

PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF METHANOLIC EXTRACT OF HOLOSTEMMA ANNULARE

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Abstracts

As per the Phytochemical investigation and literature review, *Holostemma annulare* had many active constituents especially methanolic extract have more number and quantity of active components. The methanolic extract was used for study, the extract was prepared and tested against Paracetamol induced liver damage in experimental rats. Changes were observed in the biochemical markers like Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP) and Bilirubin. The effects of the extracts were compared with that of standard drug silymarin(100 mg/kg) treated group. Phytochemical investigations showed the presence of alkaloids, flavonoids, terpenoids, saponins and absence of glycosides, the results suggest that Paracetamol (2g/kg) elevated the levels of AST, ALT, ALP and Bilirubin. Treatment of different doses (150mg/kg and 250mg/kg) of *Holostemma annulare* extract ameliorated the effects of the Hepatotoxins and significantly ($p<0.05$) reduced elevated levels of Biochemical markers

Key words: Hepatoprotective, Antioxidant, Plant extract AST, ALT, ALP, Bilirubin

INTRODUCTION

The liver plays a major role in metabolism and has a number of functions in the body, including glycogen storage, decomposition of red blood cells, plasma protein synthesis and detoxification. It performs and regulates a wide variety of high volume biochemical reactions requiring very specialized tissue. (1) Some toxic chemicals like compounds known as hepatotoxins produce varying degree of damage to the liver. They may produce morphological changes, which may be typical of the various agents. Liver damage is usually associated with elevation in the serum levels of many biochemical markers such as AST, ALT, ALP and Bilirubin. (2) The advancements in modern medicine notwithstanding, there are no synthetic drugs for the treatment of liver disorders. Herbs play an important role in the management of many liver disorders. (3, 4) In the absence of a reliable and effective hepatoprotective agent in modern medicine, a number of medicinal plant preparations have been recommended for the treatment of liver problems. (5) There are avalanche of scientific support on the efficacy of medicinal plants in the management of drug –induced and other liver disorders. (6, 7, 8) *Holostemma annulare* is belongs to the family Asclepiadaceae, the Sanskrit name of the plant is Arkapushpi, it is distributed throughout the India. A large glabrous twining shrub; stem much branched, glabrous, shining, leaves are thick, ovate-oblong with few small glands at base, is traditionally acclaimed to be very effective in the management of type 2 diabetes and liver diseases. Locally the plant is used in the treatment of ulcers, blood diseases, itching, leucoderma, gonorrhoea, diabetes, jaundice and vascular calculi (Ayurveda), no literature review available for the hepatoprotective activity of this plant, the high cure rates acclaimed in the use of H A stimulated our interest in investigating the hepatoprotective effects of the extracts in experimental drugs induced hepatic models.

MATERIALS AND METHODS

Chemicals

Silymarin was used as a standard Hepatoprotective agent and was obtained from Micro Labs. Methanol from S S Pharma distributors Warangal, formaldehyde from S. D fine Chemicals, Mumbai, olive oil from local Ayurvedic stores, Thiopental sodium (thiosol)- Neon Labs., Mumbai; and normal Phase pre- coated Chromatographic Plates – Merck, Germany.

Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP), Total Bilirubin (TBL), Total protein (TPL) and Albumin (ALB) by manual methods were purchased from Span Diagnostic Ltd., Surat, India. The biochemical analytical kits for auto analyzer were purchased from Merck specialties Private Ltd, Mumbai India.

Animals

Male albino rats, weighing about 150–200 g obtained from the Mahaveer Enterprises, Bagh amberpet, Hyderabad (CPCSEA registration no: 432/2010/CPCSEA) and the animals were kept in the animal house of Sree Dattha Institute of Pharmacy, Nagarjunasagar road Sheriguda, Ibrahimpatnam, TS. at room temperature of 25 - 30°C and at 45 - 55% relative humidity for 12 hr, each of dark and light cycle. The animals were feed with rat pellets (Hindustan Lever Limited, Bangalore, India) and filtered water.

