ISSN: 2322 - 0902 (P)
ISSN: 2322 - 0910 (O)

International Journal of Ayurveda and Pharma Research

Research Article

# ANTI-OXIDANT POTENTIAL OF *BAEL* {*AEGLE MARMELOS* (L) CORR} FRUIT IN EXPERIMENTAL GASTRIC ULCERATED RAT

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**ABSTRACT**

The present investigation of unripe fruit of *A. marmelos* was done to the study their antioxidant and free radical scavenger activity in experimental gastric ulcerated animals. The rats were given EtOH extract of herbal drug *Aegle marmelos* fruit extract (50 to 200 mg/kg) and control drug ranitidine (50 mg/kg) orally, twice daily for 5 days and on day 6 of experiment, 1 hour prior to subjecting the animal to stress or necrotizing concentration of respective irritants. Rats were then subjected to CRS in the absence and presence of cytoprotective irritant or herbal drugs. The fundic part of the stomach is homogenized (5%) in ice cold 0.9% saline with a Potter-Elvehjem glass homogenizer for 30 sec. the homogenate were centrifuged at 8,000 rpm for 10 min followed by centrifugation of the supernatant at 12000 rpm for 15 min in a sigma laboratory centrifuges 3K30 and the obtained mitochondrial fraction and further this fraction was used for the estimation of LPO product malondialdehyde. Further SOD and CAT was estimated.

The study revealed that the 50% fruit extract dose dependently protected the oxidative stress and showed a tendency to decrease in volume acid-pepsin concentration and output. Reference drug rantidine a known cytoprotective agent has little effect on volume acid and pepsin concentration and acid output but showed a significant reduced in peptic output reduced LPO level and increase SOD and CAT level. The outcome indicate that the 50% ethanolic extract of A. marmelos fruit might have protected the rat tissues from ASP, PL, CRS and alcohol induced oxidative stress through anti-oxidant mechanism.

**KEYWORDS:** ASP (Aspirin), PL (Pyloric ligation), CRS (Cold resistant stress), LPO (Lipid peroxidation), SOD (Superoxide dismutase), CAT (Catalase), EtOH (Ethanol), *A. marmelos*.

## INTRODUCTION

An anti-oxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction involving the loss of electron or an increase in oxidation state[1]. A balance between free radicals and anti-oxidants is necessary for proper physiological functions in human body. If free radicals overwhelm the body's ability to regulate them. A condition known as oxidative stress ensure and it alter lipids, proteins and DNA and trigger a number of human disease[2]. Hence application of external source of anti-oxidants can assist in copying this oxidative stress.

It has been shown that OH radical play a critical role in gastric mucosal tissues damage and causes ulceration. Thus, the results of the present study on free radical mediated lipid peroxidation and alteration in circulating enzymatic anti-oxidant, CAT and SOD indicate the involvement of these enzymes in ulcer. 50% ethanolic extract of *A. marmelos* fruit has been shown to process anti-oxidant and free radical scavenging activity[3]. The extract has been shown to protect against oxidative stress induced in gastric ulcer[4]. Furthermore the *A. marmelos* has been shown to reduce cellular damage. Thus protecting liver from ethanol induced toxicity. The results of the current study indicate that this ethanolic fruit extract provides protection against PL induced, ASP induced, and ethanol induced; CRS induced oxidative stress of rat's tissue possibly[5].

## MATERIALS AND METHODS

Total 30 Sprague-Dawley rats having weight in between 150 to 180 grams were procured from the central animal house of Central Drug Research Institute Lucknow, India. These were kept in the departmental animal house at 26 ± 2°C and relative humidity 44 –56 %, light and dark cycles of 10 and 14 h respectively for 1 week before and during the experiment for acclimatization. The animals were provided with standard rodent pellet diet (Amrut, India) and the food was withdrawn 18-24 h before the experiment, though water was allowed *ad libitum*. All studies were performed in harmony with the guide for the care and use of laboratory animals, as adopted and promulgated

by the Institutional Animal Care Committee, CPCSEA, India (Reg. No. 222/2000/CPCSEA). The standard oro-gastric cannula was used for oral drug administration in experimental animals.

