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Research Article

ANTI TOXIC POTENTIAL OF *ECBOLIUM VIRIDE* (FORSK.) ALSTON IN ACETAMINOPHEN PROVOKED HEPATOPATHY

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ABSTRACT

The present study was scrutinized to evaluate protective effect of ethanolic and aqueous extract of leaves of *Ecbolium viride* (Forsk.) Alston on acetaminophen (APAP) provoked toxic hepatitis. Oral administration of APAP at the dose of 2g/kg decreased the activities of antioxidant status (Superoxide dismutase, Catalase, Glutathione peroxidase, Glutathione-S-Transferase and Reduced Glutathione) and increased the lipid peroxidation in hepatic tissue. Furthermore, APAP intoxication diminished the hepatic levels of total ATPase (1.63-fold), Mg^{2+}ATPase, Ca^{2+}ATPase and $Na^{+}K^{+}$ATPase. Treatment with EVEE and EVAE at the dose of (200 & 400 mg/kg p.o), significantly ameliorated the toxic manifestations to normalcy. In conclusion, *Ecbolium viride* may be useful as a potential candidate preventing hepatic damage induced by APAP through its antioxidative and lipid peroxidative effect.

Keywords: Ecbolium viride, acetaminophen, hepatotoxicity, antioxidants, membrane phosphatases.

INTRODUCTION

Reactive oxygen species (ROS), a chief culprit elicits oxidative damage to biomolecules, such as nucleic acids, lipids, and proteins. Thus, the oxidative insult displayed by ROS, alters the biological functions and produces an array of disease conditions, including cancer, diabetes, cardiovascular disease, and many others (Thompson, 2004).Acetaminophen (APAP), a universally used antipyretic drug causes oxidative detoriation of biomolecules due to the generation of free radicals. APAP produces centrilobular hepatic necrosis (Mitchell et al., 1973a).Metabolism of APAP by cytochrome P4502E1 produces *N*-acetylp-benzoquinone imine (NAPQI), leading to the depletion of glutathione (GSH) and covalent adduct formation (Jaeschke et al., 2002). Furthermore, nitric oxide synthesis and superoxide plays a predominant role in APAP induced toxicity due to the generation of peroxynitrites. Our body endogenous antioxidant GSH detoxifies peroxynitrites (Sies et al., 1997), but the exhaustion of GSH by NAPQI (Mitchell et al., 1973b) make the way for peroxynitrites to attack cellular macromolecules by nitrating tyrosine (Beckman, 1996; Pryor and Squadrito, 1995). These serious of events causes distortion of lipid membrane and initiate the peroxidation of lipids (Jaeschke et al., 2002).Further, the reactive metabolite, NAPQI covalently bind to hepatic protein and alters the electron transport chain of the cell which lead to suppression of antioxidant network in the biological system. Peroxidation of membrane lipids, lead to the formation of the oxidation product malondialdehyde (MDA) which eventually affects the functional integrity of cell membranes, but also affects the activities of various membrane-bound enzymes including total ATPase, Mg^{2+}ATPase, Ca^{2+}ATPase and $Na^{+}K^{+}$ATPase (Rauchcova et al., 1995; Hazarika and Sarkar, 2001).

The World Health Organization (WHO) has long recognized and drawn the attention of many countries to the ever increasing interest of the public in the use of medicinal plants and their products in the treatment of various ailments. Plants have wide acceptability by the people and serve as cheaper alternatives to orthodox medicine (Akah & Nwabie, 1994; Akuodor et al., 2010). In this scenario, *Ecbolium viride* (Forsk.) Alston. (Acanthaceae) locally known as "Nilambari", is a perenial woody undershrub found occasional in plains of India and also found in Arabia, Srilanka and tropical Africa. In folk medicine, aqueous extracts of dried roots of the plant are used for menorrhagia, rheumatism and jaundice (Datta and Maiti, 1968; Kirtikar and Basu, 1987). The root juice of this plant was utilized by the rural people in Tirunelveli district of Tamilnadu for the treatment of jaundice by the local native practitioner . Root juice is used as anti-helmintic and also to treat premenstrual colic (Sharma and Sharma, 2010).Previous report had displayed hepaptoprotective potential of *Ecbolium viride* in CCl_4 intoxicated rats (Jayaprakash Narayanan et al., 2012). In this line, the present study was scrutinized to evaluate the phytochemical constituent, antioxidant effect of ethanolic and aqueous extract of *Ecbolium viride* on APAP induced hepatic oxidative damage.

