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Study on lipid peroxidation and *In vivo* antioxidant activity of various extract of *Dyschoriste littoralis nees* on paracetamol-induced hepatotoxicity rats

Ravi Teja P D[1], Balakrishnan M[2], Kottai Muthu A[*1]

[1]Department of Pharmacy, Annamalai University, Annamalainagar-608 002, Tamilnadu, India
[2]Seshachala College of Pharmacy, Puttur, Chittoor, Andra Pradesh, India

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ABSTRACT

The current investigation was to examine the antioxidant potential of *D.littoralis* Nees. (family Acanthaceae) On paracetamol-induced hepatotoxicity in rats. The aerial parts of *D.littoralis* Nees. The powder was extracted with various solvents (PE, EA, and methanol) through Soxhlet concentrates and various crude concentrates utilized for hepatoprotective activity. Hepatotoxicity was induced by paracetamol (2g/kg b.wt.) on the 5^{th} day of the investigational period and given orally. Paracetamol-induced rats to exhibit elevated activities of TBARS and reduction the enzymes levels such as Superoxide dismutase(SOD), catalase(CAD), Glutathione peroxidise (GPx), Glutathione S transferase(GST) and Glutathione (GSH) in liver and kidney. Furthermore, Oral administration of the ethyl acetate concentrates of *D.littoralis* (200 mg/kgb.wt.) given rats were major reduction the level of TBARS and also significantly elevated the levels of the enzyme such as Superoxide dismutase(SOD), catalase(CAD), Glutathione peroxidise(GPx), Glutathione S transferase(GST) and Glutathione (GSH) in liver and kidney as compared to other concentrates. Thus, results suggested that ethyl acetate concentrates of *D.littoralis* could be afford better antioxidant effect against paracetamol-induced hepatotoxicity rats.

[*]Corresponding Author

Name: Kottai Muthu A
Phone: +919443171712
Email: akottaimuthu@gmail.com

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INTRODUCTION

Reactive oxygen and nitrogen species participate in key roles in the normal physiological process, together with cellular life/death process, protection from pathogens, different cellular signaling pathways, and regulate the vascular tone (Valko *et al.*, 2007). Antioxidants are often used in oils and fatty foods to retard their autoxidation. The generally used synthetic antioxidants at present are BHA, BHT, PG, and TBHQ. However, these drugs are assumed accountable for liver harm and performing as carcinogens in lab animals. Therefore, the importance of the search for natural antioxidants has greatly increased in recent years (Jayaprakasha *et al.*, 2003). Ethnomedical literature contains a huge amount of herbs that may be used for the various diseases, in which ROS and free radical participate vital responsibility. A huge numerical herbs are used for strong antioxidant activity (Badami *et al.*, 2003). Current reports revealed that there is an opposite relationship between the food intake of antioxidant-rich foods and the incidence of human disorders (Halliwell, 1997).

Dyschoriste littoralis Nees. (family Acanthaceae) Leaves were used for the treatment of wounds *Dyschoriste* genus were generally known as snake herb. *D.littoralis* used as the treatment of pain, fever, and inflammation. *D.littoralis*is was used for the treatment of severe coughs along with administrated ginger. *D.littoralis*is leaves were used for asthma in the form cigarettes (Awan *et al.*, 2014), and *D.littoralis*is was used treatment of diarrhea and dysentery. (Hemant *et al.*, 2013). *D.littoralis*is have various activities like anti-microbial (Hemant *et al.*, 2013) and wound healing. (Subha *et al.*, 2017). Still, no literature available on the lipid peroxidation and antioxidant activity of *D.littoralis*. Thus, the study to assess hepatoprotective activities of *D.littoralis* in paracetamol induced rats.

MATERIALS AND METHODS

Chemicals

Paracetamol was purchased from Sigma-Aldrich, the USA utilized in the experiment. All other chemicals and reagents were utilized AR grade

Gathering and Identification of Plant materials

The aerial parts of *D.littoralis* Nees. (Acanthaceae) were gathered from Tirunelveli District, Tamil Nadu, India. Plant identification was made from Survey of Medicinal Plant Unit (SMP), Govt. Siddha Medical College, Palayamkottai, Tirunelveli, Tamil Nadu (Voucher No: 25834). The *D.littoralis* were desiccated under shadowy, segregate, crushed through a grinder. (Satheeshkumar *et al.*, 2011).