**Acute toxicity studies**

The adult male albino mice selected for acute toxicity study. The 50% ethanolic extract of fruits of *Aegle marmelos* were taken at various doses levels (100, 200, 400, 800, 1000, 1500, 2000 mg/kg body wt) dissolved in 1 % carboxymethyl cellulose orally to five mice per dose level. The control animals received 1 % carboxymethyl cellulose in distilled water (10 ml/kg) orally. The animals were observed continuously for two hour and then occasionally for further four hours and finally any mortality. Behavior (gross behavior, general motor activity, writhing, convulsion, response to tail pinching, pupil size, fecal output, water intake, feeding behavior, sedation *etc.*) of the animals and any other toxic symptoms also observed for 72 hours and the animals were kept under observation up to 14 days (OECD 423).

**Plan of study**

This study was a merely experimental and conducted with the goal and expressive the effect of anti-oxidant properties of *A. marmelos* on aspirin induced, cold-restraint stress induced, ethanol induced and pylorus ligation induced gastric ulcers estimation of free radicals complicated in the pathology of ulcer.

Animals are divided into five groups (six animals in each group). 50 % ethanolic extract of *A.marmelos* in dose of 50, 100 and 200 mg/kg and H2 receptor blocker ranitidine[4] in the dose of 50 mg/kg were administered orally twice daily at 10AM and 16 PM respectively for five days in various model. Control group of animals received suspension of 1 % carboxymethyl cellulose in distilled water (10 ml/kg).

Group I- Ulcer Control (1 % carboxymethyl cellulose suspension)

Group II- *A.marmelos* (50 mg/kg body wt.)

Group III- *A.marmelos* (100 mg/kg body wt.)

Group IV - *A.marmelos* (200 mg/kg body wt.)

Group V - Ranitidine (50 mg/kg body wt.)

After 5 days administration of drugs, according to their respective groups and subgroups, animals go for induction of ulcer by different ways.

**Estimation of free radical generation** [6]

The rats were given EtOH extract of herbal drug *Aegle marmelos* fruit extract (100 and 200 mg/kg), orally, daily for 3 days and on day 4 of experiment, 1 h prior to subjecting the animal to stress or necrotizing concentration of respective irritants. Rats were then subjected to CRS in the absence and presence of cytoprotective irritant or herbal drugs, and the ulcer index is calculated as described earlier. The fundic part of the stomach is homogenized (5%) in ice cold 0.9% saline with a Potter –Elvehjem glass homogenizer for 30 sec. the homogenate were centrifuged at 8,000 rpm for 10 min followed by centrifugation of the supernatant at 12,000 rpm for 15 min in a sigma laboratory centrifuges 3K30 and the obtained mitochondrial fraction.

**Measurement of Lipid peroxidation (LPO)**[7]

Lipid peroxidation product malondialdehyde (MDA) was estimated as 1.0 ml of sample was mixed with 0.2 ml 4 % (w/v) sodium dodecyl sulfate, 1.5 ml 20 % acetic acid in 0.27 M hydrochloric acid (pH 3.5) and 15 ml of 0.8 % thiobarbituric acid (TBA, pH 7.4). The mixture was heated in a hot water bath at 85 °C for 1 h. The intensity of the pink color developed was read against a reagent blank at 532 nm following centrifugation at 12,000 rpm for 10 min. Tetraethoxypropane was used as a standard.

**Superoxide dismutase (SOD)**[8]

SOD was estimated by as mixture contained sodium pyrophosphate buffer (0.052 M, pH 8.3), phenazine methasulfate (PMS, 6.2 M), Nitroblue tetrazolium (NBT, 30 M), potassium cyanide (KCN, 10 µm, pH 7.0) and 0.2 ml of sample fraction. Samples were preincubated for 5 min at $36^0$C prior to the addition of reduced nicotinamide adenine dinucleotide (NADH, 52 µm). Mixture was further incubated for 120 sec at $37^0$C in a water bath and the reaction was stopped by adding 1 ml glacial acetic acid (17.4 M). The violet color developed was extracted in 0.4 ml of n-butanol reagent blank. The activity was measured at 560 nm and the result has been expressed as unit (U) of SOD activity/mg protein. One unit of enzyme activity was defined as the enzyme concentration required inhibiting the chromogen production by 50 % in one min under the defined assay conditions.

