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ANTITUMOR POTENTIAL OF STANDARDIZED EXTRACT FROM *AEGLE MARMELOS* ROOT ON DALTON'S LYMPHOMA INDUCED MICE MODEL

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ABSTRACT: The antitumor property of the successive, standardized methanolic (MeOH) extract of *Aegle marmelos* roots (MAM) was investigated in Dalton's ascites lymphoma mice model. Materials and methods: Flavonoid present in MAM was quantitatively estimated using high performance thin layer chromatography. MAM (100, 300 mg/kg) was administered orally for 15 days to assess antitumor activity. The tumor was induced by DAL cell lines. The mean survival time (MST) and status of the antioxidant enzymes were analysed, 5 fluoro uracil (5FU) was used as standard drug. Results and discussion: Quercetin content in MAM was found to be as 208 mg/gm with the linearity range 200-800 ng. MAM treated tumor bearing mice exhibited significant ($p<0.05$) increase in the mean survival time. There was a significant improvement in the super oxide dismutase (SOD), catalase levels and ($p<0.01$) reduction in lipid peroxidation and nitrous oxide ($p<0.01$) levels were observed with the MAM treatment in comparison to vehicle treated tumor bearing mice.

INTRODUCTION: The search for cytotoxic agents continues to be an important topic to discover newer anticancer drugs. Among the medicinal plants, *Aegle marmelos* Correa (Rutaceae) appears to exhibit variety of therapeutic properties [1, 2]. This plant is indigenous to India, Bangladesh, Burma and Srilanka. Roots and stem bark of *Aegle marmelos* are used in Ayurvedic formulations such as Bilvadi leha, Citraka haritaki, Dasamula and Dhānvantara taila. These formulations are used to treat various ailments [3]. Previous investigations revealed that the roots exhibit antibacterial [4], antidiarrhoeal [5], antiulcer [6] and antiallergic activity. [5]

There is no report available on antitumor activity of the roots of *Aegle marmelos.* Hence, it was aimed to determine the anticancer potential of the standardized methanolic extracts of *Aegle marmelos*.

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MATERIALS AND METHODS:

Reagents, standard solutions, and materials

All solvents used were of analytical grade and obtained from Sigma Aldrich (India). Flavonoid standard quercetin was purchased from Himedia.

Plant material:

The roots of *Aegle marmelos* were collected from Udumalpet area, Tirupur district, Tamil Nadu, India. Voucher specimen was deposited at PSG College of Pharmacy, Coimbatore, Tamilnadu, India. The plant material collected along with the leaf and flower parts were authenticated by Botanical Survey of India, Coimbatore.

Preparation of the extracts: [7]

Sample of coarsely powdered roots of *Aegle marmelos* were successively extracted with solvents with increasing polarity starting from n-hexane, chloroform, ethyl acetate and methanol

using cold maceration technique. The successive extracts obtained were distilled off from the solvents and preserved in a desiccator.

Thin –layer chromatography:

TLC was performed on Silica gel $60F_{254}$ TLC plates (E Merck, Germany) with ethyl acetate - acetic acid- formic acid -water, 100:11:11:26 (v/v) as mobile phase [8] samples were applied to the plates as 5 mm wide from the bottom, by means of pressurized nitrogen gas (150 kg/cm^2) through CAMAG Linomat V fitted with a 100 µl syringe. Ascending development, with the mobile phase consisting of solvent was performed in a twin – trough glass chamber (10x10cm) obtained from CAMAG, saturated with the mobile phases for 30 minutes at room temperature (25 ± 2^o C) and relatively humidity (60 ± 5%). After the sample application, the TLC plates were developed for a distance of 8 mm. The bands were visualized in CAMAG UV cabinet at 254 nm and 366 nm.

***In vivo* pharmacological studies: [9]**

The experimental protocol received clearance from IAEC (proposal number: IAEC/204/2013). Healthy Swiss albino mice weighing (28±4) were obtained from central animal house facility of PSGIMS&R and maintained under standard conditions.

The mice were divided into five groups, each group consisting of four animals. DLA cells (1x10^{6}cells/mouse) were inoculated on day '0' and the mice were treated either with the standard drug 5 FU (20mg/kg) or MAM (100, 300 mg/kg). The treatments were started 24 hrs after inoculation. The death pattern of animals due to tumor bearing was noted and the percentage increase in life span was calculated.

Percentage increase in life span = T-C/C x100

Where "T" and "C" are mean survival of treated and control mice respectively.