Preparation of Plant Concentrates

The pulverized materials were progressively concentratesed with PE (40-60^{0}C) through hot constant percolation method in Soxhlet equipment (Harborne, 1984) for twenty-four hours. At that moment, the marc was used to EA (76-78^{0}C) for twenty-four hours & then mark was subjected to methanol for twenty-four hours. The concentrates were concentrated through the rotational evaporator and subjected to solidify drying in a lyophilizer till dry powder was acquired (Shajiselvin and KottaiMuthu, 2010).

Animals

Male Wister rats of 17-18 weeks age, weighing 160-185g, were collected from the Central Animal House, MNR College of Pharmacy, Sangareddy, Hyderabad, Telangana, India. The animals were set aside in cages, 2 per cage, with twelve: twelve hours light and dark cycle at 25 $\pm$ 2 ºC. The rats were maintained on their particular diets and water *ad libitum*. Animal Ethical Committee's clearance was approved by the Ethical Committee of MNR College of Pharmacy, Sangareddy, Hyderabad, Telangana(CPCSEA/COP/05/ 21-01-2019).

Experimental design

Acute toxicity test

Albino Wistar rats were separated in six groups, and each groups contain six animals (n = 6). Rats were fasted for four hours with free access to water only. The various concentrates of *D.littoralis* suspended in normal saline: 0.5% CMC was administered orally at a dose of 5 mg/kg at first, and mortality was noted for three days. The mortality was noted in 5/6 or 6/6 animals, and then the dose administered was measured as a toxic dose. However, the mortality was noted in less than 4 rats. Out of 6 rats, then the same dose was repeated again to confirm the toxic effect. If mortality was not noted, the procedure was repeated for higher doses i.e., 2000mg/kg.

Hepatoprotective activity

Animals were separated in six groups, and each groups contain six animals.

Group I

Animals served as Control group received with vehicle (0.5% CMC) for 7 days.

Group II

Animals served as a negative control, received 7 days only 1ml vehicle and paracetamol 2g/kg b.wt. given on the 5^{th}-day by orally

Group III

Animals received Pet. Ether concentrates of *D.littoralis* 200mg/kg b.wt, orally for seven days. On 5^{th} day onwards paracetamol 2g/kg b.wt. Administered by orally.

Group IV

Animals received ethyl acetate concentrates of *D.littoralis* 200mg/kg b.wt, orally for seven days. On 5^{th} day onwards paracetamol 2g/kg b.wt. Administered by orally.

Group V

Animals received a methanolic concentrates of *D.littoralis* 200mg/kg b.wt, orally for seven days. On 5^{th} day onwards paracetamol 2g/kg b.wt. Administered by orally.

Group VI

Animals received 25mg/kg b.wt of Silymarin by oral for seven days and on 5^{th} day onwards paracetamol 2g/kg b.wt. Administered by orally.

Groups III, IV, and V rats were orally fed with the various concentrates of *D. littoralis*(PE, EA, and

methanol), and Group VI rats were fed with silymarin. Both the *D. littoralis* concentrates and silymarin were suspended in 0.5% CMC individually and fed to the particular rats through oral intubation. Next day of experiments 8^{th} day, all the animals were sacrificed through cervical decapitation (Sivakrishnan and KottaiMuthu, 2014). Liver and kidney tissues (250mg) were sliced into pieces and homogenized in appropriate buffer under cold condition (pH 7.0) to give 20% homogenate (w/v). The homogenate was centrifuged at 1000 rpm for 10 min at 0 °C in cold centrifuge. The supernatant was used for biochemical estimations.

