered intraperitoneally for 14 days successively. Pathologically, neither gross abnormalities nor histopathological changes were observed. After calculation of LD_{50} values using graphical methods, we found a broad therapeutic window and a high therapeutic index value for A. marmelos extracts. Intraperitoneal administration of the extracts of the leaves of A. marmelos at doses of 50, 70, 90 and 100-mg/kg body wt for 14 consecutive days to male and female Wistar rats did not induce any short-term toxicity. Collectively, these data demonstrate that the extracts of the leaves of A. marmelos have a high margin of drug safety ([16]).

U.K. Das used aqueous extract of the A. marmelos as per the dose 50mg/100gm body wt resulted a significant determination in the key testicular steriogenic enzyme along with low level of plasma testosterone and relative wt of the sex organs in respect to counter without any significant alternative in general body growth ([17]).

The A. marmelos fruit pulp has been shown to posses antiprotozoal activity in chronic dysentery condition accompanied by loose stool alternately with occasional constipation. The ripe fruit used in different formulation for treatment of chronic diarrhea ([18]).

Influence of selected Indian immunostimulant herbs including Aegle marmelos against white spot syndrome virus (WSSV) infection in black tiger shrimp was studied by Citarasu T et al. Immunostimulants are the substances, which enhance the non-specific defence mechanism and provide resistance against the invading pathogenic micro-organism. In order to increase the immunity of shrimps against the WSSV, the methanolic extracts of five different herbal medicinal plants like Cyanodon dactylon, Aegle marmelos, Tinospora cordifolia, Picrorhiza kurooa and Eclipta alba were selected. The results revealed that the application of herbal immunostimulants had been effective against shrimp viral pathogenesis and they can be recommended for shrimp culture ([19]).

Screening of traditional antidiabetic medicinal plants including Aegle marmelos of Mauritius for possible alpha-amylase inhibitory effects in vitro was studied by Kotowaroo MI et al. In their investigation, seven exotic/indigenous medicinal plants of Mauritius, namely Coix lacryma-jobi (Poaceae), Aegle marmelos (Rutaceae), Artocarpus heterophyllus (Moraceae), Vangueria madagascariensis (Rubiaceae), Azadirachta indica (Meliaceae), Eriobotrya japonica (Rosaceae) and Syzigium cumini (Myrtaceae) were studied for possible effects on starch breakdown by alpha-amylase in vitro. The results showed that only Artocarpus heterophyllus significantly ($p < 0.05$) inhibited alpha-amylase activity in vitro. To confirm the observed effects, a further biochemical assay was undertaken to investigate the effects of Artocarpus heterophyllus on alpha-amylase activity using rat plasma in vitro. It was found that the aqueous leaf extract significantly ($p < 0.05$) inhibited alpha-amylase activity in rat plasma. The highest inhibitory activity (27.20 +/- 5.00%) was observed at a concentration of 1000 µg /mL. However, in both cases dose dependency was not observed. Enzyme kinetic studies using the Michaelis-Menten and Lineweaver-Burk equations were

performed to establish the type of inhibition involved. In the presence of the plant extract the maximal velocity (V_{max}) remained constant (1/150 g/L/s) whereas the Michaelis-Menten constant (K_m) increased by 5.79 g/L, indicating that the aqueous leaf extract of Artocarpus heterophyllus behaved as a competitive inhibitor. Results from the study indicated that Artocarpus heterophyllus could act as a 'starch blocker' thereby reducing post-prandial glucose peaks ([20]).

A study of hypoglycemic and antioxidant activity of Aegle marmelos in alloxan induced diabetic rats was studied by Upadhya S and other co-workers. Their study was performed to evaluate the hypoglycemic and antioxidant effect of aqueous extract of Aegle marmelos leaves (AML) on diabetic rats. Male albino rats were randomly divided into three groups: Group I: Control; Group II: Diabetic rats; and Group III: Diabetic rats administered AML. Glucose, urea and glutathione-S-transferase (GST) in plasma, glutathione (GSH) and malondi-aldehyde (MDA) levels in erythrocytes were estimated in all the groups at the end of four weeks. There was a decrease in blood glucose at the end of four weeks in-group III animals compared with group II, however it did not reach the control levels. There was an increase in erythrocyte GSH and a decrease in MDA in group III as compared to group II. The plasma GST levels were raised in diabetic rats when compared to controls. In the group III animals, there was a decrease in GST as compared to group II. Owing to hypoglycemic and antioxidant properties, AML may be useful in the long-term management of diabetes ([21]).