**Catalase (CAT)**

Decomposition of $H_2O_2$ in the presence of catalase was followed at 240 nm. A 50 µm sample was added to buffered substrate (50 m M phosphate buffer, pH 7.0 containing 10 mM $H_2O_2$ ) to make total volume 3 ml and decrease in the absorbance was monitored at $37^0$C for 2.5 min at an interval of 15 sec. the activity was calculated using extinction coefficient of $H_2O_2$, 0.041/µmole/$cm^2$ at 240 nm. Results are expressed as units (U) of CAT activity/mg protein.

**Data Documentation and Statistical Analysis**

All the data are presented as mean ± SEM and one-way analysis of variance (ANOVA) and Newman-Keuls Multiple Comparison Test were applied for determining the statistical significance between different groups. A value of $P<0.05$ was considered statistically significant.

**Table 1: Effect of *A.maemelos* extract on lipid peroxidation (LPO), superoxide dismutase (SOD), and catalase (CAT) in Aspirin-induced gastric ulcers**

| Treatment | Dose (mg/kg) | LPO | SOD | CAT |
|---|---|---|---|---|
| Control | - | 0.40 ± 0.02 | 100.2 ± 9.9 | 30.9 ± 2.0 |
| Aspirin | 200 | 0.57 ± 0.02$^{x}$ | 146.6 ± 6.3$^{x}$ | 19.9 ± 1.2$^{x}$ |
| *A.marmelos* extract | 50 | 0.49 ± 0.01$^{c}$ | 128.0 ± 5.3$^{b}$ | 24.9 ± 1.8$^{a}$ |
| *A.marmelos* extract | 100 | 0.44 ± 0.01$^{c}$ | 113.4 ± 8.5$^{c}$ | 28.5 ± 2.8$^{b}$ |
| *A.marmelos* extract | 200 | 0.39 ± 0.12$^{c}$ | 135.9 ± 7.9$^{c}$ | 30.9 ± 1.6$^{b}$ |
| Ranitidine | 50 | 0.37 ± 0.04$^{c}$ | 119.9 ± 6.7$^{c}$ | 32.1 ± 2.6$^{b}$ |

Values are mean ± SEM for 6 rats, $^{x}$ P < 0.001 compared to respective control group.

$^{a}$ P < 0.05 compared to respective aspirin induced group, $^{b}$ P < 0.01 compared to respective aspirin induced group, $^{c}$ P < 0.001 compared to respective aspirin induced group.

**Table 2: Effect of *A.marmelos* extract on lipid peroxidation (LPO), superoxide dismutase (SOD), and catalase (CAT) in Cold-restraint stress –induced gastric ulcers**

| Treatment | Dose(mg/kg) | LPO | SOD | CAT |
|---|---|---|---|---|
| Control | - | 0.37 ± 0.01 | 103.9 ± 9.9 | 39.9 ± 1.8 |
| Cold-restraint stress | - | 0.83 ± 0.01$^{x}$ | 247.5 ± 5.2$^{x}$ | 20.9 ± 1.5$^{x}$ |
| *A.marmelos* extract | 50 | 0.68 ± 0.02$^{a}$ | 207.7 ± 3.8$^{b}$ | 26.5 ± 1.6$^{a}$ |
| *A.marmelos* extract | 100 | 0.51 ± 0.01$^{c}$ | 158.9 ± 2.2$^{c}$ | 31.3 ± 1.8$^{b}$ |
| *A.marmelos* extract | 200 | 0.37 ± 0.01$^{c}$ | 116.7 ± 3.0$^{c}$ | 37.9 ± 0.8$^{c}$ |
| Ranitidine | 50 | 0.34± 0.01$^{c}$ | 113.9 ± 3.0$^{c}$ | 38.9± 0.8$^{c}$ |

Values are mean ± SEM for 6 rats. $^{x}$ P < 0.001 compared to respective control group. $^{a}$ P < 0.05, compared to respective CRS-induced group. $^{b}$ P < 0.01 compared to respective CRS-induced group. $^{c}$ P < 0.001 compared to respective CRS-induced group.

