

Phytochemical analysis and Hepatoprotective Activity of *Elytraria acaulis*

ABSTRACT

Introduction: The emerging of new diseases, resistance to contemporary using drugs and inadequate usage of commonly available drugs leading to different side effects and sometimes to mortality. So, there is need to identify efficient drugs from easily available sources. Traditional medicines from medicinal plants have been using since ancient times to treat different diseases, are easily available herbal formulations and there were still many medicinal plants were unexplored about their therapeutic potentiality. So, the current research is aimed to explore phytochemical constituent and hepatoprotective potentiality of *Elytraria acaulis* on paracetamol-induced liver toxicity.

Methodology: The root parts *Elytraria acaulis* were used for extraction through maceration procedure using hexane, ethyl acetate and hydro-alcoholic (70% ethanol). The dried extracts used for further for phytochemical analysis using standard procedures and evaluated liver protection on paracetamol-induced liver toxicity by estimating liver bio markable enzymes such as AST (SGOT), ALT (SGPT), ALP and total bilirubin levels.

Results: Qualitative phytochemical screening of *E. acaulis* extracts revealed the presence of different phytochemical constituents like steroids, terpenoids, flavonoids, alkaloids, glycosides, phenols, tannins, saponins and carbohydrates in them. The hydroalcoholic extract has more flavonoid content i.e., 23.84 ± 0.28 (mg/gm) than other two extracts. The tested three extracts of *E. acaulis* had showed concentration dependent hepatoprotective activity. Among three extracts hydro-alcoholic extract had more potentially compared to other two extracts. The percentage protection produced by the hydro-alcoholic extract on the enhancement of AST(SGOT), ALT (SGPT), ALP and total bilirubin levels were 21.54%, 21.94%, 21.20%, and 20.52%, 36.27%, 38.26%, 37.55% and 36.14%, 67.76%, 70.04%, 69.83% and 68.61% respectively.

Conclusion: The *Elytraria acaulis* root extracts had showed significant biological activities and own different phytochemical constituents. The hydroalcoholic extract possess more phenolic, flavonoid contents and the same had showed more potentiality against liver toxicity. The current results offer vital information about the traditional medicinal value of it. The further research is valuable and is under progress in evaluation of different biological activities and isolation of individual bioactive molecules from *E. acaulis*.

Keywords: Medicinal plants, *Elytraria acaulis*, inadequate, Paracetamol, Liver toxicity, Live 52.

1. INTRODUCTION

The plants have been using around the world in different traditional medicines (TMs) like Ayurveda, Unani, Siddha, traditional Chinese, Iranian, Korean medication to treat several diseases [1]. But on emerging of

allopathic medicine (AM) to treat diseases the use of TMs was reduced [2]. However, the pharmaceutical products which have been used in AM are originated from medicinal plants in TMs [3]. The emerging of new diseases and unsatisfactory treatment of AM in treatment of ailments, their side effects on chronic usage, the world is searching for new therapeutic agents from TMs [4].

The medicinal folklore in TMs is passed from one generation to another generation but they were not well documented [5]. The people around the world belief that, herbal medicines are less toxic, free from side effects, easily available from nature [6]. But there were less documented proofs about them, there is need to prove scientifically about different medicinal plants which have been using TMs to treat different diseases including liver diseases [7, 8].

Now a days, liver diseases are more common in people around the world and are one of the main causes to mortalities. There were different causes for liver diseases such as pathogens, pollutants, chemicals and different toxic substances [9, 10]. Liver is one of the main functional organs in the body, plays important role in protein synthesis, hormonal metabolisms, cholesterol homeostasis, oxidation, and detoxification. The exposure of liver diseases leads to alteration in its function, also causes variation in functions of interlinked organs, finally leads to mortality [11, 12]. As above said search for new bioactive molecules including liver diseases, many researchers reported different bioactive molecules from medicinal plants and made evidence biological activities of them, which were in traditional medicine and not mentioned [14-16]. Therefore, the current research was aimed to evaluate hepatoprotective activity of one of such medicinal plant i.e., *Elytraria acaulis* [17-19].

Elytraria acaulis is belongs to family Acanthaceae grows around wood lands, sandy lands as perennial herb around the world. *E. acaulis* has been using in traditional medicine for different ailments [17-19]. The root part has been using as paste for treatment of leucorrhoea, snake bites, abscess of mammary glands, throat compliments like tonsillitis [19]. There were few reported biological activities, but very were reported on root part [20].