Radioprotective effect by oral administration of Aegle marmelos (L.) Correa in vivo was studied by Jagetia GC and Venkatesh P. The radioprotective effect of an extract of Aegle marmelos (L.) Correa (AME), family Rutaceae, was investigated in mice exposed to different doses of gamma-radiation. Mice were administered orally AME 250 mg/kg b.w. daily for 5 consecutive days before exposure to 6, 7, 8, 9, 10, or 11 Gy of gamma-radiation. The animals were monitored daily up to 30 days after irradiation for the development of symptoms of radiation sickness or death. Treatment of mice with AME before irradiation reduced the symptoms of radiation sickness and delayed death compared to the irradiated controls given sterile physiological saline (SPS). AME provided protection against both gastrointestinal and hematopoietic toxicities. Reducing the administration schedule of AME to 1 or 3 consecutive days or increasing the schedule to 7 consecutive days was not as effective as 5 consecutive days of preradiation schedule. The administration of AME after irradiation was not effective, and no survivors could be reported 30 days after irradiation. The LD50/30 was found to be 8.1 Gy for the SPS + irradiation group and 9.7 Gy for the AME + irradiation group. The oral administration of AME resulted in an increase in radiation tolerance by 1.6 Gy, and the dose reduction factor was found to be 1.2. Preradiation treatment of mice with AME caused a significant depletion in lipid peroxidation followed by a significant elevation in glutathione concentration in the liver of mice 31 days after irradiation. The drug was nontoxic up to a dose of 6000 mg/kg b.w., the highest drug dose that could be tested for acute toxicity ([22]).

Investigation on the gastroprotective and antidiarrhoeal properties of Aegle marmelos unripe fruit extract was studied by Dhuley JN. The Study was designed to verify the gastroprotective and antidiarrhoeal effects of unripe fruit extract of Aegle marmelos Corr. The gastroprotective function of this extract was evaluated in rats against gastric mucosal damage induced by hypothermic restraint stress, absolute ethanol, and indomethacin, whereas the antidiarrhoeal activity was investigated by studying the influence on gastrointestinal transit as measured by a charcoal marker and on castor oil-induced accumulation of intestinal fluid in mice and also on contractile responses evoked by acetylcholine, histamine, serotonin, and barium chloride in isolated guinea-pig ileum, the results demonstrated that pretreatment of animals with unripe fruit extract (50 and 100 mg/kg, i.p.) produces a significant inhibition of gastric lesion induced by ethanol but not those induced by restraint stress or indomethacin and suggest a probable involvement of a prostaglandin-independent mechanism of gastroprotection. At similar doses, both the intestinal transit as well as the accumulation of intestinal fluids induced by castor oil in mice was significantly inhibited by raw fruit extract. Furthermore, the extract antagonized the contractile responses evoked by different agonists on guinea-pig ileum in vitro and its inhibitory potential for the drugs are in the order of acetylcholine > histamine > serotonin > barium chloride. Taken together, these results point out a possible antidiarrhoeal effect of unripe fruit extract of A. marmelos Corr., since inhibition of intestinal motility and secretion can control clinical diarrhoea ([23]).

Hypoglycaemic effect of water extracts of Aegle marmelos fruits in streptozotocin diabetic rats was studied by

Kamalakkannan N and Prince PS. Aegle marmelos Corr. (Rutaceae) is widely used in Indian Ayurvedic medicine for the treatment of diabetes mellitus. The hypoglycaemic effect of the water extract of the fruits of Aegle marmelos was examined in streptozotocin-induced diabetic Wistar rats. Oral administration of the water extract (125 and 250 mg kg $^{-1}$ twice a day for 4 weeks resulted in significant reductions in blood glucose, plasma thiobarbituric acid reactive substances, hydroperoxides, ceruloplasmin and alpha-tocopherol and a significant elevation in plasma reduced glutathione and Vitamin C in diabetic rats. The effect of the extract at a dose of 250mgkg(-1) was more effective than glibenclamide in restoring the values of these parameters. The result of this study clearly shows the hypoglycaemic activity of the fruit extract ($_{24}$).

