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Studies on Hepatoprotective Properties of Leaf Extracts of *Azadirachta indica* A. Juss (Meliaceae)

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ABSTRACT

The present study was carried out to evaluate the hepatoprotective role of leaf extracts of *Azadirachta indica* A. Juss. Hepatoprotective activities of ethanolic and aqueous extracts of *A. indica* 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. Phytochemical leaf extracts of *A. indica* exhibited significant hepatoprotective activity. Ethanolic and aqueous leaf extracts of *A. indica* exhibited moderate activity over carbon tetrachloride treated animals. Results confirm the traditional - ethnomedicinal use of *A. indica* as a potential source of hepatoprotective agent.

KEYWORDS

Azadirachta indica; Hepatoprotective activity; Serum Glutamate Oxaloacetate Transaminase (SGOT); Serum Glutamate Pyruvate Transaminase (SGPT); Alkaline Phosphatase (ALP) Carbon Tetrachloride (CCl_4)

INTRODUCTION

Medicinal plants are part and parcel of human, since the dawn of civilization. In India they form the backbone of several indigenous traditional systems of medicine. In recent times, phytochemicals from medicinal plants serve as lead compounds in drug discovery and design. Pharmacological studies have acknowledged the value of medicinal plants as potential source of bioactive compounds (Prusti *et al.,* 2008). 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). In recent years, haphazard use of synthetic drugs has been reported to result in side effects that hamper the process of treatment. This drives the need to screen medicinal plants for novel bioactive compounds as plant based drugs are biodegradable, safe and have fewer side effects (Prusti *et al.*, 2008).

Neem (*Azadirachta indica* A. Juss) is perhaps the most commonly used traditional medicinal plant of India. In India, Neem is known as "Divine Tree", "Heal All", "the village pharmacy", because of its healing versatility, and it has been used by various traditional means of medicine due to its multiple medicinal properties. Almost all parts of the plant are endowed with medicinal property. During the past few decades, apart from studies on chemical properties of Neem compounds, considerable progress has been made in evaluating biological activity of Neem compounds for medical applications (Puri, 1999). In the modern era, Neem is considered as a store house of natural compounds that can potentially be exploited in the development of drugs against infectious diseases and systemic disorders. Neem plant has been reported to be endowed with biochemical compounds with wide range of biological activities and medicinal properties (Biswas *et al.,* 2002).

Azadirachta indica A. Juss (syn. *Melia azadirachta*) is well known in India and its neighboring countries as one of the most versatile medicinal plants having a wide spectrum of biological activity. *A. indica* and *M. azedarach* are two closely related species of Meliaceae. The former is popularly known as Indian Neem (margosa tree) or Indian lilac, and the latter as the Persian lilac (Parrotta and Chaturvedi, 1994; Biswas *et al.*, 2002). Neem is an evergreen tree, cultivated in various parts of the Indian subcontinent. Every part of the tree has been used as traditional medicine for household remedy against various human ailments, from antiquity. Several pharmacological activities and medicinal applications of various parts of Neem have been documented in the ancient literature.

Chemical investigation on Neem compounds have extensively been undertaken in the middle of 20th century. Since, the isolation of nimbin, (a bitter compound from Neem oil), in 1942 on more than 145 compounds have been isolated from different parts of Neem. These compounds can be classified as isoprenoids and others. Isoprenoids include diterpenoids and triterpenoids containing protomeliacins, limonoids, azadirone and its derivatives, gedunin and its derivatives, vilasinin type and its derivatives and Csecomeliacins such as nimbin, salanin and azadirachtin. The non-isoprenoids include proteins (amino acids) and carbohydrates (polysaccharides), sulphurous compounds, polyphenolics such as flavonoids and their glycosides, dihydrochalcone, coumarin and tannins, aliphatic compounds, etc. Biological activities and medicinal properties of Neem compounds have been extensively reviewed by (Biswas *et al.*, 2002).

Biological activities of various Neem compounds have been reported with crude extracts from leaf,

bark, root, seed and oil (SaiRam *et al.*, 2002; Thakurta *et al.*, 2007; Rajasekaran *et al.*, 2008). Neem has been extensively used in Ayurveda, Unani, Homoeopathic and Siddha medicine and has become a cynosure of modern medicine (Varma, 1976). However, only crude extract of different parts of Neem has been used as traditional medicine for the treatment of various diseases. For instance, inhibitory potential of Neem leaves on Dengue virus type-2 replication has been shown by Parida *et al.*, (2002). The aqueous extract of Neem leaf was found to offer protection against paracetamol induced liver necrosis in rats. The elevated levels of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma glutamyl transpeptidase (GGT) indicative of liver damage were found to be significantly reduced on administration of the Neem leaf aqueous extract (Bhanwra et al., 2000). In the present study we have evaluated the hepatoprotective role of *A. indica*.