Liver marker enzymes

(Niehaus and Samuelsson, 1968) method was utilized for the estimation of thiobarbituric acid reactive substances(TBARS). (Kakkar *et al.*, 1984) method was used for the determination of Superoxide dismutase (SOD), (Sinha, 1972) method was used for the determination of Catalase (CAT), (Rotruck *et al.*, 1973) method was used for the determination of Glutathione Peroxidase (GPx), Habig, WH et al., 1974 method was used for the determination of Glutathione –S-Transferase(GST) and (Ellman, 1959) method was used for the determination of Glutathione (GSH), (Lowry *et al.*, 1951) method was used for the measurement of protein.

Statistical Analysis

The statistical investigation was conducted by ANOVA, and groups were compared through Duncan's Multiple Range Test (DMRT)using SPSS Software Package, version 10.0. Results were expressed as means ±standard deviation for six rats in each group. A value of $P \leq 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

The activity of various concentrates of *D.littoralis* on tissues TBARS in paracetamol-induced hepatotoxicity on Wistar rats

Table 1 appeared the activity of various concentrates of *D.littoralis* on tissue TBARS in normal and paracetamol-induced hepatotoxic rats. The hepatotoxic control group showed increased the concentration of TBARS. The administration of ethyl acetate concentrates of aerial parts of *D.littoralis,* and silymarin treated a group of rats attenuated the reduced the level of TBARS.

Effect of various concentrates of *D.littoralis* on tissue enzymatic antioxidant in paracetamol-induced hepatotoxicity in Wistar rats

Table 2 shows the effect of various concentrates of aerial parts of *D.littoralis* on tissue SOD in paracetamol-induced hepatotoxic rats. The hepatotoxic control group showed decreased activities of SOD in the liver (3.57±0.02), kidney(4.03±0.05). Administration of PE concentrates of *D.littoralis* showed considerably SOD in the liver (3.71±0.06) and kidney(4.20±0.03) group III rats. Administration of EA concentrates of *D.littoralis* showed considerably SOD in the liver (5.80±0.02) and kidney(5.21±0.02) in group IV rats. Administration of methanol concentrates of *D.littoralis* showed considerably SOD in the liver (4.28±0.03) and kidney(4.48±0.06) in group V rats. The administration of EA concentrates of *D.littoralis* attenuated the concentration of SOD in liver and kidney, and silymarin restored enzyme activities to normal values.

Table 3 shows the effect of various concentrates of aerial parts of *D.littoralis* on tissue CAD in paracetamol-induced hepatotoxic rats. The hepatotoxic control group showed decreased activities of CAD in the liver (1.11±0.06), kidney(1.27±0.02). Administration of PE concentrates of *D.littoralis* showed considerably CAD in the liver (1.25±0.04) and kidney(1.40±0.07) group III rats. Administration of EA concentrates of *D.littoralis* showed considerably CAD in the liver (2.84±0.04) and kidney(2.87±0.08) in group IV rats. Administration of methanol concentrates of *D.littoralis* showed considerably CAD in the liver (1.62±0.05) and kidney(1.68±0.05) in group V rats. The administration of EA concentrates of *D.littoralis* attenuated the concentration of CAD in liver and kidney, and silymarin restored enzyme activities to normal values.

Table 4 and Table 5 shows the effect of various concentrates of aerial parts of *D.littoralis* on tissue Glutathione peroxidase and GST in paracetamol-induced hepatotoxic rats. The hepatotoxic control group showed decreased activities of Glutathione peroxidase in liver (8.68 ± 0.08),kidney(9.69 ± 0.05)and GST in liver (11.49 ± 0.10),kidney(12.42 ± 0.12). Administration of PE concentrates of *D.littoralis* showed considerably Glutathione peroxidase in liver (9.42 ± 0.06),kidney(10.15 ± 0.06)and GST in liver (11.72 ± 0.18),kidney(12.76 ± 0.08) in group III rats. Administration of EA concentrates of *D.littoralis* showed considerably Glutathione peroxidase in liver (17.89±0.08),kidney(16.75 ± 0.06) and GST in liver (22.45 ± 0.12),kidney(23.43 ± 0.12) in group IV rats. Administration of methanol concentrates of *D.littoralis* showed considerably Glutathione peroxidase in liver (12.14± 0.05),kidney(12.65± 0.08)and GST in liver (13.48 ± 0.12),kidney(14.29 ± 0.13) in group V rats. The administration of EA concentrates of *D.littoralis* attenuated the concen-

