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Hepatoprotective Activity of *Holostemma ada Kodien shcult*, Extract against Paracetamol Induced Hepatic Damage in Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors JS and JYK designed the study, author PVB performed the statistical analysis, authors JS, JYK and PVB wrote the protocol and wrote the first draft of the manuscript. Author JS managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To study the hepatoprotective activity of alcoholic extract of *Holostemma ada Kodien Shcult* against paracetamol (PCM) induced liver damage in rats.

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Place and Duration of Study: Department of pharmaceutical chemistry and department of pharmacology Geethanjali College of Pharmacy, Cherryal, Keesara, Ranga Reddy District, Andhra Pradesh, India during June 2012 and Jan 2014.
Methods: Hepatotoxicity was induced by paracetamol (PCM) and various biochemical parameters were assessed to confirm the induced degrees of hepatoprotoxicity. The various extracts were tested on animals and various biochemical parameters such as serum aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP) and total serum bilirubin (SB) were investigated the hepatoprotective activity was also confirmed by histopathological studies of normal and hepatodamage and post treatment studies with the extract.
The Results: Pre-treatment of the rats with alcoholic extract prior to paracetamol (PCM) administration caused a significant reduction in the values of AST, ALT, ALP and SB (p<0.01) approximately comparable to the hepatoprotective of standard drug was silymarin and confirmed by histopathological examination of the liver tissue of control and treated animals.
Conclusion: From the results it can be concluded that *Holostemma ada Kodien Shcult possesses* the hepatoprotective effect against paracetamol (PCM) induced liver damage in rats.

Keywords: Holostemma ada kodien shcult; hepatoprotective activity; paracetamol; albino rats; histopathological studies.

ABBREVIATIONS

AEHA - Alcoholic Extraction Holostemma ada Kodien; PCM – Paracetamol; LPO - Lipid Peroxidase. GSH - Glutathione.

1. INTRODUCTION

Despite remarkable advances in modern medicine, hepatic disease remains a worldwide health problem, therefore the search for new medicines is still unending. Hepatic cells participate in a variety of metabolic activities, as a result the development of liver protective agents is of paramount importance in the protection from liver damage. The research reports have constantly shown that hepatoprotective effects are associated with plant extracts rich in antioxidants [1-4]. Several compounds and extracts from plants have thus been evaluated for hepatoprotective and antioxidant effects against chemically-induced liver damage [5,6]. Furthermore, research on hepatoprotective medicinal plants as a major indicator of the general screening systems can trigger the safety evaluation in the early phase of drug discovery because most of the toxic compounds are metabolized in liver [7-14]. Hepatotoxicity means damage to the liver by medicines and other chemicals. Liver plays a vital role in detoxification of chemicals and deemed to be more vulnerable to toxicity from toxic agents. Certain drugs when taken in overdose and administered at therapeutic dose may cause injury to liver. Other chemical agents that are used in laboratories, industries, microcystins produced by *cyanobacteria* and herbal remedies can also induce hepatotoxicity. Above 900 drugs have been concerned to cause injury, to liver [15] and it is the familiar reason for a medicine to be quitted from the market. Chemicals frequently cause subclinical injury to liver which can be detected by performing liver enzyme tests. Drug induced liver injury is responsible for 5% of all hospital admissions and 50% of all liver failure [16].

Previous studies revealed that, the systematic and scientific studies are scarce on *Holostemma ada Kodien Shcult*. Hence in the present study attempts were made to investigate the hepatoprotective activity of different doses of alcoholic and aqueous extracts of *Holostemma ada-kodien* in normal and Paracetamol (PCM) induced liver toxicity. *Holostemma ada-kodien* [17,18,19] (Syn: *Holostemma annulare*) belongs to Asclepiadaceae family. It is also called as Jivanti, Arkapushpi, Kshira, Dodi and Suryavalli. It is widely distributed in the Tropical rain forests in India [16,17]. The plant is used for maintaining vigor, strength and vitality [20]. The terpenoid sugars present in the root tubers of the plant are used as medicinal properties [21]. The plant diversity is thinly distributed in India, W. Peninsula, Ceylon and China. In India it is found in tropical Himalayas, Burma and Andhra Pradesh (AP). This species is distributed throughout the plains of AP on open hill areas, including waste lands and on the fences of Sri Venkateswara University campus in Tirupati,