2. MATERIALS AND METHODS

2.1 Chemicals and drugs

The analytical grade solutions were used in the current study. The diagnostic kits for enzymes estimation have purchased from Span diagnostics Ltd., Gujarat, India. The liver toxic inducing drug paracetamol and standard drug Liv52 were purchased from a local market, Narasaraopet, Guntur, India.

2.2 Preparation of extracts

The plant material *Elytraria acaulis* was at pulnadu region, Andhra Pradesh, India and authenticated by Dr. Prayaga Murthy. Pragada, Govt. Degree College, Yeleswaram, E. Godavari, A.P. India. The roots were separated from freshly collected plant material and wash under running tap water to remove unwanted material. The cleaned roots were shade dried and granulated into fine powder for further use. The powder was used for preparation of extracts successively with hexane, ethyl acetate and hydro-alcoholic [70%Ethanol (hyd-alc)] using maceration. The collected solvents were evaporated using rotavapor. The prepared extracts were stored in desiccator for further use.

2.3 Phytochemical Analysis

The qualitative and quantitative phytochemical analysis of *Elytraria acaulis* extracts were carried using standard procedures [21-23]

2.3 Selection of Animals

The Wistar albino rats (170-220gm) were used to evaluate the hepatoprotective activity. The animals were obtained from M/s. Mahavir Enterprises, Hyderabad and maintained at controlled environmental conditions before the experiment and also at the time of the experiment ($22\pm 2^{\circ}\text{C}$, $60\pm 5\%$ humidity). The animals were fed up with standard laboratory diet and water. The animal study was approved by the institutional ethical committee, approved CPCSEA, Govt of India (Reg No: 1987/PO/Re/S/17/CPCSEA). Before the study, the prepared extracts were tested for their toxicity as per The Organization for Economic Co-operation and Development (OECD) guidelines [24].

2.4 Hepatoprotective activity

The extracts of *Elytraria acaulis* (Hexane extracts (HE), Ethyl acetate extract (EAE), Hydro-alcoholic extracts (HAE)) at doses of 100mg/kg, 200mg/kg and 400mg/kg b.w. were tested for their hepatoprotective activity

against paracetamol-induced liver toxicity. The animals were divided into XII groups (n=6), group I as control, group II and negative control, group III as a positive group. Group I & II have treated with normal saline (2ml/Kg b.w) and, group III was treated with Liv52 (25mg/Kg b.w.) for seven days. The groups IV, V, VI were treated with HE at 100mg/kg, 200mg/kg and 400mg/kg b.w. doses. The groups VII, VIII, IX were treated with EAE at 100mg/kg, 200mg/kg and 400mg/kg b.w. doses. The groups X, XI, XII were treated with HAE at 100mg/kg, 200mg/kg and 400mg/kg b.w. doses. The paracetamol was dosed to all groups except group I on the 5th day of the experiment. On the 7th day of the experiment, after 2hrs of last dose treatment, the blood samples were collected from animals through retro-orbital plexus. The collected samples were centrifuged without any delay at 2400rpm/15min. The separated serum was used to evaluate the liver function parameters such as Aspartate aminotransferase (SGOT), alanine aminotransferase (SGPT), Alkaline phosphatase (ALP) and total bilirubin levels using an auto analyzer [25, 26].

2.4.1 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.

% Protection= (Levels in toxic group-Levels in test group)/ (Levels in toxic group-levels in control group) X100

3. RESULTS AND DISCUSSION

The natural products have different phytochemical constituents and they possess wide spectrum of therapeutic potentiality. They have been using in traditional medicine to treat different diseases and are also use as nutritional supplements around the world [27,28]. As the emerging of new diseases, resistance to current using drugs, their side effects are demanding the new research to develop high throughput efficient drugs to treat them [29, 30]. As said in introduction, many medicinal plants are still unexplored about their medicinal values. Many researchers are conducting different research on medicinal plants around the world and reporting their biological activities, identifying new bioactive molecules to treat different diseases including liver diseases [29, 30]. In recent years many medicinal plants and herbal formulations were reported about their potentiality against different liver diseases [31]. In this point of view, the current research was aimed to explore phytochemical profile and evaluate hepato-protective activity of *Elytraria acaulis*.