Comparative evaluation of hypoglycaemic activity of some Indian medicinal plants including Aegle marmelos in alloxan induced diabetic rats was studied by Kar A and other co-workers. In their experiments 30 hypoglycaemic medicinal plants (known and less known) have been selected for thorough studies from indigenous folk medicines, Ayurvedic, Unani and Siddha systems of medicines. In all the experiments with different herbal samples (vacuum dried 95% ethanolic extracts), definite blood glucose lowering effect within 2 weeks has been confirmed in alloxan diabetic albino rats. Blood glucose values are brought down close to normal fasting level using herbal samples at a dose of 250 mg/kg once, twice or thrice daily, as needed. While evaluating comparative hypoglycaemic activity of the experimental herbal samples, significant blood glucose lowering activities are observed in the 24 samples including Aegle marmelos ($_{25}$).

Aritajat S and other co-workers confirmed dominant lethal test in rats treated with some plant extracts. Their study was undertaken to investigate the toxic effect of aqueous extracts of Aegle marmelos (AM), Stevia rebaudiana (SR), Pouteria cambodiana (PC) and Clausena excavata (CE) on rats by dominant lethal test. The data of 8-week treatment suggested that none of the extracts adversely affected male body and testicular weights as well as cauda epididymal sperm counts. No notable changes in sperm morphology and motility were observed. On the other hand, sperm count in the CE group was significantly higher as compared to both control and other treatment groups. There were no abnormal changes in the number of implantation sites, number of viable fetuses and number of dead fetuses in females mated with plant extract-treated males relative to controls. Based on these results, it could be concluded that all the investigated plant extracts have no toxic effect on male rat reproduction and progeny outcome ($_{26}$).

Study of antidiarrhoeal activity of four medicinal plants including Aegle marmelos unripe fruit in castor-oil induced diarrhoea was observed by Shoba FG and Thomas M. A study was undertaken to evaluate the effect of aqueous and methanolic plant extracts of Acorus calamus rhizome, Pongamia glabra leaves, Aegle marmelos unripe fruit and Strychnos nux-vomica root bark for their antidiarrhoeal potential against castor-oil induced diarrhoea in mice. The methanolic plant extracts were more effective than aqueous plant extracts against castor-oil induced diarrhoea. The methanolic plant extracts significantly reduced induction time of diarrhoea and total weight of the faeces. The result obtained establishes the efficacy of these plant extracts as antidiarrhoeal agents ($_{27}$).

Jagetia GC et al studied effects of Aegle marmelos on the peripheral blood and small intestine of mice exposed to gamma radiation. The radioprotective effect of bael (Aegle marmelos, AME) extract was studied in Swiss albino mice against radiation-induced changes in the peripheral blood, spleen colony forming units, and intestinal mucosa. The results of study related that AME pretreatment significantly decreased lipid peroxidation accompanied by a significant elevation in the GSH concentration in the mouse intestine. The data clearly indicate that the AME significantly reduced the deleterious effect of radiation in the intestine and bone marrow of mouse and could be a useful agent in reducing the side effects of therapeutic radiation ($_{28}$).

Effect of Aegle marmelos Correa. (Bael) fruit extract on tissue antioxidants in streptozotocin diabetic rats was studied by Kamalakkannan N and Stanely Mainzen Prince P. A study was undertaken to evaluate the anti-lipid peroxidative activity of an aqueous extract of A. marmelos fruits (AMFEt) in streptozotocin diabetic rats in heart and pancreas. Oral administration of AMFEt for 30 days (125 and 250 mg kg $^{-1}$ body weight twice daily) produced a significant decrease in the elevated levels of peroxidation products, viz. thiobarbituric acid reactive substances and hydroperoxides in the tissues of diabetic rats. The depressed activities of superoxide dismutase, catalase and glutathione peroxidase and lowered glutathione content in the heart and pancreas of diabetic rats were found to increase on treatment with AMFEt. AMFEt at a dose of 250 mg kg $^{-1}$ was more

effective than glibenclamide (300 µg kg^{-1}) and both reversed all the values significantly. Thus AMFEt exhibits anti-oxidative activity in streptozotocin diabetic rats ([29]).

