Hepatoprotective Activity of *Acampe praemorsa*

Praveen Kumar Uppala¹*, G. S. N. Koteswara Rao¹, A. Rajasekhar Reddy¹, K. Umasankar¹, C. Joshitha², Kanagala Anusha³, Nadeem Siddiqui⁴ and Sarada Prasanna Mallick⁴

¹K. L. College of Pharmacy, Koneru Lakshmaiah Education Foundation, Vaddeswaram, Guntur, Andhra Pradesh, 522502, India.  
²Department of Electronics and Communication Engineering, Koneru Lakshmaiah Education Foundation, Vaddeswaram, Guntur, Andhra Pradesh, 522502, India  
³K. L. Business School, Koneru Lakshmaiah Education Foundation, Vaddeswaram, Guntur, Andhra Pradesh, 522502, India.  
⁴Department of Biotechnology, Koneru Lakshmaiah Education Foundation, Vaddeswaram, Guntur, Andhra Pradesh, 522502, India.

Authors’ contributions:

This work was carried out in collaboration among all authors. Author GSNKR has designed the study and wrote the protocol. Author UPK planned and executed the experimentation work, wrote the first draft of the manuscript. Authors ARR and KU performed the statistical analysis. Authors CJ, KA did literature review. Authors NS and SPM reviewed the manuscript. All authors read and approved the final manuscript.

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(1) Dr. Vasudevan Mani, Qassim University, Kingdom of Saudi Arabia.  
(2) Meilinah Hidayat, Universitas Kristen Maranatha, Indonesia.  
(2) Marta Mazzetti, Ospedale Di Macerata, Italy  
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ABSTRACT

**Introduction:** Liver is one of major functional organ in body, its damage can alter body metabolisms and other organs’ function. So, it is very important to maintain the healthy liver. Now a days, different chemicals and inadequate use of medicines are causing liver impairments including alcohol consumption. There is a need to identify safe hepatoprotective drugs against liver diseases from different natural resources including medicinal plants. Several medicinal plants have been using in traditional medicines against several diseases including liver disease and many of them are not scientifically proven. So, the current study was aimed to evaluate hepatoprotective nature of *Acampe praemorsa*.

*Corresponding author: E-mail: Praveen.chintu32@gmail.com;
Methodology: The hepatoprotective activity of *A. praemorsa* was carried on ethanol-induced liver toxicity on albino wistar rats by evaluating the levels of liver biomarker enzymes such as aspartate aminotransferase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP), Total protein (T.ptn), and Total bilirubin (T.Bil).

Results: The extracts of *Acampe praemorsa* were found to be safe at different doses as there were no mortality and physio-psychological changes observed in toxicity study. The extracts of *Acampe praemorsa* has showed dose dependent hepatoprotective activity on controlling the altered liver biomarker enzymes when compared along with standard drug Liv 52. The hydroalcoholic extract showed better activity compared to ethyl acetate extracts. The percentage protection on liver biomarker enzymes levels of hydro-alcoholic extract at 100, 200, and 400 mg/kg on AST, ALT, ALP, T.ptn, and T.Bil was found to be 62.72%, 60.06%, 61.77%, 63.96% and 58.58% respectively.

Conclusion: The results of recent study support traditional medicinal use of *Acampe praemorsa* and provides the information about its hepatoprotective nature. The hepatoprotective activity of *A. praemorsa* was definitely due to presence of different phytochemical compounds in it as it was compared with Liv 52 which was also an herbal drug.

Keywords: Liver; damage; ethanol; hepatoprotective; *Acampe praemorsa*; Liv 52.

1. INTRODUCTION

Liver is one of the important organs in human body which maintain different physiological metabolisms [1]. The liver controlling functions are chemical levels of body, storage of vitamins, fats, sugars, minerals and directly or indirectly it involves every metabolism [2]. So, the maintenance of a healthy liver is very important for its normal functioning and to other organs [3]. If any injuries happen to liver, it impacts the normal body physiological parameters such as enzymes levels, protein content, imbalance in detoxification etc., and finally leads to mortality [4]. Now a days, liver diseases have been increasing around the world due to different reasons like uncontrolled diet, lack of exercise, emerging of new diseases, pollution, chemicals, inadequate usage, because of side effects from current day drugs and also alcoholic consumption [5,6].