Qualitative phytochemical screening of *E. acaulis* extracts revealed the presence of different phytochemical constituents like steroids, terpenoids, flavonoids, alkaloids, glycosides, phenols, tannins, saponins and carbohydrates. The hexane extract gave positive results for phytosterols, terpenoids, glycosides, flavonoids, tannins, phenols, oils and negative results for saponins, carbohydrates, amino acids, quinones, alkaloids. The ethyl acetate extract gave positive results for phytosterols, glycosides, saponins, flavonoids, tannins, alkaloids, phenols and negative results for terpenoids, carbohydrates, amino acids, oils, quinones. The hydro-alcoholic extract gave positive results for phytosterols, glycosides, saponins, flavonoids, tannins, alkaloids, phenols, terpenoids, carbohydrates and negative results for amino acids, oils, quinones (Table 1).

Table 1. Qualitative phytochemical analysis of *Elytraria acaulis* root extracts

Name of the Phytochemicals	Extracts of <i>Elytraria acaulis</i>		
	Hexane	Ethyl Acetate	Hydro-Alcoholic
Phytosterols	+	+	+
Terpenoids	+	-	+
Glycosides	+	+	+
Saponins	-	+	+
Flavonoids	+	+	+
Tannins	+	+	+
Carbohydrates	-	-	+
Alkaloids	-	+	+
Amino acids	-	-	-
Oils	+	-	-
Quinones	-	-	-
Phenols	+	+	+

+ = Present; - = Negative

The Quantified phenolic contents of *E. acaulis* extracts were ranging from 2.04±0.12 to 20.48±0.84 (mg/gm). The hydro-alcoholic extract has more phenolic content i.e., 20.48±0.84 (mg/gm) than other extracts. The

quantified flavonoid content was ranging from 4.18±0.73 to 23.84±0.28 (mg/gm). The hydroalcoholic extract has more flavonoid content i.e., 23.84±0.28 (mg/gm) than other extracts (Table 2).

Table 2. Total phenolic and flavonoid contents (mg/gm) of *Elytraria acaulis* extracts

Name of the extract	Total phenolic content	Total flavonoid content
Hexane	2.04±0.12	4.18±0.73
Ethyl acetate	16.24±0.36	20.92±0.66
Methanol	20.48±0.84	23.84±0.28

n=3; mean±SEM

The previous studies from us reported that *Elytraria acaulis* extracts possess antibacterial and antioxidant potentiality [32, 33]. Based on the previous results from us, the current study was aimed to evaluate hepatoprotective activity on paracetamol-induced liver toxicity. The acute toxicity study of *Elytraria acaulis* extracts discloses that those are safe up to tested higher dose i.e., 2000mg/kg b.w. and observed there was no physio-psychological changes in tested animals.

Now a days, the people around the world has been using different medicines inadequately and are leading to various side effects and sometimes causing mortality [34-36]. Paracetamol is on of such drug using as analgesic, and it is effectively work in its suggestable doses. But the over dose and chronic usage is leading to different side effects including liver toxicity. The over usage of paracetamol will affect liver function and cause different illnesses like yellowish skin, blood clotting problems, and confusion [37, 38]. So, there is need to identify new bioactive moieties which are act as broad therapeutic values, be antidotes for these types of drugs, are easily available, and at low cost.

The results of current study reveal that *Elytraria acaulis* extracts possess concentration dependent hepatoprotective activity against liver toxicity caused by paracetamol. The extracts effectively reduced the elevated liver functioning parameters such as AST (SGOT), ALT (SGPT), ALP, and total bilirubin levels (P<0.05). Group I has treated with vehicle showed no significant changes, Group II has treated paracetamol, there is a significant change in levels of biomarker enzymes, group III was administered with paracetamol (200 mg/kg b. w., s.c.) and Liv 52 (25mg/kg per day, p.o.) have significant changes in biomarker enzymes levels compared to group II rats enzymes levels and the percentage protection to be had by Liv 52 against changes in AST (SGOT), ALT (SGPT), ALP and total bilirubin levels were 93.58%, 92.83%, 94.67% and 93.57% respectively. The percentage protection produced by HE in Groups IV, V and VI at doses of 100mg/kg, 200mg/kg and 400mg/kg b.w. on varied AST (SGOT), ALT (SGPT), ALP and total bilirubin levels were 10.20%, 9.70%, 11.55%, and 9.95%, 18.64%, 18.28%, 21.04%, and 18.38%, 36.52%, 35.58%, 38.98%, and 36.45% respectively. The percentage protection produced by the EAE at doses of 100mg/kg, 200mg/kg and 400mg/kg b.w. on varied of AST(SGOT), ALT (SGPT), ALP and total bilirubin levels were 18.39%, 18.00%, 18.25% and 17.46%, 35.52%, 34.60%, 34.18% and 34.00%, 60.20%, 63.29%, 60.92% and 62.17% respectively. The percentage protection produced by the HAE at doses of 100mg/kg, 200mg/kg and 400mg/kg b.w. on AST(SGOT), ALT (SGPT), ALP and total bilirubin levels were 21.54%, 21.94%, 21.20%, and 20.52%, 36.27%, 38.26%, 37.55% and 36.14%, 67.76%, 70.04%, 69.83% and 68.61% respectively. The results were shown in Table 3, Table 4, and Fig. 1.