Determination of antioxidant enzyme activities, reduced glutathione (GSH), and lipid peroxide (LPO) levels:

The antioxidant enzymes superoxide dismutase, catalase, reduced glutathione, lipid peroxidase and nitric oxide were estimated in haemolysate.

Estimation of superoxide dismutase (SOD) [10]

SOD activity was determined spectrophotometrically by measuring inhibition of nicotinamide adenine nucleotide (reduced) – phenazine methosulphate -nitro blue tetrazolium (NBT) reaction system. Superoxide radical involved in the NBT reduction leading to the formation of blue farmazan. The colour intensity of the chromogen was read at 560 nm using spectrophotometer. Enzyme activity was expressed as 1 Unit = 50% inhibition / minute/mg of protein.

Catalase estimation (CAT): [11]

Catalase measurement was done based on the ability of catalase to inhibit oxidation of hydrogen peroxide (H_2O_2). The change in absorbance at 240 nm was measured for 3 minutes. dy/dx for every minute for each assay was calculated and the results are expressed as CAT units of protein

CAT (U) in 100µl of sample = dy/dx × 0.0003/ 38.3956 × 10^{-6}

Reduced glutathione (GSH): [12]

Glutathione (GSH) content was estimated by using Jollow et al. (1974) method. The values were expressed in nanomoles/mg protein.

Glutathione peroxidase (GPx): [13]

GPx activity was measured according to the method described by Paglia and Valentin (1967). GPx activity was expressed in units/g Hb.

Lipid peroxidation assay (TBARS) [14]

Lipid peroxidation was evaluated by measuring the TBARS content according to the TBA test described by Ohkawa & co workers. The absorbance of the supernatant was determined at 532 nm using spectrophotometer against the blank. TBARS content were expressed in nanomoles / mg protein.

Nitric oxide assay (NO) [15]

Nitric oxide was evaluated by Green et.al (1982). The colour intensity of the chromogen was read at 540 nm.

Statistical analysis:

The antioxidant data were subjected to one-way analysis of variance (ANOVA) followed by post hoc Tukey's multiple comparison tests. p value less

than 0.05 was considered as significant. The analysis was carried out using Graph pad Prism software (Version 4.03).

RESULTS AND DISCUSSION:

The mobile phase ethyl acetate-acetic acid-formic acid-water (100:11:11:26, v/v/v/v) eluted the MAM and also the reference standard quercetin. A defined peak at Rf = 0.34 for the standard flavonoid quercetin (**Fig. 3**) was obtained. The 3D display of the densitogram and chromotogram (**Fig: 1, 2**) indicates the presence of quercetin in the MAM. The limit of detection was found to be 20 ng/spot on silica gel $60F_{254}$ and the linearity range were validated for the range of 200-800 ng (**Table 1**). Polynomial regression curve based on the peak height Versus concentration of the standard flavonoid (quercetin) with the standard deviation 0.37% and r value 0.9999 were documented (**Fig. 4**). The quantity of quercetin present in the MAM was found to be 0.208 mg/gm.