Now a days, alcohol consumption is very common around the world but, excessive consumption is causing a global problem with enormous economic, social and clinical consequences [7]. The over drinking of alcohol causes different liver diseases, initially alcoholic fatty liver (accumulation of fat), next is alcoholic hepatitis (inflammation of liver) and advanced is alcoholic cirrhosis (irreversible liver failure). The world health organization (WHO) records more than one million deaths due to the alcohol consumption and its consequences [8].

Prevention of or treatment for liver diseases is very crucial to control mortality and provide healthy life to people [9,10]. Different medications are available for treatment on liver diseases condition. Many researchers and studies suggest natural medications (phytochemicals) are more effective in treatment of liver diseases and are using as medical supplements to improve their immunity against oxidative stress due to hepatic diseases [11-13]. The phytochemicals from different medicinal plants were playing very crucial role in development of new drugs with broad spectrum usage including liver diseases [14,15]. Many bioactive compounds were reported from medicinal plants and about their protective nature against liver diseases [16-19]. But still many plants are available to know about their medicinal value.

*Acampe praemorsa* is one such medicinal plant that belongs to the family Orchidaceae grows in tropical and subtropical regions. It is an erect plant grows up to 40cm with thick leaves, less branches and more flowers. There were few studies reported with evidence on its traditional usage in bone fractures, anti-typhoid and some other reports about the orchids have potential medicinal values like enhancing white blood cells, reducing headache, fatigue etc. [20-24].

But there were no earlier reports on its hepatoprotective activity. So, the current study was aimed to evaluate hepatoprotective nature of *Acampe praemorsa*.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

The chemicals and reagents used in current study were analytical grade. The diagnostic kits used in study were purchased from Span.
Diagnostics Ltd., Gujarat, India. The standard drug Liv 52 was purchased from local medical shop.

2.2 Preparation of Plant Extracts

The plant material *Acampe praemorsa* (Voucher specimen number: 23342) was collected at Araku valley region, Visakhapatnam and was authenticated by Prof. S. B. Padal, Department of Botany, College of Science & Technology, Andhra University, Andhra Pradesh, India. The collected aerial parts were cleaned under running tap water to remove debris and was shade dried. The dried material was made into bristly powder. The powder was used for extraction by maceration process successively using ethyl acetate and hydro-alcoholic (70% ethanol in water v/v). The collected solvents were evaporated using rotavap and extracts [Acampe praemorsa ethyl acetate extract (APEAE) and Acampe praemorsa hydro-alcoholic extract (APHAE)] were stored in desiccator for further usage.

2.3 Selection of Animals

Albino wistar rats (200-250 g) of 60-90 days age were procured from M/S Mahaveer Enterprizes, Hyderabad, India to study the toxicity and hepatoprotective activity of *Acampe praemorsa* extracts. The animals were maintained with controlled conditions (12 h light/dark, 24±2°C, 40-70% relative humidity) by providing necessary food and water.

2.4 Toxicity Study

The extracts were tested for their toxicity at 1000 and 2000 mg/kg body weight (b.w.) as per Organization for Economic Co-operation and Development (OECD) guidelines [25]. The animals were divided into four groups (n=6) administered with APEAE to groups I (1000 mg/kg), II (2000 mg/kg) and APHAE to groups III (1000 mg/kg), IV (2000 mg/kg) with metal oropharyngeal cannula and animals were observed at regular intervals for any physiological and psychological changes and finally mortality.

2.5 Hepatoprotective Activity

The hepatoprotective activity of *Acampe praemorsa* extracts was carried on ethanol-induced liver toxicity as per method described by Shukla et al. 2001 [26]. The animals were divided into nine groups (n=6).

- Group I is control - treated with drug vehicle (2%v/v tween 80).
- Group II is toxic - administered with ethanol 3.76 g/kg p.o. twice a day.
- Group III - treated with Liv 52 (25 mg/kg).
- Groups IV, V, VI treated with APEAE at 100, 200 and 400 mg/kg b.w. respectively for 21 days after 1 hr of ethanol administration and
- Groups VII, VIII, IX treated with APHAE at 100, 200 and 400 mg/kg b.w. respectively for 21 days after 1 hr of ethanol administration.