Table 3. The effect of *Elytraria acaulis* extracts on liver enzymes in the paracetamol-induced liver toxicity

Name of the Drug	AST (U/L)	ALT (U/L)	ALP (U/L)	T. bil (mg/dl)
Control	84.33±1.45	51.50±0.99	215.00±1.46	0.25±0.01
Paracetamol	216.67±1.65	170.00±1.93	531.00±2.78	1.35±0.02
Liv 52 25mg/kg b.w.	92.83±1.08	60.00±1.06	231.83±2.01	0.34±0.01
HE 100mg/kg b.w.	203.17±1.28	158.5±0.85	494.5±1.77	1.25±0.01
HE 200mg/kg b.w.	192±0.58	148.33±1.58	464.5±1.52	1.15±0.00
HE 400mg/kg b.w.	168.33±1.12	127.83±0.83	407.83±1.22	0.96±0.01
EAE 100mg/kg b.w.	192.33±1.20	148.67±0.99	473.33±1.76	1.16±0.01
EAE 200mg/kg b.w.	169.67±0.95	129±0.86	423±1.13	0.98±0.01
EAE 400mg/kg b.w.	137±1.53	95±0.86	338.5±2.13	0.68±0.01
HAE 100 mg/kg b.w.	188.17±1.11	144±1.15	464±1.03	1.13±0.01

HAE 200mg/kg b.w.	168.67±0.99	124.67±1.33	412.33±2.03	0.96±0.01
HAE 400mg/kg b.w.	127±1.53	87±1.77	310.33±1.58	0.61±0.01

n=6 and Mean±SD; HE-Hexane extract, EAE- Ethyl acetate extract, HAE-Hydro-alcoholic extract

Table 4. Percentage (%) protection on enzymes levels due to the effect of *E. acaulis* extracts at different doses on Paracetamol-induced liver toxicity

Name of the enzyme	Amount of the extract								
	100mg/kg b.w			200mg/kg b.w			400mg/kg b.w		
	Name of the extracts			Name of the extracts			Name of the extracts		
	HE	EAE	HAE	HE	EAE	HAE	HE	EAE	HAE
AST (U/L)	10.20	18.39	21.54	18.64	35.52	36.27	36.52	60.20	67.76
ALT (U/L)	9.70	18.00	21.94	18.28	34.60	38.26	35.58	63.29	70.04
ALP (U/L)	11.55	18.25	21.20	21.04	34.18	37.55	38.98	60.92	69.83
T. Bil (mg/dL)	9.95	17.46	20.52	18.38	34.00	36.14	36.45	62.17	68.61

HE-Hexane extract, EAE- Ethyl acetate extract, HAE-Hydro-alcoholic extract. All groups were compared with paracetamol group. Values in the parenthesis indicate percent protection in individual biochemical parameters from their elevated values caused by the hepatoprotection.