In vitro antiviral activity of bael (Aegle marmelos Corr.) upon human coxsackieviruses B1-B6 was studied by Badam L et al. The in-vitro antiviral activity of a series of compounds in samples extracted from various parts of the Indian holy tree, Bael (Aegle marmelos corr.).The inhibitory concentrations (IC50) for leaves (L1 and L2) stem and stem bark (S1, S2, S3 and S4) fruit (F1 and F2 micro) root and root bark (R1 and R2) and pure compound, the marmelide were 1000 µg /ml (for L1 and L2), 1000 µg/ml (for S1, S2, S3 and S4), 1000 µg/ml (for F1) and 500 µg /ml (for F2) 250 µg /ml (for R1) and 500 µg/ml (for R2) and 62.5 µg/ml for marmelide respectively by plaque inhibition assay at 96 hrs. On the other hand, the corresponding value for Ribavirin, a standard antiviral drug, was 2000 µg/ml for the same viruses at the same time period. These concentrations did not exhibit any toxicity to Vero cells, the host subtoxic concentrations were 5000 µg/ml for leaf and stem fractions 2000 µg/ml for fruit fractions 500 and 1000 µg/ml for root fractions 250 µg/ml for marmelide and 2000 µg/ml for Ribavirin. The cytotoxic concentrations were 8000 µg /ml for leaf and stem compounds 4000 mg/ml for fruit; 1000 µg /ml and 2000 µg/ml for root 500 µg/ml for marmelide and 4000 µg/ml for ribavirin at 96 hrs. These were also confirmed by trypan blue dye exclusion test and further passaging of cells. Additionally pretreatment of host cells, virus inactivation, yield reduction and effect of time of addition assays against coxsackievirus B3 suggested that marmelide was most effective as a virucidal agent besides interfering at early events of its replicative cycle like adsorption, penetration, at various steps in single cycle growth curve and effect of time of addition ([30]).

The antioxidant activity and the membrane effects of linear furanocoumarin marmesinin isolated from Aegle marmelose was evaluated by Vimal V and Devaki T during experimental myocardial injury. Isoproterenol (150 mg kg^{-1} intraperitonially twice at an interval of 24 h) caused increase in the levels of serum marker enzymes via creatinekinase (CK), creatinekinase-MB (CK-MB) isoenzyme, lactatedehydrogenase (LDH) and lactatedehydrogenase isoenzyme (LDH1). It also produced electrocardiographic changes such as increased heart rate, reduced R amplitude and ST elevation. Marmesinin at a dose of 200 mg kg^{-1} , when administered orally, demonstrated a decrease in serum enzyme levels and restored the electrocardiographic changes towards normalcy. Myocardial injury was accompanied by the disintegration of lipidperoxides and the impairment of natural scavengers. Marmesinin oral treatment for 2 days before and during isoproterenol administration decreased the effect of lipidperoxidation. It was also shown to have a membrane stabilizing action by inhibiting the release of beta-glucuronidase from the subcellular fractions. Thus, linear furanocoumarin marmesinin could have the protective effect against the damage caused by experimental myocardial injury ([31]).

The effect of Aegle marmelos fruit extract in streptozotocin diabetes: a histopathological study was done by Kamalakkannan N and Prince PS. Aegle marmelos Correa. (Bael) fruit exhibit antidiabetic, antihyperlipidaemic and antioxidant properties. This study was designed to elucidate the protective effect of an aqueous extract of Aegle marmelos fruits on the histopathology of the pancreas in streptozotocin-induced diabetic rats. Oral administration of Aegle marmelos fruit extract at doses of 125 and 250 mg/kg twice daily to diabetic rats for a period of 30 days resulted in a significant increase in body weight, weight of the pancreas and insulin levels associated with a significant decrease in fasting blood glucose levels. The fruit extract treated groups showed improved functional state of the pancreatic ss-cells and partially reversed the damage caused by streptozotocin to the pancreatic islets. The findings of our study indicate that Aegle marmelos fruit extract exhibits protective effects on the pancreas. The effects observed in the fruit extract treated animals were better those in animals treated with glibenclamide (300µg/kg) ([32]).

