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Hepatoprotective Activity Of Aqueous Extract Of Roots Of Anisomeles Malabarica Against Paracetamol Induced Intoxication In Rats.

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ABSTRACT

Keywords:

Hepatoprotective,
Anisomeles
Malabarica,
Aqueous Extract,
Roots, Rats.

This study has been designed to find out hepatoprotective activity of aqueous extract of roots of Anisomeles Malabarica (AERAM) against paracetamol induced hepatotoxicity in albino rats. The three doses of (AERAM) i.e., low (100 mg/kg), medium (200 mg/kg) and high (400 mg/kg) were selected to find out hepatoprotective activity in hepatotoxic rats. The paracetamol (2g/kg p.o.) and Silymarin (100 mg/kg) were used as toxicant and standards in this study. The hepatotoxicity was induced by administration of paracetamol in rats. The biochemical parameters (AST, ALT, ALP and total Bilirubin) and physical parameters (liver weight and volume) were estimated. In this study AERAM has exhibited a significant hepatoprotection activity by controlling normal levels of biochemical and physical parameters.

1. Introduction

Modern food habits, over dose of medications, exposure of pollutants besides and other factors have become to most serious diseases including liver failure¹. Liver is the largest gland which plays crucial role in the process of digestion, intermediary metabolism and detoxification of toxic products. The liver failure such as hepatitis & cirrhosis occurs due to environmental pollutants and drugs². A raise of asymptomatic elevation of liver enzymes leads to failure of hepatic tissue due to toxic dose of drugs which is highly variable. Paracetamol is well known as Acetaminophen for antipyretic & Analgesic activities but in excessive dose produces serious nephrotoxicity & hepatotoxicity³. Cytochrome P450 enzymes metabolize and convert Paracetamol to toxic metabolite NAPQI (N-acetyl-p-benzoquinoneimine) which causes oxidative stress and depletion of glutathione⁴. The toxic metabolite NAPQI which is left over after exhaustion of glutathione produces necrosis of centrilobular hepatocytes, ^{5, 6} which alters

both the cytosolic and the mitochondrial compartments. If GSH is exhausted, the remaining NAPQI formed will react with cellular proteins which are alternative targets (Jollow et al., 1973)⁷. Number of deaths was reported due to two major hepatic diseases like Jaundice and hepatitis. N-Acetylcysteine (NAC) is an antidote for the prevention of hepatotoxicity produced by paracetamol but some cases do not respond and adverse anaphylactoid reactions were reported (Mant et al., 1984)⁸ & (Tripathi, 2008)⁹.

The allopathic medicines against hepatotoxicity are may be useless but causes serious unwanted effects which made the thrust to explore medicinal plants and found herbal products in the treatment of liver diseases. The natural phenolic compounds of medicinal plants are well known anti-oxidant molecules to subsides the released free radicals and finally protect the living beings from different type of disorders.

A. malabarica is an aromatic, perennial herb, densely pubescent, 1.2-2.0 meter in height (figure 1). Leaves are very thick, opposite, simple, oblong-lanceolate, aromatic, acute, pale above, white below, crenate-serrate, and woolly; flowers purple, in dense whorls of more or less interrupted spikes; fruits nutlets, bearing ellipsoid and compressed seeds (Joshi, 2000; Singh et al., 2003)^{10& 11}.

The common vernacular names of the plant are Peimiratti, Malabar catmint, Bhutan kusham and Peyameratti. The taxonomic classification of *A. malabarica* is as follows:

Division Spermatophyta

Sub-division Angiospermae

Class Dicotyledone

Sub-class Gamopetalae

Series Bicarpellatae

Order Lamiales

Family Labiales or Lamiaceae

Genus *Anisomeles*

Species *Malabarica* R.Br.