Tirumala and Talakona [22,23]. Traditionally the plant is used as an alternative, astringent to the bowels; cures ulcers, diseases of the blood, worms [19], itching, leucoderma; useful in gonorrhea [24,25]. Roots are used for diabetes [26,27,28],. Cough, gonorrhea, as tonic and stomachic, Aphrodisiac Agent [29].Though some of the plants are reputed in the indigenous systems of medicine for their activities [30,31]. it requires scientific evaluation. To the best of our knowledge, the hepatoprotective effect of *Holostemma ada Kodien Shcult* against paracetamol-induced liver injury in mice has not been demonstrated. Hence, the present study focused on evaluating the potential l hepatoprotective effect of methanolic extract from *Holostemma ada Kodien Shcult* on paracetamol-induced liver injury in mice [18-64].

2. MATERIALS AND METHODS

Holostemma ada Kodien Shcult plant material was collected from Tirumala hills in the month of December 2011 from Chitoor district, Andhra Pradesh [22] and identified by a Plant Taxonomist Dr. K. Madhava Chety from Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India. A specimen was deposited in their herbarium against voucher number 2189. Then after the plants were washed thoroughly to remove adhering soil and earthy matter, later on sliced to thin chips and dried in shade at room temperature and ground to optimal coarse powder.

3. CHEMICALS

The materials used for the experiment were of analytical grade. Paracetamol and coconut oil were purchased from the local supplier (GSN Pharmaceuticals Private Limited Kukatpally, Hyderabad, Andhra Pradesh). These biochemical parameters were analyzed by Silymarin, purchased from Micro labs, Tamilnadu. India. AST, ALT, ALP, Bilirubin and the Protein kits were procured from Span Diagnostics, Surat, India. The rest chemicals were of analytical grade and obtained from Sisco research laboratories, Mumbai, India. The experimental rats and food supplies were obtained Mahaveer Enterprises, Madipally, Hyderabad, India

4. PREPARATION OF EXTRACTS

The powdered material was subjected to successive solvent extraction with Ethylacetate (50-70°C), chloroform (50-60°C), alcohol (95%) (50-60°C) and water. Extraction was carried out for 16 h with each solvent by Soxhlet extractor. The yield of extracts was as follows Table 1.The alcohol extract was screened pharmacologically by Paracetamol (PCM) induced hepatotoxicity.

4.1 Preliminary Phytochemical Studies

The phytochemical constituents present in extract of *Holostemma ada Kodien* was determined according to the standard method [19,30]. The results of Preliminary phytochemical screening are given Table 2.

5. EXPERIMENTAL ANIMALS

Animal experiments were performed on albino rats weighing about 200-250 g. Divided into 5 groups of 6 animals per cage and each group contain an equal ratio of male and female rats (3 male and 3 females) were used. Animals were maintained under standard laboratory aseptic conditions (12-h light/dark cycle, 24 hrs). The food in the form of dry pellets and water was provided using ad libitum. All the animals were approved by the institutional ethics approval committee.

5.1 Paracetamol (PM) Induced Liver Toxicity

The PCM was diluted with saline before oral administration (o.p). The animals were divided into following 5 groups of 6 each. Group 1: vehicle (saline) for 9 days. Group 2: vehicle + PCM (1 g/kg) on the ninth day, Group 3: Silymarin (100 mg/kg/day, po) + PCM (1 g/kg, p.o) on ninth day, Group 4: AEHA (250 mg/kg/day, p.o) + PCM (1 ml/kg, i.p) on the ninth day, Group 5: AEHA (500 mg/kg/day, p.o) + PCM (1 ml/kg, p.o) on the ninth day. To enhance the acute liver damage in animals groups 2, 3, 4 and 5, the food was withdrawn 12 h before PCM administration. The experimental animals were sacrificed after 24 h of administration of PCM. Blood samples were collected by puncturing the retro-orbital plexus under light ether anesthesia and allowed to coagulate for 30 min at 37°C. Serum was separated by centrifugation at 2500 rpm at 37°C for 15 min and analyzed for various biochemical parameters [2,5,44,58-60,62].

Percentage of inhibition = 100 X (value of toxic control – value of test sample)/ (value of toxic control – value of control).