MATERIALS AND METHODS

Chemicals and Reagents

Acetaminophen and Silymarin were purchased from Sigma, St. Louis,USA. Other chemicals used for the investigation were of analytical grade.

Plant Material

The leaves of *E. viride* were collected in the month of September 2010 from, Tamilnadu, South India. The plant material was taxonomically identified and authenticated by Prof.Dr.P.Jayaraman, Director, Plant Anatomy Research Centre, West Tambaram, Chennai. The voucher specimen No PARC/2011/1037 has been preserved in our department for future reference. The plant materials were dried under shade, sliced into small pieces, pulverized using a mechanical grinder, passed through 40-mesh sieve, and stored in an airtight container for further use.

Preparation of Plant Extract

The powdered leaves of *E. viride* were extracted with ethanol and water successively at room temperature. After exhaustive extraction, the solvent was collected and filtered. The solvent was concentrated under reduce pressure at 50-55°C. The concentrated *E. viride* ethanolic extract (EVEE) and *E. viride* aqueous extract (EVAE) were kept in desiccator for further use.

Animals

Male Wistar Albino rats weighing between 100-200 g were used for this purpose. The animals were housed in polypropylene cages and maintained at 24 ± 2°C under 12h light dark cycle and were fed *ad libitum* with standard pellet diet and had free access to water maintenance and use of animals as per the experiment was approved by the institutional Animal Ethics Committee.

Preliminary Phytochemical Screening

The extracts of the plant material were screened for the presence of alkaloids, carbohydrates, phenols, gums and mucilage, flavonoids and terpenes, steroids, proteins, tannins and saponins using standard qualitative methods as described by Harborne (1973).

Study Design

The hepatoprotective activity was tested on seven groups of male albino wistar rats,each group consisting of six animals.
Group I- Served as control, received vehicle 0.5% CMC (1ml/kg ;p.o) for 14 days.
Group II- Toxic control received vehicle for 14 days and on 14th day received APAP (2g/kg; p.o) suspended in 0.5% CMC.
Group III- Received EVAE (200mg/kg; p.o) suspended in 0.5% CMC for 14 days.
Group IV- Received EVAE (400mg/kg; p.o) suspended in 0.5% CMC for 14 days.
Group V- Received EVEE (200mg/kg; p.o) suspended in 0.5% CMC for 14 days.
Group VI- Received EVEE (400mg/kg; p.o) suspended in 0.5% CMC for 14 days.
Group VII- Received standard Silymarin (100mg/kg; p.o) suspended in 0.5% CMC for 14 days.
Similarly, Group III-VII received APAP (2g/kg; p.o) suspended in 0.5% CMC on 14th day 30 min after the administration of extract.
The dose of the extract was selected based on the previous study carried out in our research lab by malarvizhi (2012**)**
At the end of the study, animals were sacrificed by cervical decapitation, 24 h after the last dosage of extract or vehicle administration. The hepatic tissue (homogenized in 0.1M Tris–HCl buffer) obtained from the animals were used for the estimation of the biochemical parameters.

Biochemical Analysis

Lipid peroxidation:

Tissue lipid peroxide (LPO) level was determined as MDA (Ohkawa et al., 1979). The absorbance was measured photometrically at 532 nm and the concentrations were expressed as nmol malondialdehyde (MDA) min/mg/protein.