On 22nd day blood was collected from all the group animals through retro orbital puncture under isoflurane anesthetic condition. After collection of blood, immediately serum was separated for estimation of liver profile enzymes such as aspartate aminotransferase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP), Total protein (T.ptn), and Total bilirubin (T.Bil) using diagnostic kits on semi-autoanalyzer.

2.6 Statistical Analysis

The enzyme levels were presented as mean±SEM and liver protection as percentage with below formula. The significance was analyzed with two-way ANNOVA followed by Dunnett’s multiple comparison test.

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\% \text{ Protection} = \frac{(\text{Levels in toxic group} - \text{Levels in test group})}{(\text{Levels in toxic group} - \text{levels in control group})} \times 100
\]

3. RESULTS AND DISCUSSION

The identification of new bioactive molecules from natural resources is always a typical and interesting research [27]. Now a days, emerging new diseases and side effects of current day using drugs have driven researchers to identify new therapeutics from natural resources including medicinal plants [28]. The medicinal plants have been playing vital role in traditional medicine and are sources for identification of new bioactive molecules in modern medicines [29,30]. New diseases and side effects are increasing mortality rate around the world. Liver diseases including alcohol-induced liver damage are one of them [31]. Many research studies
have stated the application of medicinal plants in traditional medicine to cure different diseases including liver diseases [32,33]. But many medicinal plants are still not scientifically screened for their therapeutic value. So, the current study was aimed to evaluate hepatoprotective activity of *Acampe praemorsa* extracts on ethanol-induced liver toxicity in rats.

During the toxicity study, the extracts of *A. praemorsa* (APEAE and APHAE) were found to be safe at 1000 and 2000 mg/kg doses. There were no observed mortality, physiological and psychological changes. The phytochemical analysis exposed the variation in their phytochemical constituents i.e., the both extracts posses' sterols, terpenoids, glycosides, flavonoids, tannins, alkaloids, carbohydrates, phenols and gave negative results for amino acids. APHAE gave positive results for saponins and oils but PAEAE gave negative results. As there is variation in their phytoconstituents, the extracts showed concentration dependent hepatoprotective activity and APHAE has more activity compared to PAEAE.

The hepatoprotective activity of *A. praemorsa* was evaluated by estimation of different liver biomarker enzymes (AST, ALT, ALP, T.Bil and T.Ptn) by comparing the treated groups with control group. Group I is served as control and there were no discrepancies observed in the enzymes’ level. Group II served as toxic group administrated with ethanol, observed the alterations in enzymes’ levels with related to control group i.e., increased AST, ALT, ALP, T.Bil and decreased T.Ptn levels. Group III treated with Live 52 served as positive control, the altered biomarker enzymes as in toxic group were kept back in this group because of treatment with Live 52 [34]. Liv 52 is a naturally developed herbal formulation which is clinically proved in hepatoprotective nature by protecting hepatic parenchyma and promotes hepatocellular regeneration. The tested extracts of *A. praemorsa* exhibited concentration dependent hepatoprotective nature in restoration of altered liver biomarker enzymes levels when compared with toxic and control groups levels. The results of enzymes levels and percentage protection were showed in Table 1 and Fig. 1.

The Liv 52 showed 92.23%, 90.38%, 92.63%, 92.31% and 89.35% protection on enzymes levels of AST, ALT, ALP, T.Bil and T.Ptn respectively. The APEAE treated groups IV, V, and VI showed variation in percentage protection, the extract at 400 mg/kg showed more protection on AST, ALT, ALP, T.Bil and T.Ptn levels. The percentage protection at