**Table 3: Effect of *A.marmelos* extract on lipid peroxidation (LPO), superoxide dismutase (SOD), and catalase (CAT) in Ethanol –induced gastric ulcers**

| Treatment | Dose(mg/kg) | LPO | SOD | CAT |
|---|---|---|---|---|
| Control | - | 0.40 ± 0.02 | 105.9 ± 3.7 | 32.5 ± 2.5 |
| Ethanol | - | 0.79 ± 0.03$^{y}$ | 189.9 ± 7.2$^{x}$ | 20.1 ± 0.5$^{y}$ |
| *A.marmelos* | 50 | 0.61 ± 0.03$^{b}$ | 156.9 ± 4.8$^{a}$ | 24 ± 2.6$^{a}$ |
| *A.marmelos* | 200 | 0.46 ± 0.01$^{c}$ | 130.9 ± 3.9$^{c}$ | 29.9 ± 0.7$^{c}$ |
| *A.marmelos* | 400 | 0.39 ± 0.06$^{c}$ | 102.9 ± 3.2$^{c}$ | 31.2 ± 1.2$^{c}$ |
| Ranitidine | 50 | 0.38 ± 0.01$^{c}$ | 100.4 ± 2.8$^{c}$ | 31.6± 1.1$^{c}$ |

Values are mean ± SEM for 6 rats. $^{x}$ P < 0.01 compared to respective control group. $^{y}$ P < 0.001 compared to respective control group. $^{a}$ P < 0.05, compared to respective pylorus ligated group. $^{b}$ P < 0.01 compared to respective pylorus ligated group. $^{c}$ P < 0.001 compared to respective pylorus ligated group.

**Table 4: Effect *A.marmelos* extract on lipid peroxidation (LPO), superoxide dismutase (SOD), and catalase (CAT) in Pylorus ligation-induced gastric ulcers**

| Treatment | Dose(mg/kg) | LPO | SOD | CAT |
|---|---|---|---|---|
| Control | - | 0.53± 0.01 | 98.7 ± 9.6 | 36.1 ± 2.1 |
| Pylorus ligation | - | 0.71 ± 0.3$^{x}$ | 224.2 ± 11.2$^{x}$ | 20.3 ± 1.9$^{x}$ |
| *A.marmelos* | 50 | 0.64 ± 0.02$^{b}$ | 180.2 ± 6.1$^{b}$ | 27.7 ± 2.1 $^{a}$ |
| *A.marmelos* | 100 | 0.57 ± 0.01$^{c}$ | 146.9 ± 4.3$^{c}$ | 32.5 ± 2.3$^{b}$ |
| *A.marmelos* | 200 | 0.52 ± 0.02$^{c}$ | 120.1 ± 4.7$^{c}$ | 37.0 ± 1.9$^{b}$ |
| Ranitidine | 50 | 0.50 ± 0.02$^{c}$ | 115.4 ± 4.4$^{c}$ | 36.2 ± 1.8$^{b}$ |

Values are mean ± SEM for 6 rats. $^{x}$ P < 0.001 compared to respective control group. $^{a}$ P < 0.05, compared to respective pylorus ligated group. $^{b}$ P < 0.01 compared to respective pylorus ligated group.

**Table 5: Effect of ethanolic extract of *A.marmelos* on gastric secretion in 4 hr PL**

| Treatment | Dose (mg/kg) | Volume (ml/100g) | Acid Concentration (µEq/ml) | Acid Output (µEq/ml) | Peptic Concentration (µmol/ml) | Peptic Output (µmol/h) |
|---|---|---|---|---|---|---|
| Control | - | 2.46±0.16 | 97.9±15.4 | 284.3±38.8 | 295.2±31.4 | 729±3.89.2 |
| *A.marmelos* extract | 200 | 2.03±0.27 | 70.5±11.8 | 149±30.6 | 285.9±33.5 | 383.5±79.2 |
| Ranitidine | 50 | 2.10±0.18 | 86.0±7.9 | 181.4±29.2 | 204.7±29.5 | 453.1±69.6 |

Values are mean ± SEM of 6 rats in each group. $^{a}$P<0.05 compared to respective control group

## DISCUSSION AND RESULT

**Table No.1:** Pretreatment with *Aegle marmelos* fruit extract at dose level of 50, 100 and 200 mg/kg significantly reduced the LPO and SOD level by 0.57 ± 0.02- 0.39 ± 0.12 and 146.6 ± 6.3 – 104.9 ± 7.9, as compared to elevated level in ASP induced ulcerated gastric tissue 0.57 ± 0.02 and 146.6 ± 6.3, respectively. Level of CAT was decreased significantly in ASP-induced ulcers. AME also increased the level of CAT by 24.9 ± 1.8 – 30.9 ± 1.6, in gastric tissue as compared to ASP control 30.9 ± 2.0, respectively. Ranitidin at the dose of 50 mg/kg significantly reduced the LPO, SOD and increased CAT.