Animal studies in the work have been strictly performed as per the Institutional Animal Ethical Committee (IAEC) constituted under the guidelines of the Committee for the Purpose of Control and Supervision on experimental Animal (CPCSEA), Ministry of an Environment, and Govt. of India.

Collection of plant materials

The leaves of *Holostemma annulare* (5KG) collected from Tirupati Seshachalam Hills Andhra Pradesh India between November and December; the plant was authenticated by the Prof. S. K. Mahmood, Head of Botanical Department, Osmania University Hyderabad, Telangana, India. A voucher specimen (OU 20119/23/11) has been preserved in laboratory. The plants were washed thoroughly in tap water, shade dried and powdered.

Extraction

the fine powdered plant material was subjected to the soxhlet extraction by the solvent methanol until the clear liquid appear in the tube, after completion of extraction, the extract was treated with charcoal for removing the pigments and then it was evaporated by applying vacuum pump finally we got black colored compound

Determination of acute toxicity

Acute toxicity study was conducted for Methanol extract of *Holostemma annulare* by stair case method following OECD guidelines .There was no lethality up to a dose of 1000 mg/kg, p. o. nearly one tenth of the maximum dose of the extract that is 150, 250 mg/kg (p. o) was selected as the plant extract in all experiments

Hepatoprotective activity

In the present study, the animals were pretreated with test extract before inducing liver damage with PCM Seven days after acclimatization, the rats were divided into 5 groups each group consisting of six animals. All animals were kept on same diet for 7 days.

Study design

- **Group – I** served as a control and received 1 ml/kg of 2% w/v gum acacia in distilled water p. o. for seven days.
- **Group – II** treated with vehicle (1 ml/kg of 2% w/v gum acacia in distilled water p. o.) daily for 15days followed by PCM.
- **Group – III** (standard Silymarin) animals were administered with 100 mg/kg of Silymarin p. o. for seven days followed by PCM administration p. o.
- **Group – IV** treated 150mg/kg methanolic extract

- **Group – V** treated with 250 mg/kg of methanolic extract followed by PCM administered p. o on the seventh day.

All the rats were anaesthetized with thiopental sodium (60 mg/kg intraperitoneally), 36 hrs after administration of PCM, blood was collected from common carotid by carefully opening the neck region of the rats. After blood collection, the blood samples were allowed to coagulate at room temperature for at least one hour.

Serum was separated by centrifugation at 3000 rpm for 30 minutes and then analyzed for TB, ALT, AST, ALP, TP and ALB levels. The animal was dissected the livers were carefully removed, washed with 0.9% saline solution and preserved in formalin solution (10% formaldehyde) for histopathological studies.

$$\text{Percentage protection} = 1 - \frac{T - V}{C - V} \times 100$$

Where” T” is the mean value of the drug and PCM “C” is the mean value of PCM alone and V is the mean value of the vehicle treated animals