Table 1. The yield of extract of AEHA

Solvent extraction with	Ethylacetate	Chloroform	Alcoholic	Aqueous
% Yield	3.2% w/w	2.9% w/w	15.5% w/w	5.5% w/w

Table 2. Preliminary phytochemical screening of extract of AEHA

Type of phytochemical constituents	Ethylacetate extract	Chloroform extract	Ethanol extract	Aqueous extract
Alkaloids	–	–	+	–
Carbohydrates	–	–	+	+
Flavonoids	–	–	+	–
Glycosides	–	–	–	–
Tannins/phenols	–	+	+	+
Protein	–	–	+	+
Steroids	+	+	+	–

- Absent, + Present

5.2 Assessment of Liver Functions

The hepato-protective effect of extract was evaluated by the assay of liver function, biochemical parameters such as Alanine amino transferase (ALT) [45,49,58-60], Aspartate amino transferase (AST) [45,49,58-60], Alkaline phosphatase (ALP) [52,58-60], and Total serum bilirubin (SB) [50,51] following standard protocols.

5.3 Measurement of Antioxidant Activity

From all the experimental groups, the portion of the liver was collected and rinsed with 0.15 M Tris-HCl (pH 7.4). A 10% w/v of liver homogenate was prepared in 0.15 M Tris-HCl buffer and processed for the estimation of lipid peroxidation in the form of malondialdehyde (MDA) in the liver. And the supernatant was used for reduced glutathione (GSH) estimation [1,5,32,38,48].

5.4 Histopathological Studies

For the histopathological study, the live of 5 animals from each group were immediately removed after autopsy and the tissues were fixed in bouin's solution for 12 h, then embedded in paraffin using conventional methods [5,50] and cut in to 5 µm thick section and stained using haematoxylin-eosin dye and finally mounted in diphenyl xyline. The sections were observed under microscope for histopathological change. (The live of 5 animals in every group were examined with 10 different microscopes).

6. STATISTICAL ANALYSIS

The statistical analysis was performed by using One Way ANOVA followed by Dunnet's comparison test and student t-test (unpaired). The values were expressed as mean ± SEM and the $P<0.05$ were considered significant.

7. RESULTS AND DISCUSSION

Alcoholic extracts of *Holostemma* used in the study preserved the structural integrity of the hepatocellular membrane in a dose dependent manner as evident from the protection provided similar to that produced by Silymarin (100 mg/kg; p.o), a well known hepatoprotective agent [5]. It is well known fact that PCM induces liver injury through its toxic metabolite, N-acetyl-P-benzoquinone imine [54,55], produced by the action Cytochrome P-450. This metabolite causes depletion of glutathione (GSH) leading to cell death. However it is more obvious that the AEHA was able to reduce all the elevated levels of Serum levels of alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP) and Total serum bilirubin (SB) of group 1 to 5 were estimated quantitatively and tabulated in Table. 3. Figs. 1 and 5 were Histopathology [microscopic] view of liver tissue of normal rat [53]. It is interesting to note that the relationship between dose and percentage PCM (1 ml/kg.p.o) intoxication in normal rats produced elevated levels of serum biochemical parameters as follows ALT (292.4±0.49), AST (256.5±0.9), ALP

(251.5±1.26), S.B (2.13±0.13) compare to control (Group-1) animals having ALT (156.6±0.62), AST (98.9±1.34), ALP (189.6±.89) and Total Serum Bilirubin (0.83±0.06) Fig 2. The percentage reduction of various serum biochemical parameters in case of standard drug Silymarin (100 mg/kg/day, po) (1 ml/kg) in PCM intoxicated rats were as follows ALT (90.07%), AST (89.47%), ALP (92.46%) and S.B (94.93%) levels Fig 3. Conversely, when compared to the PCM toxic control group, the group treated with the plant extract at doses of 250 and 500 mg/kg in PCM intoxicated rats exhibited a significant reduction of ALT (55.47%, 74.25%), AST (54.73%, 84.18%), ALP (64.16%, 81.13%) and S.B (65.83%, 73.33%) levels Figs. 4 & 5.