Effect of Aegle marmelos fruits on normal and streptozotocin-diabetic Wistar rats was studied by Kamalakkanan N and other co-workers. They would like to evaluate the antidiabetic effect of an aqueous extract of Aegle marmelos fruits (AMFEt) in diabetes. Female albino Wistar rats were randomly divided into five groups: normal (untreated), normal + AMFEt, streptozotocin (STZ)-treated, STZ-treated + AMFEt, and STZ-treated + glibenclamide. Rats were rendered diabetic by STZ (45 mg/kg) administered intraperitoneally. AMFEt (250 mg/kg) was given twice daily for 1 month. Blood glucose, plasma insulin, glycosylated hemoglobin, liver glycogen, and change in body weight were determined. Food intake and water intake were monitored daily. An oral glucose tolerance test was also performed to determine the effect of this

extract. The results show that glucose level and glycosylated hemoglobin were increased and plasma insulin and liver glycogen were decreased in diabetic rats, and that treatment with AMFEt reversed the effects of diabetes on these biochemical parameters to near-normal levels ([33]).

Evaluation of the radioprotective effect of Aegle marmelos (L.) Correa in cultured human peripheral blood lymphocytes exposed to different doses of gamma-radiation: a micronucleus study was studied by Jagetia GC et al. The radioprotective effect of a hydroalcoholic extract of Aegle marmelos (AME) was evaluated in cultured human peripheral blood lymphocytes (HPBLs) by the micronucleus assay. The optimum protective dose of the extract was selected by treating HPBLs with 1.25, 2.5, 5, 6.25, 10, 20, 40, 60, 80 and 100 µg /ml AME before exposure to 3 Gy gamma-radiation and then evaluating the micronucleus frequency in cytokinesis blocked HPBLs. Treatment of HPBLs with different doses of AME reduced the frequency of radiation-induced micronuclei significantly, with the greatest reduction in micronucleus induction being observed for 5 µg /ml AME. Therefore, this dose of AME was considered as the optimum dose for radioprotection and further studies were carried out treating the HPBLs with 5 µg/ml AME before exposure to different doses (0, 0.5, 1, 2, 3 and 4 Gy) of gamma-radiation. The irradiation of HPBLs with different doses of gamma-radiation caused a dose-dependent increase in the frequency of lymphocytes bearing one, two and multiple micronuclei, while treatment of HPBLs with 5µg/ml AME significantly reduced the frequency of lymphocytes bearing one, two and multiple micronuclei when compared with the irradiated control. The dose-response relationship for both groups was linear. To understand the mechanism of action of AME separate experiments were conducted to evaluate the free radical scavenging of OH, O_2(-), DPPH, ABTS(+) and NO in vitro. AME was found to inhibit free radicals in a dose-dependent manner up to a dose of 200 µg /ml for the majority of radicals and plateaued thereafter. Our study demonstrates that AME at 5 µg/ml protected HPBLs against radiation-induced DNA damage and genomic instability and its radioprotective activity may be by scavenging of radiation-induced free radicals and increased oxidant status ([34]).

Modulation of doxorubicin-induced genotoxicity by Aegle marmelos in mouse bone marrow: a micronucleus study was done by Venkatesh P et al. The effect of various concentrations of Aegle marmelos (AME) on the doxorubicin (DOX)-induced genotoxic effects in mice bone marrow was studied. Treatment of mice with different concentrations of DOX resulted in a dose-dependent elevation in the frequency of micronucleated polychromatic (MPCE) as well as normochromatic (MNCE) erythrocytes in mouse bone marrow. The frequencies of MPCE and MNCE increased with scoring time, and the greatest elevation for MPCE was observed at 48 hours post-DOX treatment, whereas a maximum increase in MNCE was observed at 72 hours post-DOX treatment. This increase in MPCE and MNCE was accompanied by a decline in the polychromatic erythrocytes-normochromatic erythrocytes (PCE/NCE) ratio, which showed a DOX-dose-dependent decline. Treatment of mice with 200, 250, 300, 350, and 400-mg/kg body weight of AME, orally once daily for 5 consecutive days before DOX treatment, significantly reduced the frequency of DOX-induced micronuclei accompanied by a significant elevation in the PCE/NCE ratio at all scoring times. The greatest protection against DOX-induced genotoxicity was observed at 350mg/kg AME. The protection against DOX-induced genotoxicity by AME may be due to inhibition of free radicals and increased antioxidant status ([35]).