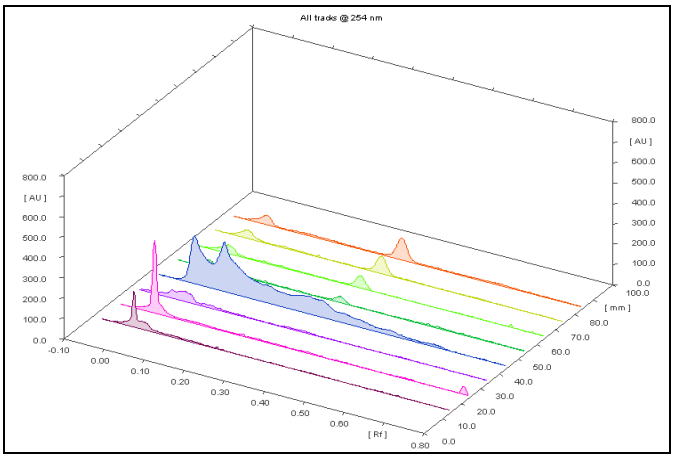

FIG: 1

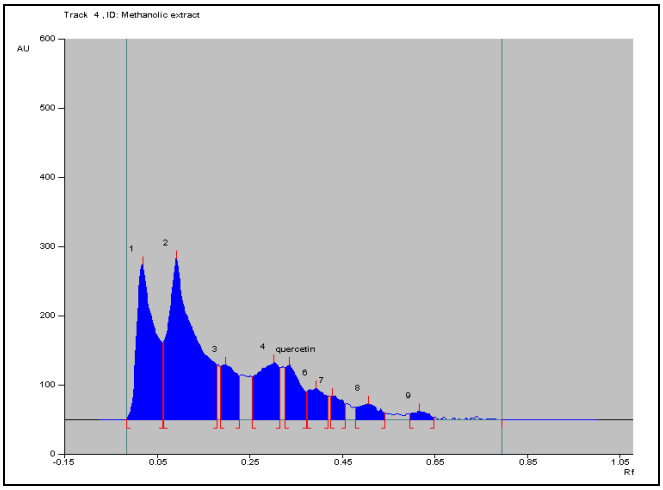

FIG: 2

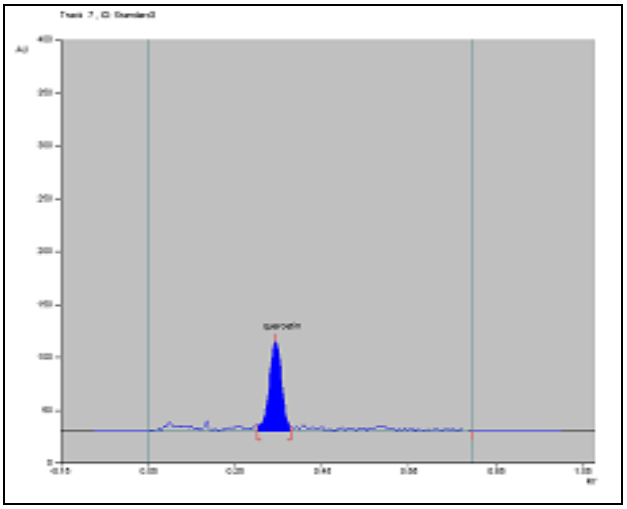

FIG: 3

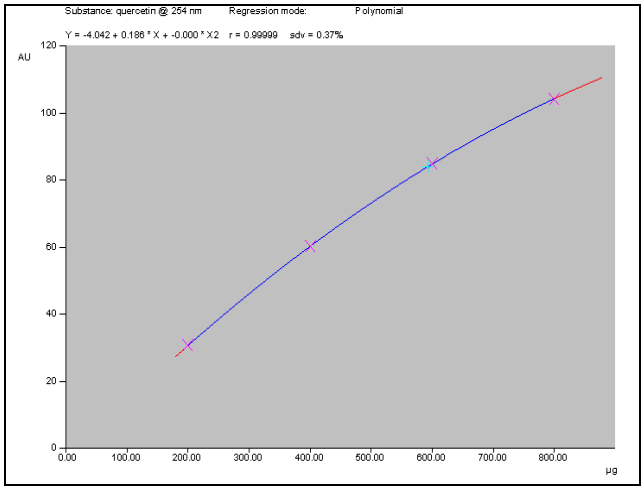

FIG: 4

TABLE 1:

S. No	Method property	Quercetin
1.	Linearity Range (ng/spot)	200-800
2.	Correlation coefficient (r)	0.9999
3.	Limit of detection(ng/spot)	20
4.	Methanolic extract (mg/gm)	0.208

In vivo pharmacological activity:

The DLA induced ascites tumor bearing mice survived for a period of 14 ± 1.2 days.

Administration of standard 5FU (20mg/kg body weight) improved the life span of the mice to 22 ± 1.4 days. The MAM treated mice (100, 300 mg/kg) had average life span of animals to 16 ± 1.7 and 19 ± 1.5 days, respectively. The percentage increase in the life span was found to be 66.66 % for 5 FU and MAM treated mice showed 28.32% and 35.89% respectively (**Table 2**)

TABLE 2:

Design of treatment	Survival Time (in days)	Increase in life span (%)
DLA + 5-FU	22 ± 1.4	66.66
DLA+ methanolic Am (100 mg)	16 ± 1.7	28.32
DLA+ methanolic Am (300 mg)	19 ± 1.5	35.89
DLA induced	14 ± 1.2	-

Antioxidant evaluation:

Super oxide dismutase (SOD):

The effect of MAM and DLA induced tumor bearing mice have indicated intracellular depletion of SOD in haemolysate (**Fig 5**). In comparison to control group, DLA induced tumor bearing mice have shown ($p<0.001$) significant decrease in intracellular SOD levels. Treatment with 5FU significantly increased the ($p<0.01$) intracellular SOD level. The MAM treated mice showed significant increase in the SOD levels with high dose (300 mg/kg b.w).