Assay of antioxidants:

SOD was assayed by the method of (Misra and Fridovich, 1972). Catalase (CAT) level was estimated by the method described by (Sinha, 1972). Glutathione peroxidase (GPx) was assayed by the method of (Rotruck et al., 1973). Glutathione-S-transferase (GST) was assayed by the method of (Habig et al., 1974). Total reduced glutathione (GSH) was determined by the method of (Ellman, 1959).

Assay of membrane bound ATPases in liver homogenate:

The amount of phosphorus liberated by the enzymes were assessed to quatify the activities of total ATPase (Hokins et al., 1973), Ca2+ATPase (Hjerten and Pan, 1983), Mg2+ATPase (Ohinishi et al., 1982) and Na+K+ATPase (Jorgensen, 1988).

Protein content was estimated by the method of (Lowry et al, 1951).

STATISTICAL ANALYSIS

The results were expressed as Mean±SD for six animals in each group. Differences between groups were assessed by one-way analysis of variance (ANOVA) using the SPSS 13.0 software package for Windows. Post hoc testing was performed for inter-group comparisons followed by Tukey's Kramer comparison test. P-values<0.05 have been considered as statistically significant.

RESULTS

Preliminary Phytochemical Screening

Preliminary phytochemical screening of EVAE revealed the presence of alkaloids, carbohydrates phenols, tannins, flavanoids, gums and mucilage, glycosides while all other constituents were absent, while EVEE showed the presence of alkaloids, carbohydrates, phenols, tannins, flavanoids, gums, mucilage, glycosides , sterols , saponins , terpenes, phenols, gums and mucilage, flavonoids and terpenes (Table 1).

Table 1: Preliminary Phytochemical screening of ethanolic and aqueous extract of Ecbolium viride

S. No	Constituents	EVEE	EVAE
1.	Alkaloids	-	+
2.	Carbohydrates	-	+
3.	Protein	-	-
4.	Steroids	-	-
5.	Phenols	+	+
6.	Tannins	+	+
7.	Flavanoids	+	+
8.	Gums and Mucilage	+	+
9.	Glycosides	+	+
10.	Sterols	+	-
11.	Saponins	+	-
12.	Terpenes	+	-

(+) Indicate present; (-) Indicate absent

Effect of APAP, EVEE and EVAE on lipid peroxidation and reduced glutathione

Injection of APAP (2g/kg body weight) induced a significant ($p<0.001$) increase in the level of lipid peroxidation (Fig. 2), which was paralleled by significant ($p<0.001$) reduction in the level of GSH (Fig. 1) in the liver tissue of Group II rats as compared to normal control rats (Group I). Glutathione plays an important role in the regulation of variety of cell functions and in cell protection from oxidative injury. Depletion of GSH results in enhanced lipid peroxidation and excessive lipid peroxidation can cause increased GSH consumption, as observed in the present study. Treatment with EVEE (200 & 400 mg/kg) and EVAE (200 & 400 mg/kg) significantly ($P < 0.001$) counteracted the APAP induced lipid peroxidation and restored the level of GSH to near normalcy.

Effect of APAP, EVEE and EVAE on antioxidant enzymes.

Activities of glutathione-dependent antioxidant enzymes (Gpx and GST) and anti peroxidative enzymes (SOD and CAT) were significantly ($p<0.01$) lower in the liver tissue of APAP intoxicated rats (group II) as compared to control rats (group I). In the present study, treatment with EVEE (200 & 400 mg/kg) and EVAE (200 & 400 mg/kg) significantly ($P < 0.001$) reversed the APAP induced alterations in the activities of antioxidant enzymes (SOD, CAT, GPx and GST) to a near normal status. (Table.2)

Effect of APAP, EVEE and EVAE on levels of membrane-bound ATPases

APAP intoxication resulted in a significant ($P < 0.001$) decrease in total ATPase, Ca^{2+}ATPase, Mg^{2+}ATPase and $Na^{+}K^{+}$ATPase as measured by the levels of phosphorus liberated by the enzymes. Treatment with EVEE (200 & 400 mg/kg) and EVAE (200 & 400 mg/kg) significantly ($P < 0.001$) attenuated the levels of membrane-bound enzymes and protected the animals against APAP induced membrane damage. (Table.3).

