

Investigations on Hepatoprotective Activity of Leaf Extracts of *Aegle marmelos* (L.) Corr. (Rutaceae)

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Issued 04 January 2009

ABSTRACT

The present study was carried out to screen and evaluate the hepatoprotective activity of leaf extracts of *Aegle marmelos* (L.) Corr. Hepatoprotective activities of ethanolic and aqueous extracts of *A. marmelos* were examined against carbon tetrachloride induced liver damage in mice using silymarin as control. Enzyme activities of Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT) and Alkaline Phosphatase (ALP) were analyzed. Results indicate that ethanolic and aqueous leaf extracts of *A. marmelos* had moderate activity over carbon tetrachloride treatment as compared to control. Results of the present investigation confirm the traditional uses of this plant as a potential hepatoprotective agent.

KEYWORDS: *Aegle marmelos*; Hepatoprotective activity; Serum Glutamate Oxaloacetate Transaminase (SGOT); Serum Glutamate Pyruvate Transaminase (SGPT); Alkaline Phosphatase (ALP).

INTRODUCTION

Medicinal plants form the backbone of traditional system of medicine in India. Pharmacological studies have acknowledged the value of medicinal plants as potential source of bioactive compounds (Prusti et al., 2008). Phytochemicals from medicinal plants serve as lead compounds in drug discovery and design. Medicinal plants are rich source of novel drugs that forms the ingredients in traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates, bioactive principles and lead compounds in synthetic drugs (Ncube, 2008). WHO pointed out that more than 80% of world's population depends on plants to meet their primary health care needs. However, overexploitation of the selected medicinal

plant species lead to the reduction in number of plants in the wild and inclusion of their name in the red data book (Ahmedullah and Nayar 1999).

Aegle marmelos (L.) Corr., belongs to the family Rutaceae, and is popularly known as Bael tree (Eng.) (Gamble, 1935; Mathew, 1983). In Hindu mythology leaves and wood of *Aegle marmelos* are used to worship Lord Shiva. This is a sacred tree amongst the Hindus. This tree is commonly found in Hindu sacred grooves. It is considered sacrilegious to destroy it; enormous quantities of the leaves are gathered for use during ritual ceremonies. In ancient time it is frequently alluded to as an emblem of fertility (Jain and Sastry, 1979). Hindu physicians regard the unripe or half ripe fruit as astringent, digestive, and stomachic, and prescribe it for diarrhoea and dysentery (Bakhru, 1997). The ripe fruit is aromatic, cooling and is used as laxative. The root bark is used as a remedy in hypochondriasis, melancholia and palpitation of the heart. The fresh juice of the leaves is taken with honey as a laxative and febrifuge; it is used in asthmatic complaints. Addition of black pepper in anasarca is used to treat costiveness and jaundice; moreover, in external inflammations it is given to correct the supposed derangement of the humours. Small unripe fruit is consumed with fennel seeds and ginger in decoction for piles (Kamalakkannan and Prince, 2005). The fruit is used as a remedy for diarrhoea. Two tolās of bark juice is given with a cummin in milk to increase the quality of seminal fluid.

The tribals in Salem, Dharmapuri, Vellore regions, Tamilnadu, India offer leaves in the month of July/ August, to god to overcome sterility problem and subsequent year the couples are blessed with the child. Therefore, this tree is considered as an emblem of fertility. Beverages prepared with fruit pulp are used to relive body heat. Cologne is obtained by distillation from flowers. The wood is used for carving, small-scale turnery, tool and knife handles, pestles and combs, taking a fine polish. The ripen fruit, tamarind and sugar in mixture is used as laxative to overcome constipation and body heating problems (Jain and Sastry, 1979). Mature but still unripe fruits are made into jam. A firm jelly is made from the pulp alone or better still, combined with guava to modify the astringent flavor. The pulp is also pickled (Bakhru, 1997). The shell of hard fruits has been fashioned into pill- and snuff boxes, sometimes decorated with gold and silver. The gum from seeds is used as household glue and as an adhesive by jewelers. It is used to wash the silver ornamentals and shields. The fruit pulp is used as detergent for washing clothes. The fruit pulp is used as shampoo. The dried pulp is also used in local based hair cosmetics along with mehendi and amla. In the present study we have evaluated the hepatoprotective potential of *A. marmelos*.

MATERIALS AND METHODS

Collection of Plant Material

Mature leaves of *A. marmelos* were collected from Vellore, Tamilnadu, India during Apr 2008. The Flora of Presidency of Madras (Gamble, 1935) and The Flora of Tamil Nadu Carnatic (Matthew, 1983) were used for identification and authentication of the plants. Collected material was washed thoroughly in running tap water, rinsed in distilled water and shade dried in open air and grounded into powder.

Preparation of Phytochemical Extracts

The leaves were dried under shade and coarsely powdered. The powder was successively extracted using soxhlet apparatus with ethanol and water. These extracts were condensed using rotary

vacuum evaporator followed by vacuum evaporator and stored in desiccator. The powder of all the extracts was suspended in appropriate solvent systems and was subjected to further analysis.

Experimental animals

The cross breed albino mice weighing 20-25g were housed in clean propylene cages and maintained at $30\pm 2^{\circ}\text{C}$ under natural light/ dark conditions. They were fed with standard pellet diet and water was given *ad libitum*. The animals were acclimatized to laboratory conditions for 2 weeks. Animals were divided into seven groups of six mice each. The body weight of each of the animals was recorded initially. The period of experimentation was 5 days.