Botanical name *Anisomeles malabarica*

As per previous phytochemical investigation of *A. malabarica* revealed the presence of anisomelic acid, anisomelolide, 2-acetoxymalabaric acid, anisomethyl acetate anisomelin, betulinic acid, β -sitosterol, Citral, geranic acid, malabaric acid, ovatodiolide, and triterpenebetulinic acid (Guha Bakshi et al., 1999; Choudhary et al., 2001).^{12 & 13}

A. malabarica is one of the medicinal plants, prominent as a folkloric medicine to control anorexia, fevers, swellings, amentia and rheumatism. The plant *A. malabarica* is potential to control intermittent fever, curing wounds and colic dyspepsia (Jeyachandran et al., 2007)¹⁴. The crude extract of medicinal plants used as medicaments in some cases. The bioactive molecules & their lead structures of medicinal plants and modified for enhanced activity. Some naturally occurring botanical compounds contain a broad range of chemical active ingredients which can intervene in all biological processes of the mosquito, thus interrupt its life cycle and dispersal and reduce harms to humans and animals.

Many medicinal plants were tested for their pesticide and repellent potential, as crude material, essential oils or individual active ingredients (Ramaraj, 2005)¹⁵. Consequently, this paper highlight the above, provides an overview of the classes of molecules present in plants and gives some examples of the types of molecules and secondary metabolites that have led to the development of these pharmacologically active extracts. Therefore, this study also presents some data on the significance of *A.malabarica* against malarial vector (*Anopheles stephensi*). In addition, the medicinal values of *A.malabarica* plant details were shortly demonstrated.

2. EXPERIMENTAL SECTION

Preparation of Aqueous Extract: The roots of *A.malabarica* were collected from forest area of Tirupathi, Andhra Pradesh in the month of June-July and dried under sunshade and authenticated by Dr K Madhava Chetty, Asst. Prof., Dept. of Botany, Sri Venkateswara University, Tirupathi (Voucher No.1891). The roots of

A.malabarica were cleaned with water to wipe away the adhering dirt including sand, dried and powdered under shade. The roots of the selected was made coarse powder and extracted with chloroform water by maceration technique under reduced pressure and the extract, concentrated form was obtained by using rotary evaporator. AERAM was in dark brown colored solid mass and the yield was calculated as 09.13 % w/w (Harborne JB 1973)¹⁶.

Preliminary Phytochemical Investigation: The aqueous extract was subjected for phytochemical investigation according to standard procedure (Kokate CK 1994)¹⁷.

Acute Toxicity Studies: The acute toxicity studies were conducted for extract in mice at limit test dose of 2000 mg/kg according to OECD 423 guidelines. The mice were fasted and administered the plant extract as per the procedure and observed for mortality for 24 hrs and found no death. (Kandelwal KR 2000, Bugar GT & Miller CL 1989)^{18,19}

Experiment: The albino rats (150-250g) were selected randomly and separated into 6 groups, each of six animals. The standard conditions were maintained (Room temperature at $25 \pm 20^\circ\text{C}$, 12 h light/dark and free accessible to food with water up to 2 weeks before start of the experiment to accustom to laboratory conditions. The animals were fasted for 18 h and water allowed ad libitum prior to experiment. (Shenoy AK, 2002)²⁰

Group A: Received vehicle for 10 days.

Group B: Received paracetamol (2g/kg) for first 3 days and vehicle for from 4th day to 10th day.

Group C: Received paracetamol (2g/kg) for first 3 days and Silymarin (100 mg/kg) from day 4 to 10th day.

Group D: Received paracetamol (2g/kg) for first 3 days and AERAM 100 mg/kg from day 4 to 10th day.

Group E: Received paracetamol (2g/kg) for first 3 days and AERAM 200 mg/kg from day 4 to 10th day.

Group F: Received paracetamol (2g/kg) for first 3 days and AERAM 400 mg/kg from day 4 to 10th day.

Estimation of Biochemical Parameters: The blood samples were collected from retro-orbital plexus and centrifuged. The serum was analyzed for biochemical parameters such as AST, ALT, ALP and total bilirubin and recorded for its sleeping time. Then the animals were sacrificed for wet & dry liver weights.