Table 2: Effect of APAP, EVEE and EVAE on antioxidant enzymes

Groups	SOD	CAT	GPx	GST
Control- I	3.46 ± 0.03	61.08 ± 0.62	16.30 ± 0.27	53.45 ± 0.41
APAP(2g/kg)-II	1.83 ± 0.04^{a***}	45.63 ± 0.25^{a***}	10.57 ± 0.16^{a***}	38.15 ± 0.56^{a***}
EVAE- III (200mg/kg)+ APAP	2.35 ± 0.02^{b***}	51.15 ± 0.42^{b***}	12.63 ± 0.19^{b***}	43.10 ± 0.37^{b***}
EVAE- IV (400mg/kg)+ APAP	3.11 ± 0.03^{b***}	56.05 ± 0.25^{b***}	14.33 ± 0.29^{b***}	48.53 ± 0.43^{b***}
EVEE-V (200mg/kg))+APAP	2.48 ± 0.03^{b***}	53.45 ± 0.38^{b***}	13.17 ± 0.29^{b***}	46.72 ± 0.69^{b***}
EVEE-VI (400mg/kg))+APAP	3.25 ± 0.03^{b***}	58.73 ± 0.32^{b***}	15.37 ± 0.23^{b***}	51.13 ± 0.50^{b***}
Silymarin-VII (100mg/kg))+APAP	3.31 ± 0.03^{b***}	57.65 ± 0.31^{b***}	15.77 ± 0.17^{b***}	52.25 ± 0.26^{b***}

Comparison were made between a=> Group I vs II, b=> Group II vs III, IV, V, VI and VII. Values are represented as mean ± SEM of 6 animals. The statistical significance at **p < 0.01 and ***p < 0.001.Units: SOD (units/min/ mg of protein); CAT (n moles of H_2O_2 decomposed / min / mg of protein); GST (n moles of CDNB conjugate formed / min / mg of protein); GPx (n moles of GSH oxidised / min / mg of protein)

Table3: Effect of APAP, EVEE and EVAE on membrane bound phosphatases.

Groups	Total ATPase	Na^+K^+ATPase	Mg^{2+}ATPase	Ca^{2+}ATPase
Control- I	4.80± 0.19	9.40 ± 0.15	17.77 ± 0.26	1.21 ± 0.02
APAP(2g/kg)-II	3.38 ± 0.18^{a***}	5.00 ± 0.04^{a***}	11.78 ± 0.29^{a***}	2.50 ± 0.04^{a***}
EVAE- III (200mg/kg)+ APAP	4.03 ± 0.16^{b*}	6.06 ± 0.18^{b*}	13.42 ± 0.14^{b**}	2.31 ± 0.01^{b**}
EVAE- IV (400mg/kg)+ APAP	4.40 ± 0.12^{b***}	8.567± 0.14^{b***}	15.88 ± 0.30^{b***}	1.82 ± 0.02^{b***}
EVEE-V (200mg/kg))+APAP	4.12 ± 0.04^{b**}	7.43 ± 0.46^{b***}	14.02 ± 0.43^{b***}	2.30 ± 0.02^{b**}
EVEE-VI (400mg/kg))+APAP	4.48 ± 0.03^{b***}	9.06 ± 0.21^{b***}	16.53 ± 0.32^{b***}	1.62 ± 0.03^{b***}
Silymarin-VII (100mg/kg))+APAP	4.54 ± 0.04^{b***}	9.21 ± 0.04^{b***}	16.93 ± 0.24^{b***}	1.50 ± 0.03^{b***}

Comparison were made between a=> Group I vs II, b=> Group II vs III, IV, V, VI and VII. Values are represented as mean ± SEM of 6 animals. The statistical significance at *p < 0.05, **p < 0.01 and ***p < 0.001.Units:µmoles of phosphorous liberated/min/mg protein.