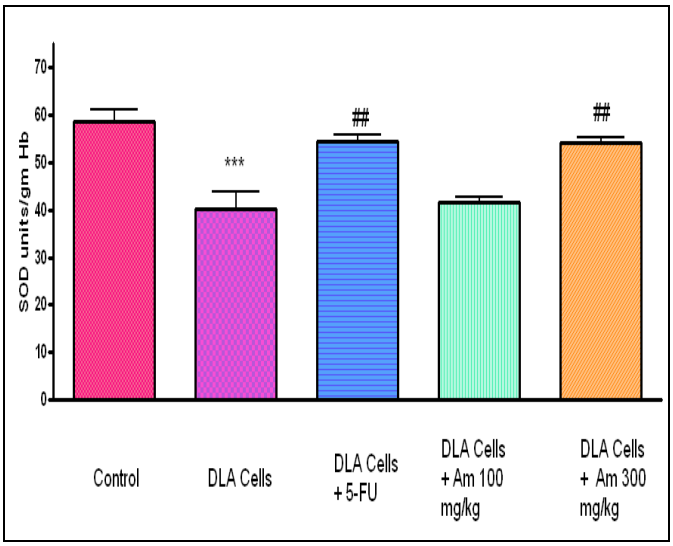

FIG: 5 EFFECT OF MAM (100, 300mg/kg) ON SOD ACTIVITY OF DLA TUMOR BEARING MICE

Glutathione peroxidase (GPx):

The reduced level of GPx was observed with the DLA tumor mice ($p<0.001$) GPx when compared to control mice. The significant increase in the GPx level ($p<0.001$) was observed with the standard drug treatment 5FU and MAM treatment ($p<0.05$, $p<0.01$) at different dose levels.

Catalase enzyme (CAT):

Evaluation of Catalase enzyme levels in DLA induced tumor bearing mice, 5FU treated and MAM were recorded in **Fig. 7**. Significant reduction in the Catalase level ($p<0.001$) were observed with the haemolysate of DLA induced tumor bearing mice group. The 5 FU treated mice showed significant reversal ($p<0.01$) of the enzyme levels. Simila.

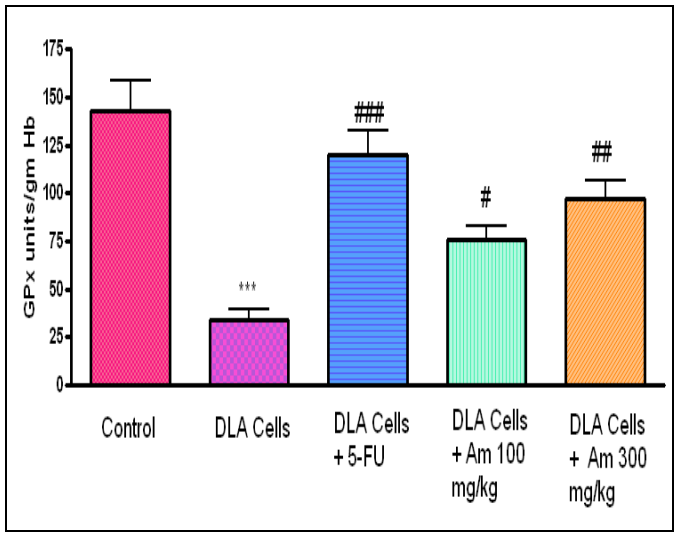

FIG.6: EFFECT OF MAM (100, 300mg/kg) ON GPx LEVEL ON DLA TUMOR BEARING MICE.

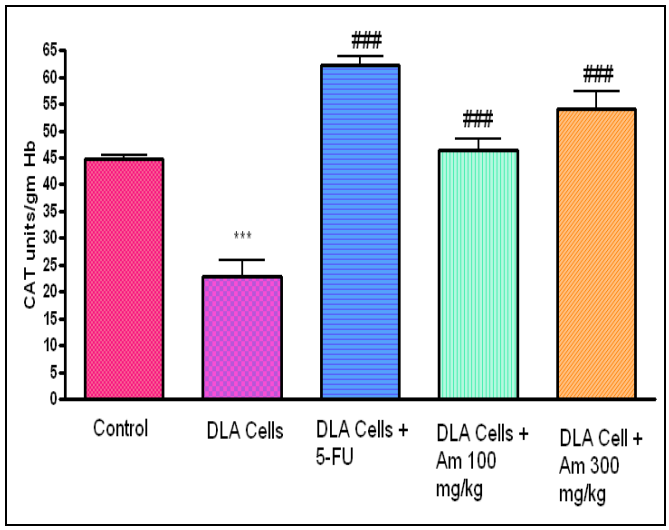

FIG.7: EFFECT OF MAM (100, 300mg/kg) ON CAT LEVEL ON DLA TUMOR BEARING MICE.