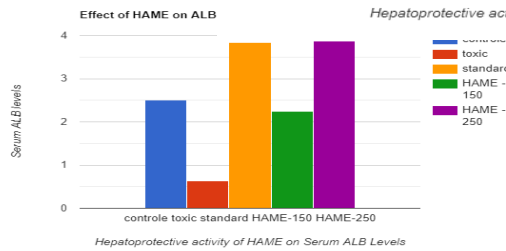
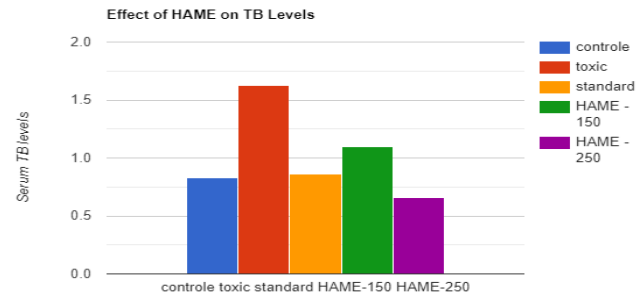
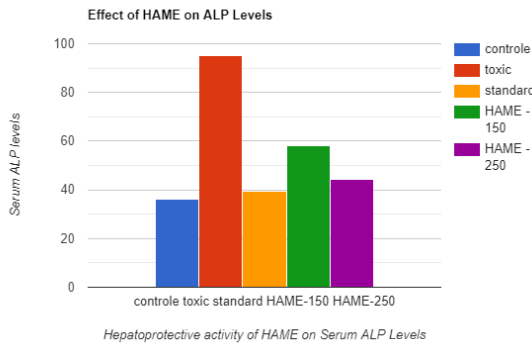
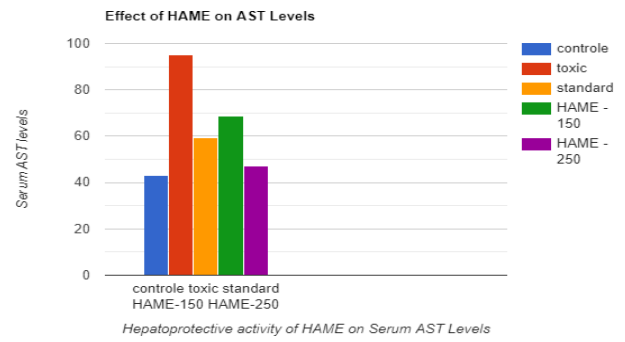
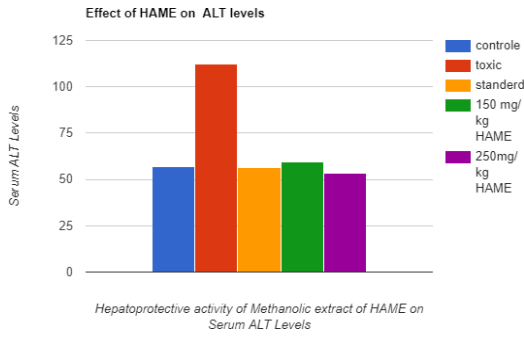
Estimation of biochemical parameters

The following are the biochemical parameters estimated to evaluate the effect of the test materials against the experimentally induced hepatotoxicity caused by different agents: Alanine aminotransferase (ALT) Aspartate amino transferase (AST) Serum alkaline phosphatase (ALP), Total protein levels (TP) Total serum bilirubin (TB) Albumin levels (ALB).

Hepatoprotective activity of Methanolic extract of *Holostemma annulare* leaves:

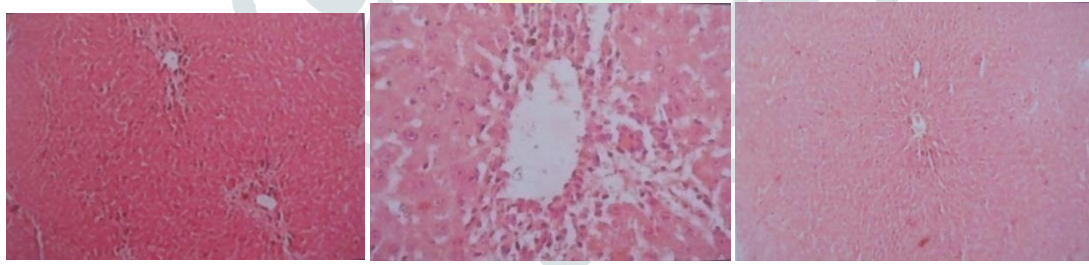
Biochemical parameters: The elevated serum AST, ALT, ALP and TB levels were significantly ($P < 0.001$) reduced by the standard. The test groups HAME-150mg/kg and HAME-250mg/kg.b.w also exhibit a significant protective effect on the serum levels and also increase the reduced serum TP and ALB levels. The HAME-250 showed a better hepatoprotective activity ($P < 0.001$) than HAME-150. The high percentage protection was observed with HAME-250 was also comparable to the reference standard drug Silymarin with to all the parameters.