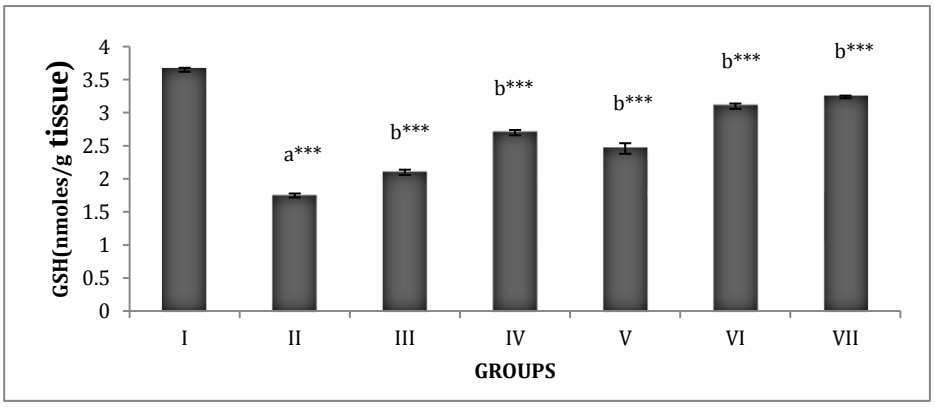

Figure1: Effect of APAP, EVEE and EVAE on reduced glutathione
Results are expressed as Mean±SEM for 6 rats. Comparisons are made between: [a] Group I and Group II; [b] Group II and Group III, IV, V, VI and VII. ***Statistically significant (p<0.001)

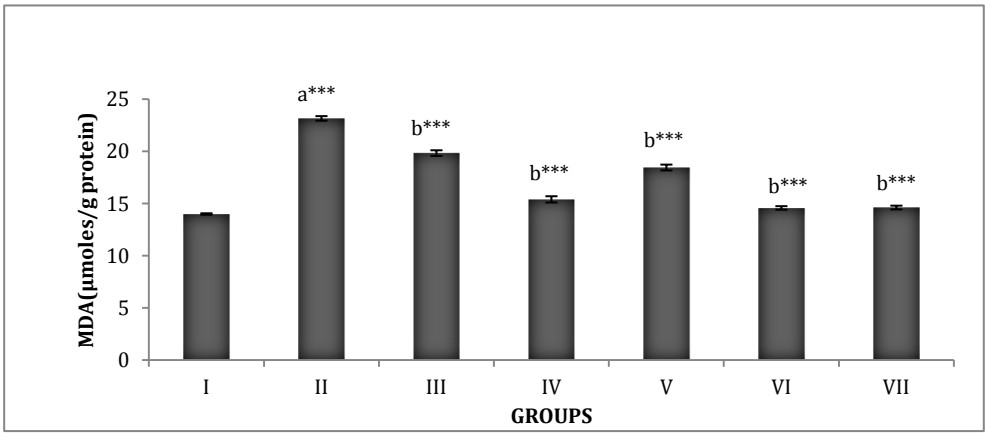

Fig.2. Effect of APAP, EVEE and EVAE on lipid peroxidation.
Results are expressed as Mean±SEM for 6 rats. Comparisons are made between: [a] Group I and Group II; [b] Group II and Group III, IV, V, VI and VII. ***Statistically significant (p<0.001)