**Table No. 2:** Pretreatment with AME at dose level of 50, 100 and 200 mg/kg significantly reduced the LPO and SOD level by $0.68 \pm 0.02^{a}$ - $0.37 \pm 0.01^{c}$ and $207.9 \pm 3.8^{b}$ – $116.7 \pm 3.0^{c}$, as compared to elevated level in CRS $0.83 \pm 0.01^{x}$ and $247.6 \pm 5.2^{x}$, respectively. Level of CAT was decreased significantly in CRS-induced ulcers. AME also increased the level of CAT by $26.5 \pm 1.6^{a}$ – $37.9 \pm 0.8^{b}$, in gastric tissue as compared to CRS $20.9 \pm 1.5^{x}$, respectively. Ranitidine at the dose of 50 mg/kg significantly reduced the LPO, SOD ($0.34 \pm 0.01^{c}$ and $113.9 \pm 3.0^{c}$), and further increased CAT ($38.9 \pm 0.8^{b}$) respectively.

**Table No. 3:** Pretreatment with AME at dose level of 50, 100 and 200 mg/kg significantly reduced the LPO and SOD level by 0.79 ± 0.03 - 0.39 ± 0.06 and 156.9 ± 4.8 – 102.9 ± 3.2, as compared to elevated level in EtOH 0.60 ± 0.03 and 106 ± 3.8, respectively. Level of CAT was decreased significantly in EtOH-induced ulcers. AME also increased the level of CAT by 24 ± 2.6 – 31.2 ± 1.2, in gastric tissue as compared to EtOH 20.1± 0.5, respectively. Ranitidine at the dose of 50 mg/kg significantly reduced the LPO, SOD (0.38 ± 0.01and 100.4 ± 2.8), further increased CAT ($31.6 \pm 1.1^{)}$ respectively**.**

**Table No.4:** Pretreatment with *Aegle marmelos* fruit extract at dose level of 50, 100 and 200 mg/kg significantly reduced the LPO and SOD level by 0.64 ± 0.02 - 0.52 ± 0.02 and 180.2 ± 6.1 – 120.1 ± 4.7, as compared to elevated level in Pylorus ligation 0.71 ± 0.03 and 224.2 ± 11.2 respectively. Level of CAT was decreased significantly in Pylorus ligation-induced ulcer. AME also increased the level of CAT by 27.7 ± 2.1 – 37.0 ± 1.9 in gastric tissue as compared to Pylorus ligation 20.3 ± 1.9, respectively. Ranitidine at the dose of 50 mg/kg significantly reduced the LPO, SOD (0.50 ± 0.02 and 115.4 ± 4.4), further increased CAT (36.2 ± 1.8) respectively.

**Table No.5:** The effect of *A.marmelos* fruits EtOH extract of (200 mg/kg) when administered orally, twice daily for 5 days was studied for their effect on volume, acid and pepsin secretion in 4 hour pylorus ligation rats. *A.marmelos* fruit**s** EtOH extract showed a tendency to decrease in volume, acid-pepsin concentration and output. However, reference drug Ranitidine a known cytoprotective agent has little or no effect on volume, acid and pepsin concentration and acid output but showed a significant decrease in peptic output.

## CONCLUSION

The present investigation showed that the 50% ethanolic extract of *A. marmelos* fruit showed potent ant-oxidant activity as indicated the SOD and CAT where significantly increased and LPO levels reverted back in rat gastric mucosa.

## ACKNO WLEDGMENT

The authors are grateful to Dr. Ch.V.Rao, Principal scientist of pharmacognosy and Ethanopharmacology division of N.B.R.I., Lucknow (U.P.) for providing constant support and guidance. I am also thankful to N.B.R.I. laboratory, N.B.R.I. Library, C.D.R.I. Library, Lucknow for providing the most necessary materials and journals as reference of this research work.

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**Cite this article as:**
Singh Madhu, Prasad Kamleshwar, Shukla Amit Kumar. Anti-Oxidant Potential of Bael {Aegle Marmelos (L) Corr} Fruit in Experimental Gastric Ulcerated Rat. International Journal of Ayurveda and Pharma Research. 2016;4(1):23-26.
***Source of support: Nil, Conflict of interest: None Declared***

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