Fruit extract of Aegle marmelos protects mice against radiation-induced lethality was studied by Jagetia GC and other co-workers. The radioprotective effect of a hydroalcoholic extracted material from the fruit of Aegle marmelos (AME) was studied in mice exposed to different doses of gamma radiation. The optimum dose for radioprotection was determined by administering 0, 5, 10, 20, 40, or 80 mg/kg body weight of AME intraperitoneally (ip) once daily, consecutively for 5 days before exposure to 10 Gy of gamma radiation. A total of 20 mg/kg of AME for 5 consecutive days before irradiation was found to afford maximum protection as evidenced by the highest number of survivors after 30 days postirradiation. Animals from all groups were monitored for 30 days postirradiation for development of symptoms of radiation sickness and mortality. Treatment of mice with AME before exposure to different doses of gamma radiation reduced the severity of symptoms of radiation sickness and mortality with all exposure doses. This was accompanied by an increase in number of survivors in the AME + irradiation group when compared with the concurrent sterile physiological saline (SPS) + irradiation group. AME pretreatment protected mice against the gastrointestinal as well as bone marrow deaths, as evidenced by the greater number of survivors on day 10 or 30, respectively. LD50/30 was found to be 8.2 Gy for the

SPS + irradiation group, while it was 8.8 Gy for AME + irradiation. The dose-reduction factor (DRF) was found to be 1.1 for AME + irradiation group. The acute toxicity study of AME showed that it was nontoxic up to a dose of 6 g/kg body weight, the highest drug dose that could be administered. Irradiation of animals resulted in a dose-dependent elevation in lipid peroxidation in liver, kidney, stomach, and intestine of mice. Conversely, GSH concentration declined in a dose-dependent manner. Treatment of animals with AME before irradiation caused a significant decrease in the lipid peroxidation accompanied by a significant elevation in the GSH concentration in liver, kidney, stomach, and intestine of mice determined at 31 days postirradiation ($_{36}$).

The effect of hydroalcoholic (80% ethanol, 20% water) extract of leaves of Aegle marmelos was examined on carcinogen-metabolizing phase-I and phase-II enzymes, antioxidant enzymes, glutathione content, lactate dehydrogenase and lipid peroxidation, using two doses of dried extract (50 and 100 mg kg $^{-1}$ daily for 14 days), in the liver of mice. The modulatory effect of the extract was also examined on extrahepatic organs (lung, kidney and fore-stomach) for effects on the activity of glutathione S-transferase, DT-diaphorase, superoxide dismutase and catalase. Extract treatment significantly increased the basal levels of acid-soluble sulphydryl (–SH) content, cytochrome P_{450}, NADPH-cytochrome P_{450} reductase, cytochrome b_5, NADH-cytochrome b_5 reductase, glutathione S-transferase, DT-diaphorase, superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase in the liver. Aegle acted as a bifunctional inducer since it induced both phase-I and phase-II enzyme systems. Both doses significantly decreased the activity of lactate dehydrogenase and formation of malondialdehyde in liver, suggesting a role in cytoprotection as well as protection against pro-oxidant-induced membrane damage. Butylated hydroxyanisole (positive control) induced almost all the antioxidative parameters measured in this study. The extract was effective in inducing glutathione S-transferase, DT-diaphorase, superoxide dismutase and catalase in lung, glutathione S-transferase, DT-diaphorase and superoxide dismutase in fore-stomach, and DT-diaphorase and superoxide dismutase in lung. These significant changes in the levels of drug-metabolizing enzymes and antioxidative profiles are strongly indicative of the chemopreventive potential of this plant, especially against chemical carcinogenesis ($_{37}$).

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