7.1 Effect of *Holostemma ada* Kodien on Antioxidant Activity

There was a significant increase in MDA content and reduction in GSH activities of PCM intoxicated animals Table 4. Pre-treatment with silymarin (100 mg/kg) and *Holostemma ada Kodien* (250 and 500 mg/kg) significantly (P< 0.01 prevented the increase in MDA levels and brought them near to normal level, whereas GSH levels were significantly (P< 0.01) raised, thus providing protection against paracetamol toxicities.

The *Holostemma ada kodien* extract has been reported to contain different types of terpenoids, the phytochemical screening [19]. Several compounds belonging to the class of terpenes have been reported to possess antioxidant [30]. Pretreatment of animals with alcoholic extract of *Holostemma ada* Kodien and silymarin prevented the Paracetamol induced rise in serum level of transaminases and total serum bilirubin, confirming the protective effects of alcoholic extract of *Holostemma ada* Kodien against carbon tetrachloride and Paracetamol induced hepatic damage. The hepatoprotective activity of *Holostemma ada Kodien* (500 mg/kg) was compared with the activity of standard silymarin (100 mg/kg). However there was no effect on rise in serum alkaline phosphatase levels by the test extract and silymarin. A comparative histopathological study of the livers from different groups further corroborated the hepatoprotective potential.

Table 3. Effect of alcoholic extract of *Holostemma ada Kodien* (AEHA) on ALT, AST, ALP and SB in Paracetamol (PCM) induced liver toxicity in rats

Treatment	Dose	ALT (U/L)	AST (U/L)	ALP (U/L)	SB (mg/dl)
Group-I					
Vehicle (saline)	1 ml/kg	156.6±0.62	98.9±1.34	189.6±0.89	0.83±0.06 [38]
Group-II					
Control (PCM)	1 ml/kg	292.4±0.49	256.5±0.9	251.5±1.26	2.13±0.13 [44]
Group-III					
PCM+ Silymarin	100 mg/kg	174.9±1.98**	115.5±1.25**	190.9±1.95**	0.89±0.04**[5,44]
Group-IV					
PCM+ AEHA	250 mg/kg	218.0±1.75*	171.7±3.65*	212.5±1.37*	1.24±1.05* [44,59]
Group-V					
PCM+ AEHA	500 mg/kg	192.1±4.15**	124.4±3.08**	201.5±4.05**	1.15±2.25**[60]

*Each value represents the mean ± SEM. n = 6 number of animals in each group. *P<0.01, **P<0.001 Compared to respective PCM treated control groups*

Table 4. Effect of alcoholic extraction of *Holostemma ada Kodien* (AEHA) on lipid peroxidation (LPO), glutathione (GSH), PCM induced hepatic damage in rats [5,38,48]

GROUP	DOSE	LPO (nM MDA/mg protein)	GSH (µg/mg protein)
Vehicle	1 ml/kg	0.98±0.12	5.23±0.54
PCM + control	1 ml/kg	0.99±0.75	4.75±1.17
PCM + silymarin	100 mg/kg	1.09±0.21	4..96±0.26
PCM + AEHA	250 mg/kg	4.24±029*	2.94±0.25*
PCM + AEHA	500 mg/kg	3.94±029**	5.54±0.15**

*Each value represents the mean ± SEM. n = 6 number of animals in each group. *P<0.01, **P<0.001 Compared to respective PCM treated control groups*