Serum marker enzymes of liver function: Serum was separated by centrifugation at 3000 rpm at 4°C for 10min and used for measurement of various biochemical markers like aspartate and alanine aminotransferase (AST and ALT) activities, alkaline phosphatase (ALP) activity and total bilirubin using commercially available kits. (Crest Bio Systems, A division of Coral Clinical Systems, Verna, Goa-403 722, India)

Barbiturate Sleeping Time: After collection of blood samples the rats were administered with thiopental sodium (40 mg/kg) to estimate sleeping time of rats (Brauer RW, Root MA, 1946; Fujimoto JM, 1960 & Vogel G, 1977)^{27, 28, 29}.

Statistical Analysis: All the experimental data was subjected to one-way analysis of variance (ANOVA) followed by Dunnett's 't' test and $P < 0.05^*$, $P < 0.01^{**}$, $P < 0.001^{***}$, were considered as statistically significant.

Table1. Effect of AERAM on Biochemical & Physical Parameters in albino rats

Treatment	Biochemical parameters				Physical parameters	
	AST (U/L)	ALT (U/L)	ALP (U/L)	T.BIL (mg/dl)	Liv. Wt. (g/100g)	Liv. Vol. (ml/100)
Group A	88.14±0.95	53.88±1.23	124.04±1.32	0.43±0.00	4.50±0.04	4.98±0.06
Group B	244.14±1.37**	145.97±1.29	321.55±1.57**	3.45±0.11**	7.42±0.05**	8.18±0.13**
Group C	110.60±0.56**	57.52±1.57**	137.93±0.58**	0.67±0.02**	5.36±0.08**	5.4±0.05**
Group D	207.25±2.46	141.78±1.33	291.88±1.66	2.91±0.01	6.38±0.15	7.91±0.10
Group E	171.95±1.97	82.55±1.46**	226.64±1.33	2.48±0.05**	5.76±0.05**	7.05±0.07
Group G	133.51±1.10**	65.66±0.88**	186.14±0.95**	1.06±0.02**	5.16±0.02**	6.26±0.06**

Table2. Effect of AERAM on sleeping time in rats

Group	Time in min
Normal	54.28±1.31
Toxicant	271.02±1.41**
Sylimarin	56.18±0.99**
AERAM 100 mg/kg	218.37±1.58
AERAM 200 mg/kg	90.27±1.19**
AERAM 400 mg/kg	59.37±0.98**

n=6 Values are expressed as mean± SEM. Significant at $P < 0.05^*$, $P < 0.01^{**}$, $P < 0.001^{***}$

3. RESULTS

As per the phytochemical instigation, the plant extract contains phenolic compounds, flavonoids, tannins, glycosides, carbohydrates and saponins.

The results of hepatoprotective activity of AERAM against paracetamol intoxicated rats are shown in Table.1. The toxicant (Paracetamol) treated rats has shown biochemical parameters of ALT (145.97 ± 1.29 U/L), AST (244.14 ± 1.37 U/L), ALP (321.55 ± 1.57 U/L) and bilirubin (3.45 ± 0.11 mg/dl) and physical parameters such as liver weight (7.42 ± 0.05 g/100g) and liver volume (8.18 ± 0.13 g/100g) were significantly increased when compared to normal group.

The elevated biochemical parameters of ALT (65.66 ± 0.88 U/L), AST (133.51 ± 1.10 U/L), ALP (186.14 U/L), bilirubin ($2.06 \pm 0.02 \pm 0.01$ mg/dl) and physical parameters of liver weight (5.16 ± 0.02 g/100g) and liver volume (6.26 g/100g) were significantly decreased against paracetamol intoxicated rats after treatment with AERAM. The plant extract has exhibited significant & promised hepatoprotective activity.