Table 1: Effect of various extract of *Dyschoriste littoralis* Nees on tissues TBARS in paracetamol-induced hepatotoxicity on Wistar rats

Groups	TBARS (n mol of MDA formed/g tissue)	
	Liver	Kidney
Group I	1.36±0.06	1.37±0.04
Group II	2.72±0.05a*	2.91±0.02a*
Group III	2.62±0.04 **	2.81±0.03 **
Group IV	1.47±0.04 **	1.54±0.02 **
Group V	2.46±0.04 *	2.38±0.05 *
Group VI	1.39±0.05 **	1.41±0.02 **

#Data be articulated as mean ± SEM., n = six rats each group.
P values, *P<0.05; **P<0.01; ns= not significant; compared to Paracetamol group. One way ANOVA followed by Dunnett's test. a→ Group II compared to Group I; b→ Group II compared to Group III, IV, V, and VI

Table 2: Effect of various extract of *Dyschoriste littoralis* Nees on tissues SOD in paracetamol-induced hepatotoxicity on Wistar rats

Groups	SOD (unit min/mg/protein)	
	Liver	Kidney
Group I	6.34 ±0.03	5.68±0.05
Group II	3.57±0.02a*	4.03±0.05 a*
Group III	3.71±0.06 **	4.20±0.03 **
Group IV	5.80±0.02 **	5.21 ±0.02 *
Group V	4.28±0.03 **	4.48±0.06 *
Group VI	5.85±0.07 **	5.94±0.03 **

#Statistical data and details of group I-VI are the same as in Table 1

Table 3: Effect of various extract of *Dyschoriste littoralis* Nees on tissues CAT in paracetamol - induced hepatotoxicity on Wistar rats

Groups	CAT (μ moles of H_2O_2 consumed min/mg/protein)	
	Liver	Kidney
Group I	3.26±0.08	3.42 ± 0.04
Group II	1.11±0.06 a*	1.27±0.02 a**
Group III	1.25±0.04 **	1.40±0.07 **
Group IV	2.84±0.04 **	2.87±0.08 **
Group V	1.62±0.05 *	1.68±0.05 **
Group VI	2.97±0.02 **	3.21±0.06 *

#Statistical data and details of group I-VI are the same as in Table 1

tration of Glutathione peroxidase in liver and GST in liver and kidney, and silymarin restored enzyme activities to normal values. Table 5 as follows,

The various concentrates of D.littoralis on GSH in tissue from normal and paracetamol-induced hepatotoxic rats were depicted in Table 6. The hepatotoxic control group showed a reduction of GSH in liver 0.65 ± 0.08and kidney as 0.52 ± 0.04. Oral administration of PE concentrates showed considerable GSH in liver 0.72 ± 0.06and kidney as 0.64 ± 0.06 in group III rats. Oral administration of ethyl acetate concentrates showed a marked reduction in GSH in liver 1.22 ± 0.11 and kidney as 1.12 ± 0.10. Oral administration of methanol concentrates showed reduced the level of GSH in liver 0.89 ± 0.10 and kidney as 0.78 ± 0.12 in group V rats. The administration of EA concentrates of *D.littoralis* and silymarin treated rats were significantly elevated the level of GSH in the liver and kidney as compared to PE and methanol concentrates.

In the present study shown that a lethal dose of various concentrates of *D.littoralis* were showed the safety of concentrates. Administration of various concentrates of *D.littoralis* in rats did not change

Table 4: Effect of various extract of *Dyschoriste littoralis* Nees on tissues Glutathione peroxidase (GPx) in paracetamol-induced hepatotoxicity on Wistar

Groups	GPx (mg of glutathione consumed/min/mg protein	
	Liver	Kidney
Group I	21.29 ± 0.04	23.85±0.05
Group II	8.68±0.08 a*	9.69±0.05a*
Group III	9.42±0.06 *	10.15±0.06 **
Group IV	17.89±0.08 **	16.75±0.06 *
Group V	12.14±0.05 **	12.65±0.08 **
Group VI	19.58±0.08 *	19.12±0.04 **