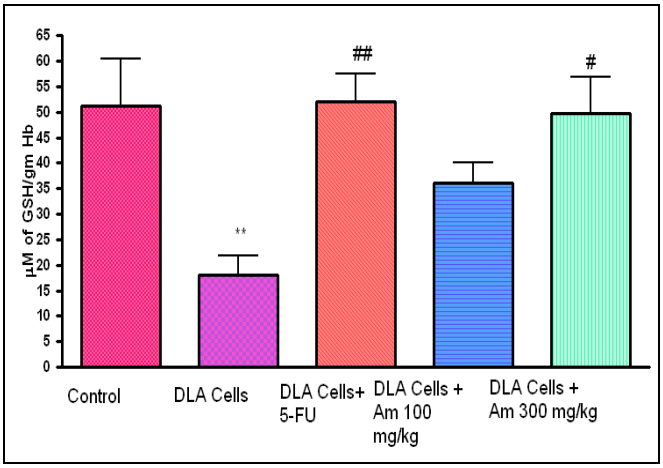

FIG.8: EFFECT OF MAM (100, 300MG/KG) ON GSH LEVEL ON DLA TUMOR BEARING MICE.

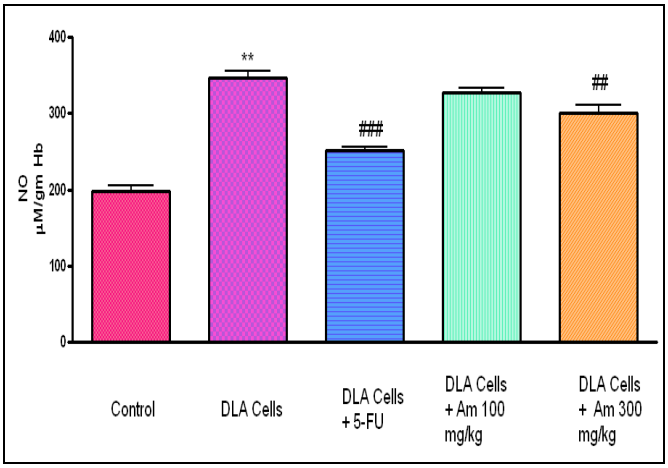

FIG.9: EFFECT OF MAM (100, 300MG/KG) ON NO LEVELS ON DLA TUMOR BEARING MICE

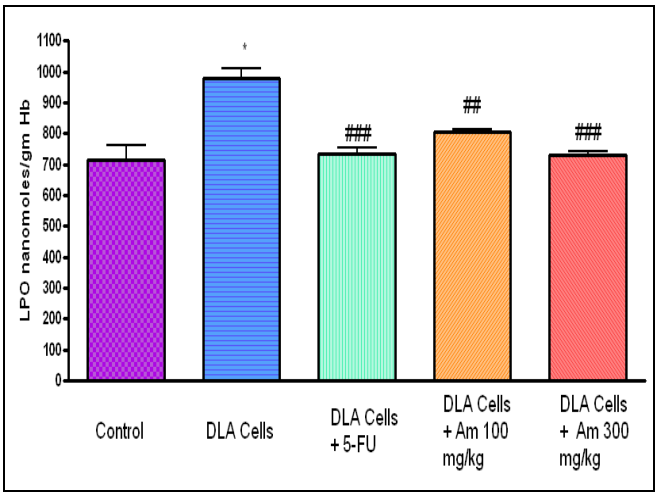

FIG.10: EFFECT OF MAM (100, 300MG/KG) ON LPO LEVEL ON DLA TUMOR BEARING MICE.

Glutathione reductase (GSH):

The GSH level in DLA induced tumor mice was significantly decreased when compared to the control mice. The standard drug 5FU significantly ($p<0.01$) increased the GSH levels. MAM (300 mg /kg) treated have shown significant elevation in GSH level (**Fig. 8**)

Lipid peroxidation and NO levels:

The release of TBARS and NO levels were significantly ($p<0.01$) high in DLA induced tumor bearing mice when compared to control group. There was a significant decrease in the lipid peroxidation level was observed with 5FU treated mice. The MAM treated mice had significantly decreased TBARS and NO level ($p<0.05$, $p<0.01$) (**Fig. 9, Fig. 10**)

CONCLUSION: In conclusion, the anti tumor potential of the flavonoidal rich extract of *Aegle marmelos* have been studied and its effect may be due to antioxidative stress mechanisms. The presence phenoxy radical scavengers in *Aegle marmelos* might also be responsible for the antitumor potential. Further bioactivity guided fractionation could be done to identify and characterize the potential leads.

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