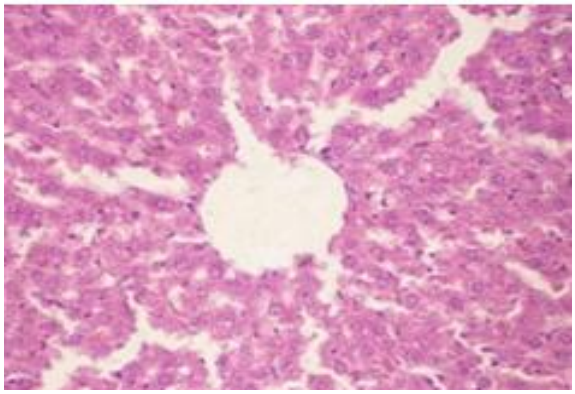

Fig. 1

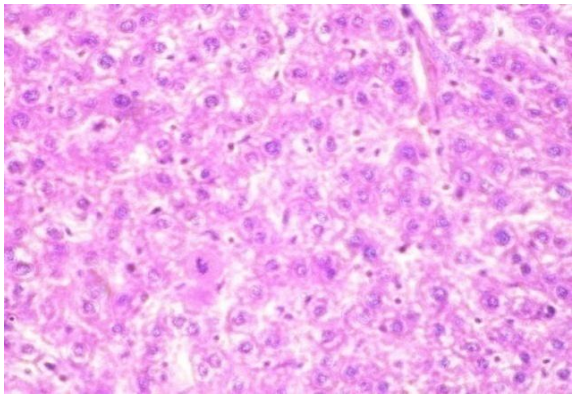

Fig. 2

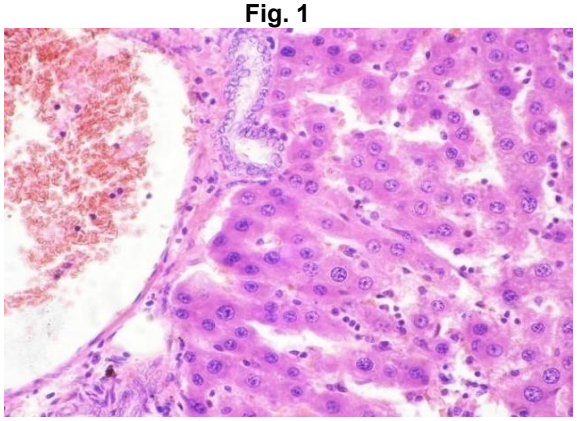

Fig. 3.

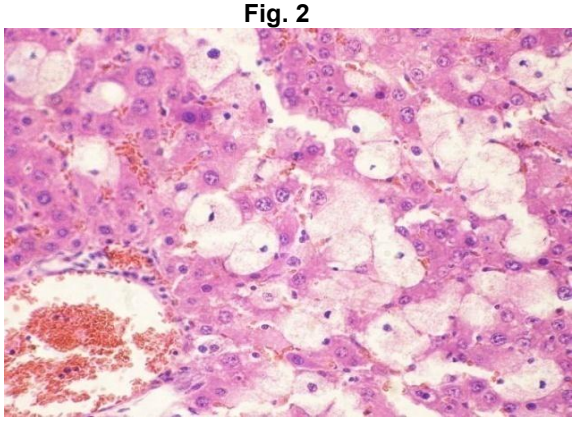

Fig. 4.

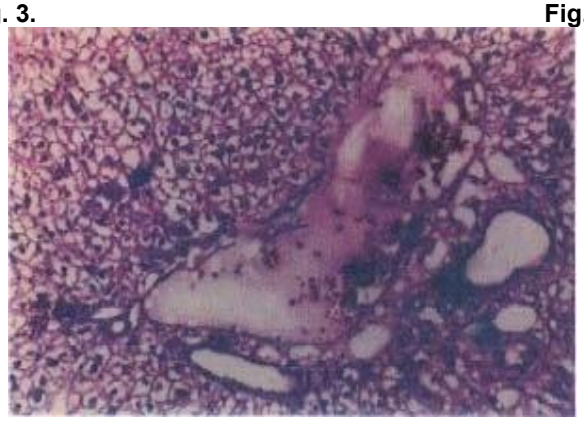

Fig. 5.

Figs. 1-5. Histopathology of liver tissue of alcoholic extract of *Holostemma ada Kodien* (AEHA) on ALT, AST, ALP and SB in PCM induced liver toxicity in rats

Fig. 1. Histopathology of liver tissue of Normal rat (46), Fig. 2. Histopathology of liver tissue of PCM, Fig. 3. Histopathology of liver tissue of PCM + Silymarin, Fig. 4. Histopathology of liver tissue of PCM + 250 mg/kg, p.o plant extract, Fig. 5. Histopathology of liver tissue of PCM + 500 mg/kg, p.o plant extracts

8. CONCLUSION

The results of the present study clearly demonstrates various biochemical, (serum AST, ALT, ALP, and SB) histopathological alterations produced by Paracetamol in the serum and tissue were reversed significantly by the pretreatment of extracts of *Holostemma ada Kodien* (AEHA) and Silymarin. This study confirms its use as hepatoprotective as per the ethno pharmacological claims (50).

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CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the Cameroon National Ethical Committee (Reg No: 1648/PO/A/12/CPCSEA).

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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