| Name of the drug | Name of enzymes | AST(U/L) | ALT(U/L) | ALP(U/L) | T. bil (mg/dl) | T. ptn (gm/dl) |
|------------------|-----------------|---------|---------|---------|---------------|---------------|
| GROUP-I Control (Drug Vehicle) | | 85.00±1.03 | 46.33±1.28 | 129.00±0.82 | 0.24±0.01 | 6.97±0.07 |
| GROUP-II Ethanol | | 327.33±2.16 | 159.00±0.63 | 483.83±2.47 | 2.17±0.06 | 4.15±0.05 |
| GROUP-III Liv 52 25mg | | 103.83±0.65 | 57.17±0.60 | 155.17±1.42 | 0.39±0.04 | 6.67±0.08 |
| GROUP-IV (APEAE 100mg) | | 308.00±0.73 | 150.17±0.70 | 456.67±1.61 | 2.02±0.03 | 4.35±0.06 |
| GROUP-V (APEAE 200mg) | | 284.67±1.69 | 139.33±0.99 | 421.67±1.98 | 1.87±0.04 | 4.63±0.06 |
| GROUP-VI (APEAE 400mg) | | 242.00±1.46 | 117.83±1.49 | 350.67±1.31 | 1.53±0.04 | 5.17±0.03 |
| GROUP-VII (APHAE 100mg) | | 294.67±1.43 | 142.67±1.61 | 440.83±1.83 | 1.90±0.04 | 4.52±0.07 |
| GROUP-VIII (APHAE 200mg) | | 252.83±2.01 | 123.33±1.12 | 375.00±1.77 | 1.60±0.07 | 5.03±0.06 |
| GROUP-IX (APHAE 400mg) | | 175.33±2.04 | 91.33±1.33 | 264.67±2.11 | 0.93±0.07 | 5.80±0.07 |

The values were expresses as mean±SEM
Fig. 1. Percentage protection of A. praemorsa extracts at different doses and Live 52 on Ethanol-induced liver toxicity.

Results were analyzed with Two-way ANOVA followed by Dunnett’s multiple comparison test with control group. ***p<0.001; **p<0.01; *p<0.05; ns=Nonsignificant.

400 mg/kg treated with APEAE on AST, ALT, ALP, T.Bil and T.Ptn levels was and 35.21%, 36.54%, 37.53%, 32.84% and 36.09% respectively. The APHAE showed more hepatoprotection compared to APEAE on liver biomarker enzymes but it is less than the standard drug Liv 52. The percentage protection of APHAE on AST, ALT, ALP, T.Bil and T.Ptn at 400 mg/kg doses was 62.72%, 60.06%, 61.77%, 63.96% and 58.58% respectively.

The results of current study reveal that A. praemorsa possess hepatoprotective nature against ethanol-induced liver toxicity. Different phytochemicals were extractable with different solvent depends on their polarity. So, different solvents were used for extraction of compounds from A. praemorsa. Among two extracts hydro-alcoholic extract has shown better activity as standard drug Liv 52. As said earlier, alcohol consumption causes different health problems like liver damage with different side effects. Liv 52 is one of the herbal drugs mainly used in liver disease therapy around the world [34]. The main component of Liv 52 was a mixed extract formulation of chicory and caper bush, both having potent antioxidant activity i.e., reduction of free radicals’ formation and reduces the elevation of malonaldehyde [35]. The early reports on them says that they contain different phytochemical bioactive compounds including flavonoids [36]. Several researchers have been reported about different phytochemical compounds from medicinal plants and their biological activities including hepatoprotective activity. The A. praemorsa may be also have phytochemical components those can reduce free radicals and protect the cell from oxidative stress. The preliminary phytochemical analysis on A. praemorsa extracts showed presence of different phytochemical constituents in them and variation was observed in the qualitative analysis between APEAE and APHAE. The results of present study support the presence of biologically active compounds in A. praemorsa extracts with hepatoprotective activity. The compounds in them may have potency against free radicals i.e., playing important role in cell membrane damage in oxidative stress condition which helps in protection and regeneration of hepatocellular membrane and possess the compounds which helps in breakdown of lipids which controls the fat formation in ethanol-induction liver damage (fatty liver) [37,38]. So, the result of current study was an evidence for hepatoprotective activity of A. praemorsa and the plant may be useful as food supplement in protection against free radicals and chemical-induced liver impairments.

4. CONCLUSION

The current study was aimed to evaluate hepatoprotective activity of A. praemorsa extracts against ethanol-induced liver toxicity.
The results clearly indicated that it possesses good hepatoprotective nature as standard drug Liv 52. The hydro-alcoholic extracts have more protective capacity compared to ethyl acetate extract on restoration of the altered liver biomarker enzymes levels compared to toxic group. The further research was worthwhile and is under progress to know the mode of action and isolation of pure phytochemical components in them.

CONSENT

It’s not applicable.

ETHICAL APPROVAL

The animal studies were approved by institutional ethical committee of Santhiram Medical College and General Hospital, Udumalpuram, Nandyal, Andhra Pradesh, India (897/PO/RE/S/05/CPCSEA).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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