#Statistical data and details of group I-VI are the same as in Table 1

Table 5: Effect of various extract of *Dyschoriste littoralis* Nees on tissues Glutathione-S-Transferase (GST) in paracetamol-induced hepatotoxicity on Wistar rats

Groups	GST ((m mole of CDNB – GSH – Conjugate to /min/mg protein)	
	Liver	Kidney
Group I	26.48 ± 0.12	28.64± 0.14
Group II	11.49 ± 0.10 a*	12.42 ± 0.12 a*
Group III	11.72 ± 0.18 **	12.76 ± 0.08 **
Group IV	22.45 ± 0.12 *	23.43 ± 0.12 **
Group V	13.48 ± 0.12 **	14.29 ± 0.13 **
Group VI	24.64 ± 0.10 **	25.16 ± 0.12 *

#Statistical data and details of group I-VI are the same as in Table 1

Table 6: Effect of various extract of *Dyschoriste littoralis* Nees on tissues Glutathione (GSH) in paracetamol-inducedhepatotoxicity on Wistar rats

Groups	GSH(mg/g tissue)	
	Liver	Kidney
Group I	1.66 ±0.10	1.42 ±0.12
Group II	0.65 ±0.08 a*	0.52 ±0.04 a*
Group III	0.72 ±0.06 **	0.64 ±0.06 *
Group IV	1.22 ±0.11 **	1.12 ±0.10 **
Group V	0.89 ±0.10 *	0.78 ±0.12 **
Group VI	1.35 ±0.02 **	1.23 ±0.10 *

#Statistical data and details of group I-VI are the same as in Table 1

any autonomic or behavioral reaction. There was no mortality of various concentrates of *D.littoralis* was recorded at 2000mg/kg. Therefore we have calculated and selected for 200 mg/kg b.wt. *D.littoralis* concentrates was taken for further investigation.

In addition, paracetamol-induced liver injure due to increased lipid peroxidation, as indicated by the major increase in TBARS. Increased TBARS level in paracetamol-treated rats indicates the major responsibility of lipid peroxidation in the initiation of oxidative stress. Excessive ROS generation triggers the process of lipid peroxidation in cell membranes and destroys cell components and cell death. (Hasanein and Sharifi, 2017) reported that hepatic lipid peroxidation increases through paracetamol toxicity. In the present study, *D.littoralis* administered rats found that the reduced formation of TBARS in paracetamol-induced rats and thereby protected the liver tissue through antioxidant capacity.

The antioxidant enzymes such as SOD, CAT, and GPx are the most important defense systems against O_2- and H_2O_2 mediated damage (Birben *et al.*, 2012). The levels of first-line antioxidant enzymes were significantly exhausted in paracetamol-induced rats. This lacking functioning of free radical-scavenging

enzymes leads to the gathering of highly reactive free radicals and resulting in deteriorating alterations (Madkour and Daim, 2013). However, pretreatment with *D.littoralis* and silymarin significantly elevated antioxidant enzymes SOD, CAT, and GPx in paracetamol-induced rats. Glutathione (GSH) is a major antioxidant against oxidative stress (Fernández-Checa *et al.*, 1993). GSH also assists as a scavenger to toxic electrophiles such as NAPQI (Guo *et al.*, 2019). A significant decrease in the GSH level in paracetamol-induced rats suggests that the elevated levels of NAPQI may covalently bind to cell protein, leading to cell necrosis. The cellular GSH depletion is also closely related to the lipid peroxidation and disturbance of intracellular Ca^{2+} induced by toxic agents (Fu *et al.*, 2018). In the present study, the treatment of *Dyschoriste littoralis* clearly enhanced GSH levels and enabled the rapid and efficient depletion of ROS production in paracetamol-induced rats, which may be involved in the counteraction of free-radical species.

CONCLUSIONS

The current study showed that EA concentrates of *D.littoralis* against paracetamol-induced hepatic tissue damage by reducing the activities of TBARS and increase the activities of antioxidant enzymes. From our results, ethyl acetate concentrates of *D.littoralis* had a better antioxidant effect compared to that of two concentrates in paracetamol-induced in rats.

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