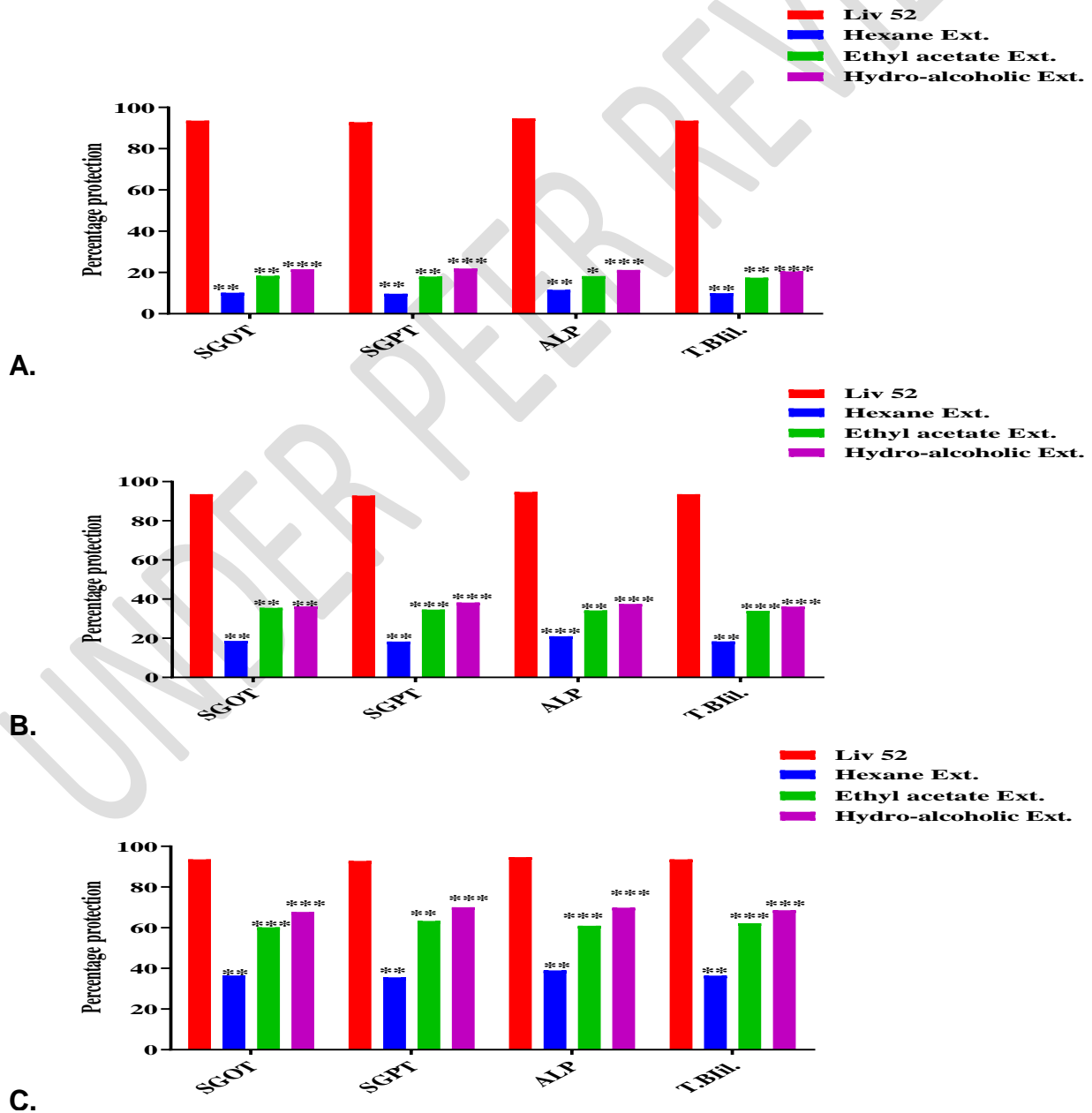


Fig 1. Percentage protection produced by different extracts of *E. acaulis* at a dose of 100mg/kg (A), 200 mg/kg (B) and 400 mg/kg. Results were analysed by using Two-way ANOVA followed by Dunnet's multiple comparison test. All groups were compared with Liv 52 group. ***p<0.001; **p<0.01; *p<0.05; ns= Non significance. ■ Liv 52; ■ hexane extract; ■ ethyl acetate extract; ■ hydro-alcoholic extract

Many medicinal plants are still available and are unexplored about their biological activities. The current study is evidence that provide that *E. acaulis* has bioactive compounds such as phenols, flavonoids, alkaloids, steroids etc., and it has potential antioxidant and hepatoprotective activities. In recent years different medicinal plant formulations were reported with their component compounds like flavonoids, alkaloids and other such bioactive compounds from medicinal plants [39, 40]. The results supports that the extracts of *E. acaulis* will alter the damaged liver functional biomarker enzymes levels as standard drug Liv 52. Liv 52 is an herbal formulation drug having different components that controls liver damage by reducing toxicity other molecules and oxidative stress in the body. The current study provides the biological activities evidence for *E. acaulis* to its; traditional usage and presence of different phytochemical components in them as depends on their extraction solvents to isolate different bioactive components from it and to explore the mechanism of their action on reduction of liver toxicity. The further research is worthful and is under going on our laboratory to isolate individual molecules from *E. acaulis*.

4. CONCLUSION

The present study was aimed to explore the phytochemical profile and evaluate hepatoprotective activity based on our previous antioxidant, antibacterial potentiality of *E. acaulis*. The *E. acaulis* root extracts had showed significant biological activities and the current results offer vital information about the TM value of it. The hydroalcoholic extract possess more phenolic, flavonoid contents and the same extract had showed more potentiality against liver toxicity.

NOTE:

The study highlights the efficacy of "Ayurved" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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