Groups	ALT(IU/L)	AST(IU/L)	ALP(KA/dL)	TB(mg/dL)	TP(gm%)	ALB(gm%)
Control	55.84±4.51	43.22 ±0.11	36.22±0.57	0.83±0.30	4.74±0.06	2.51±0.11
Toxic	110.00±3.25	95.20 ±4.01	95.01± 3.00	1.63±0.11	0.78±0.17	0.64±0.09
Standard	57.23±4.18*	59.19 ±2.50	39.56±2.18*	0.86±0.18*	4.18±0.17*	3.83±0.15**
HAME – 150	60.36±1.98*	68.65 ±1.96	58.29±2.98*	1.10±0.20*	3.42 ±0.33*	2.24±0.37*
HAME – 250	55.21±3.21*	46.98 ±3.19	44.28±2.19*	0.66±0.11**	4.56±0.19*	3.87±0.28**



HISTOPATHOLOGICAL STUDY:

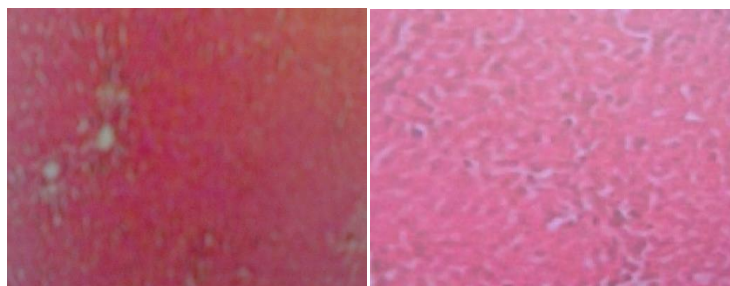
The histopathological study indicated that the hepatic damage induced by PCM were remarkably reduced by the standard Silymarin, HAME-250 showed a reduced fatty changes, necrosis and broad infiltration of lymphocyte produced by PCM



Control

PCM Toxicity

Standard



HA ME 150MG/KG

HAME 250MG /KG

Statistical analysis

Results were expressed as mean \pm SEM. Statistical analysis was performed with one way analysis of variance (ANOVA) followed by student's t-test. P values less than 0.05 was considered to be statistically significance when compared with the control.

DISCUSSION

Holostemma annulare is widely used in folk medicine for the treatment of liver diseases but there was no literature available for the evidence that's why we selected this plant for evaluation, this plant was collected from Tirupati Seshachalam forest and then authenticated by Head of Botanical Department from Osmania University

As per the laboratory test and the thin layer chromatograph the methanolic extract had the much compounds than other extracts hence methanolic extract was subjected to the all Pharmacological effect

The hepatotoxin is also known to interfere with the phospholipids synthesis, and to decrease liver plasma membrane phospholipids content resulting in fatty liver. In the PCM model, the drug is said to be eliminated mainly as sulfate and glucuronide, a small amount is metabolized via the cytochrome P450 enzyme system to the alkylating metabolite N-acetyl-p-benzoquinone imine (NAPQI), which is responsible for the toxic side effects of PCM. However, up on administration of toxic doses of PCM, the sulfation and glucuronidation routes become saturated, and hence, higher percentage of PCM molecules are oxidized to highly reactive NAPQI. Higher dose of PCM and NAPQI can alkylate and oxidize intracellular glutathione (GSH) and protein thiol groups, which result in the depletion of liver GSH pool subsequently, lead to increased lipid peroxidation and Liver damage

150 mg/kg, 250mg/kg doses of extract orally administrated to the IV, V group of rats and the standard Silymarin 100mg /kg dose administrated to the III group rats, after 7 th day of treatment all biochemical parameters were evaluated for the hepatoprotective activity 250mg / kg dose was shown drastic changes in biochemical parameters in blood stream which is comparable with the standard and histopathological evaluation shown better reduction in the damaged cell count

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