MATERIALS AND METHODS

Collection of Plant Material

Mature leaves of *A. indica* 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 desiccators. 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±2^{o}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) 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) 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) 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 37^0C for 30 min, and subjected to centrifugation at 2500 rpm. Serum samples were stored at 2-8^0C 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. indica* 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 ± 11.5^a, 100 ± 8.7^a, 12.0 ± 0.2^a) in serum. Ethanolic extract (500 mg/kg) (91.3 ± 5.8^c, 64.0 ± 2.3^c, 8.9 ± 0.5^c) and AEE (600 mg/kg + CCl_4) (90. 6 ± 5.4^c, 62.3 ± 1.9^c, 8.7 ± 0.4^c) were found to have moderate activity as compared to silymarin (200 mg/kg + CCl_4) 54 ± 5.7^a, 35.3 ± 1.7^a and 4.4 ± 0.3^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. indica* revealed that ethanolic and aqueous extracts were moderately effective when compared to silymarin treatment. Further, the $P<0.01$ values in the case of AEE (500 mg/kg) was significant indicating that ethanolic extracts of *A. indica* 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. Off late, interest in a large number of traditional natural products has increased. Much work has been done on Neem in India. It has been suggested that phytochemical extracts from *A. indica*, can be used as potential source for antidiabetic, antitumoral and antimicrobial compounds (Rajasekaran *et al.*, 2008). The results of the present study would certainly help to ascertain the potency of the crude extracts of Neem as potential source of natural hepatoprotective agents. Results indicate that ethanolic extracts of *A. indica* holds an impending to be used as a hepatoprotective agent. However, further research is needed to identify individual components responsible for hepatoprotective activity and develop their application for food and pharmaceutical industries. Further, the present study acknowledges the ethnobotanical uses and hepatoprotective nature of *A. indica.*

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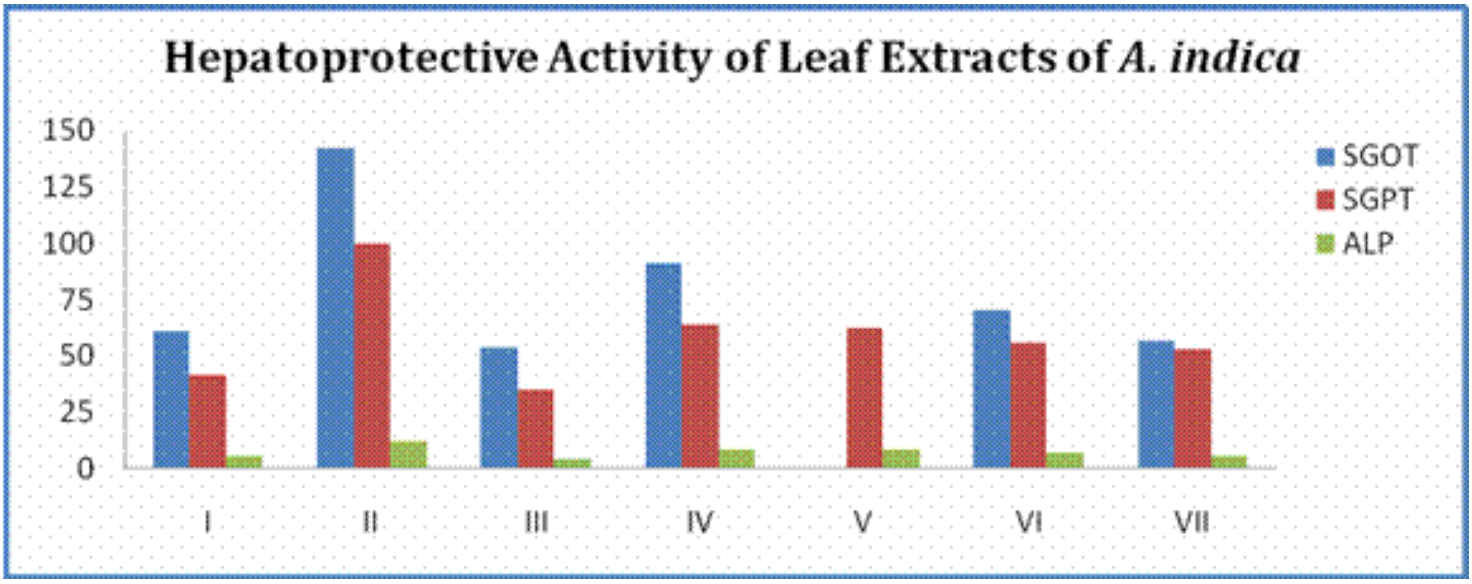
Table 1. Analysis of hepatoprotective activity of leaf extracts of *A. indica.*

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
II	CCl_4 treatment	142.0 ± 9.5[a]	100.0 ± 8.7[a]	12.0 ± 0.2[a]
III	Silymarin (200 mg/ kg) + CCl_4	54.0 ± 5.7[a]	35.3 ± 1.7[a]	4.4 ± 0.3[a]
IV	AEE (500 mg/kg) + CCl_4	91.3± 5.8[c]	64.0± 2.3[c]	8.9 ± 0.5[c]
V	AEE (600 mg/kg) + CCl_4	90. 6 ± 5.4[c]	62.3 ± 1.9[c]	8.7 ± 0.4[c]
VI	AAE (500 mg/kg) + CCl_4	70.0 ± 3.0[c]	56.0 ± 1.1[c]	7.3 ± 0.1[b]
VII	AAE (600 mg/kg) + CCl_4	56.6± 6.5[c]	53.3±2.9[c]	5.5 ± 0.3[b]

Values are expressed in Mean ± SE for three animals in each group; [a]P<0.001; [b]P<0.01; [c]P<0.1.

Group I Control
Group II CCl_4 treated
Group III Silymarin (200 mg/ kg) treated
Group IV AEE (500 mg/ kg) treated
Group V AEE (600 mg/ kg) treated
Group VI AAE (500 mg/ kg) treated
Group VII AAE (600 mg/ kg) treated

Fig. 1. Hepatoprotective activity of leaf extracts of *Azadirachta indica.*



I Control

II	CCl_4 treated	V	AEE (600 mg/ kg) treated
III	Silymarin (200 mg/ kg) treated	VI	AAE (500 mg/ kg) treated
IV	AEE (500 mg/ kg) treated	VII	AAE (600 mg/ kg) treated