The toxicant (Paracetamol) received rats has shown a prolonged thiopental sodium induced sleeping time (271.02 ± 1.41 min) when compared with normal (54.28 ± 1.31 min) and it has been reversed with silymarin (56.18 ± 0.99 min) and AERAM treated groups (59.37 ± 0.98 min)

4. DISCUSSION

The preliminary phytochemical analysis confirmed the presence of glycosides, carbohydrates and phenolic compounds such as flavonoids, Tannins in the extract. Paracetamol bio-transformed in the hepatic cells by cytochrome P-450 into highly reactive toxin, N-acetyl-P-benzoquinoneimine (NAPQI)²¹ and depletion of glutathione is produced due to excessive release of NAPQI. In-addition, NAPQI binds to cellular macromolecules covalently and net result is cell damage. Paracetamol is well known to be used as an analgesic & antipyretic agent which causes hepatic necrosis at toxic doses. Paracetamol induced hepatotoxicity is estimated by the induction of cytochrome & depletion of glutathione.

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The developed countries are also confirmed one of the leading liver failures is due to acute paracetamol poisoning. Paracetamol at the toxic levels induces hepatic necrosis which can be fatal. AST exists mainly in mitochondria of hepatocytes and ALT more particularly present in liver, hence latter one is more specific for detecting liver injury. The bilirubin and ALP levels are associated with liver cell injury. The AST, ALT & ALP activity and bilirubin level are largely used as common biochemical markers to evaluate liver injury.^{22, 23}

Hepatocytes damage due to hepatotoxicants leads to leakage of enzymes such as AST, ALT and ALP. ALT is the specific enzyme for liver and important enzyme for detection of liver damage. The increased serum enzyme and bilirubin levels as these biochemicals are released into blood circulation due to damaged structural integrity of hepatocytes, an indication of liver toxicity^{24, 25}. The serum levels of AST, ALT and ALP return to normal with the treatment of hepatic parenchyma and the regeneration of hepatocytes²⁶.

The estimation of thiopental sodium sleeping period is the measurement of activity of hepatic enzymes (CYP's). The free radicals of xenobiotics produce damage to components of cell including CYP's. During destruction of hepatic CYP's thiopental sodium induced sleeping time is prolonged which is the fair indication of hepatic destruction. The hepatoprotective drugs prevent damage of CYP's and decreases prolongation sleeping time of thiopental sodium. The AERAM has reversed thiopental sodium induced sleeping time by protecting hepatic CYP 420 family. The time of lost reflex is significantly prolonged by hepatic damage which indicates functional status of enzymes, responsible for drug metabolism in liver (Brauer RW, Root MA, 1946; Fujimoto JM, 1960 & Vogel G, 1977)^{27, 28, 29}. Barbiturates are exclusively metabolized by enzymes of liver, therefore thiopental sodium, a short acting barbiturate shows intensified sleeping activity during hepatotoxicity condition. If liver damage is produced due to drug toxicity, the metabolizing capacity of liver per unit time is reduced, thus sleeping time of thiopental sodium is augmented in animals.

5. CONCLUSION

The AERAM exhibited a dose dependent activity in liver protection against Paracetamol induced hepatotoxicity which is more significant effect at a dose of 400 mg/kg. The liver protection against Paracetamol induced hepatotoxicity may be due to the presence of phytoconstituents like phenolic and flavonoids that are present in the AERAM and further these chemical constituents are already reported for their hepatoprotective activities.

Anisomeles Malabarica

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Effect of aqueous extract of roots of *A.malabarica* on AST levels against Paracetamol induced intoxication in Rats.

Effect of aqueous extract of roots of *A.malabarica* on ALT levels against Paracetamol induced intoxication in Rats.

Effect of aqueous extract of roots of *A.malabarica* on ALP levels against Paracetamol induced intoxication in Rats.

Effect of aqueous extract of roots of *A.malabarica* on Bilirubin levels against Paracetamol induced intoxication in Rats.

Effect of aqueous extract of roots of *A.malabarica* on Liver Weight against Paracetamol induced intoxication in Rats.

Effect of aqueous extract of roots of *A.malabarica* on Liver Volume against Paracetamol induced intoxication in Rats.

Effect of aqueous extract of roots of *A.malabarica* on Thiopental Induced Sleeping Time against Paracetamol induced intoxication in Rats.