DISCUSSION

Reactive Oxygen Species (ROS) include superoxide anion, hydroxyl radical, alkoxyl radical, peroxyl radical, hydrogen peroxide (H_2O_2) and singlet oxygen (Halliwell *et al*., 1995; Simon *et al*., 2000). Superoxide anion itself is not a strong oxidant, but it reacts with protons in water solution to form H_2O_2, which can serve as a substrate for the generation of hydroxyl radicals and singlet oxygen (Stief, 2003). The prevalent free radical states, or so-called oxidative stress, initiate the oxidation of polyunsaturated fatty acid**s** (PUFA), proteins, DNA and sterols. Free radicals generated through APAP metabolism, cause membrane damage by initiating LPO, which leads to impairment in the integrity and function of hepatic membranes. The obtained data reveal that APAP exposure produced a marked oxidative impact as reflected by elevated LPO, measured in terms of MDA level in the hepatic tissue. EVEE and EVAE treated rats showed decreased MDA level, due to significant inhibition of LPO, which is in line with earlier studies (Ref). This might be due to the presence of flavanoids, terpenoids and phenolic compound in the extract, which have been reported to possess anti-lipid peroxidation and/or free radical scavenging properties (Sathiavelu et al., 2009; Singh et al., 2011; Saravanan et al., 2006).

Cells orchestrate an impressive network of antioxidant defensive system (Fang et al., 2002). The present communication shows that the APAP provokes LPO levels that is is accompanied by concomitant decline in the activities of cellular antioxidants. This may be due to the inactivation of cellular antioxidants by lipid peroxides and ROS (Halliwell and Gutteridge, 1984). SOD is inhibited by H_2O_2, while GPx and CAT by an excess of superoxide radical (Pigeolet *et al*., 1990). The diminish in endogenous antioxidant enzymes might affect the hepatic tissue to increased free radical damage, because SOD catalyzes the dismutation of superoxide anion to H_2O_2, while CAT and GPx are involved in cellular detoxification and can convert H_2O_2 into water and oxygen (Konorev *et al*., 1999). GPx is the most important H_2O_2 removing enzyme existing in the membrane. If the activity of CAT or GPx is not adequate to degrade H_2O_2, more H_2O_2 could be converted to toxic hydroxyl radicals and may contribute to the APAP induced oxidative damage. Administration of EVEE and EVAE replenished the antioxidant levels, which might be attributed to the free radical scavenging/antioxidant properties of the extract which is in line with the previous reports (Jayaprakash Narayanan et al., 2012).

Reduced glutathione (GSH), the first line of defense against ROS, is a readily available source of endogenous sulfhydryl (-SH) groups. APAP exposure caused a dramatic decline in GSH level, due to the utilization of GSH during its biotransformation, thereby interfering with the antioxidant functions. The activities of CAT, SOD and GPx were significantly reduced in GSH depleted condition due to pronounced oxidative stress and accumulation of H_2O_2, making the cells more vulnerable to oxidative stress (Rajasekaran et al., 2002). Furthermore, APAP treated rats displayed decreased activities of GSH metabolizing enzyme, GST and it offers protection against LPO by promoting the conjugation of toxic electrophiles with GSH (Jakoby, 1988). EVEE and EVAE treatment restored the normal activities of these enzymes, thereby confirming its protective action. ATPases are lipid dependent, membrane-bound enzymes involved in active transport process and have been implicated in the pathogenesis of liver cell injury (Isreal et al., 1975). Enhanced susceptibility of membrane to lipid peroxides can lead to loss of protein thiol, thereby changing membrane functions (Adhirai and Selvam, 1997). Further, toxic insult of liver can promote a variety of chemical reactions including depletion of GSH, which affect membrane-bound ATPases (Kaplowitz, 2002) as they require SH group to maintain their structure and function (Garner and Garner, 1983). In the present study, Decreased activities of total ATPase, Mg2+ATPase, Ca2+ATPase and Na+K+ATPase were observed in APAP intoxicated rats, which is in corroboration with previous report (Raghavendran et al.,2007). Treatment with EVEE and EVAE restored the levels of membrane bound enzymes to normalcy by upregulating the levels of GSH and diminishing the lipid peroxidation levels, which is in line with previous reports.

CONCLUSION

It is evident from this study that ethanolic and aqueous leaf extract of *E.viridae* attenuated APAP induced hepatic oxidative damage possibly by modulating the activities of the antioxidant status and membrane bound phosphatases and lipid peroxidation, by acting as a potent scavenger of free radicals.

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