Carbon tetrachloride induced hepatotoxicity

Group I animals received 1% CMC in distilled water (2ml/ kg body weight) for five days with olive oil (2ml/kg body weight i.p) on second and third day. Group II animals received 1% CMC (2ml/ kg body weight) for 5 days with 1: 1 mixture of olive oil and CCl_4 (2ml/kg body weight i.p.) on 2nd and 3rd day. Group III animals served as positive control and were given silymarin (200mg/kg) for five days. Group IV and Group V animals were given ethanolic extract of AM (500mg/kg) and (600mg/kg) body weight respectively. Group VI and Group VII animals received respectively 500mg/kg and 600mg/kg body aqueous extract of AM weight. In addition to ethanolic and aqueous extracts, 1:1 mixture of olive oil and CCl_4 (2ml/kg body weight i.p.) were given to Group IV to Group VII animals on 2nd and 3rd day.

Enzyme Assay

On the fifth day, blood was collected from the retro orbital plexus of the animals and serum was allowed to coagulate at 37°C for 30 mins, and subjected to centrifugation at 2500 rpm. Serum samples were stored at $2-8^{\circ}\text{C}$ until further use. The enzyme assay was determined for Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT) (Reitman, 1957), and Alkaline Phosphatase (ALP) (modified method of Kind, 1954) using commercially available enzyme kit (Crest Biosystems, Goa, India).

RESULTS AND DISCUSSION

Levels in CCl_4 induced hepatotoxicity with respect to SGOT, SGPT and ALP in mice using aqueous and ethanolic extracts of *A. marmelos* is given in Table 1. As compared to the control (61.3 ± 5.2 , 41.3 ± 2.9 , 5.7 ± 0.4), CCl_4 treated animals exhibited significantly higher levels of enzyme activities ($142 \pm 11.5^{\text{a}}$, $100 \pm 8.7^{\text{a}}$, $12.0 \pm 0.2^{\text{a}}$) in serum. Ethanolic extract (500mg/kg) ($99.3 \pm 7.8^{\text{b}}$, $53.3 \pm 2.9^{\text{b}}$, $6.0 \pm 0.3^{\text{a}}$) was found to have moderate activity as compared to silymarin $54 \pm 5.7^{\text{a}}$, $35.3 \pm 1.7^{\text{a}}$ and $4.4 \pm 0.3^{\text{a}}$ for SGOT, SGPT and ALP respectively. Analysis of SGOT, SGPT and ALP levels in carbon tetrachloride induced hepatotoxicity in mice against aqueous and ethanolic extracts of *A. marmelos* revealed that ethanolic and aqueous extracts were moderately effective when compared to silymarin treatment. Further, the $P < 0.01$ values in the case of AmEE (500 mg/kg) was significant indicating that ethanolic extracts of *A. marmelos* holds a potential to be used as an hepatoprotective

agent.

Plants are known to have beneficial therapeutic effects documented in Traditional Indian System of Medicine. Much work has been done on ethnomedicinal plants in India. Interest in a large number of traditional natural products has increased. It has been suggested that phytochemical extracts from *A. marmelos*, antidiabetic, antitumoral and antimicrobial agents (Rajsekaran et al., 2008). Results indicate that ethanolic extracts of *A. marmelos* holds a potential to be used as hepatoprotective agent. Further, this investigation acknowledges the ethnobotanical uses and hepatoprotective nature of *A. marmelos*.

ACKNOWLEDGEMENTS

The authors are thankful to VIT Management for their constant support and encouragements. Thanks are due to Prof. Lazar Mathew for his valuable comments and suggestions to carry out this research successfully.

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Table 1. Analysis of hepatoprotective activity of leaf extracts of *Aegle marmelos*.

| Group | Treatment | SGOT (U/ml) | SGPT (U/ml) | ALP (KA units) |
|-------|------------------|----------------|----------------|-------------------|
| I | Control (1% CMC) | 61.3 ± 5.2 | 41.3 ± 2.9 | 5.7 ± 0.4 |

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|-----|--|--------------------------|-------------------------|-------------------------|
| II | CCl ₄ treatment | 142 ± 11.5 ^a | 100 ± 8.7 ^a | 12.0 ± 0.2 ^a |
| III | Silymarin (200 mg/kg) + CCl ₄ | 54 ± 5.7 ^a | 35.3 ± 1.7 ^a | 4.4 ± 0.3 ^a |
| IV | AmEE (500 mg/kg) + CCl ₄ | 99.3 ± 7.8 ^b | 53.3 ± 2.9 ^b | 6.0 ± 0.3 ^a |
| V | AmEE (600 mg/kg) + CCl ₄ | 115.3 ± 8.5 ^c | 80 ± 4.1 ^c | 9.8 ± 0.2 ^c |
| VI | AmAE (500 mg/kg) + CCl ₄ | 120.2 ± 6.1 ^c | 85.6 ± 4.7 ^c | 9.6 ± 0.4 ^b |
| VII | AmAE (600 mg/kg) + CCl ₄ | 111.3 ± 6.3 ^c | 82 ± 5.2 ^c | 9.0 ± 0.3 ^b |

| | | | |
|-----------|--------------------------------|-----------|---------------------------|
| Group I | Control | Group V | AmEE (600 mg/ kg) treated |
| Group II | CCl ₄ treated | Group VI | AmAE (500 mg/ kg) treated |
| Group III | Silymarin (200 mg/ kg) treated | Group VII | AmAE (600 mg/ kg) treated |
| Group IV | AmEE (500 mg/ kg) treated | | |

Values are expressed in Mean ± SE for three animals in each group; ^aP<0.001; ^bP<0.01; ^cP<0.1.