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Effect of *Dillenia indica* Linn on Hepatic Phase II Antioxidant Enzymes and Lipid Peroxidation in Swiss Albino Mice.

Borah B[1], and Bharali R[1]*.

Department of Biotechnology, Gopinath Bordoloi Nagar, Gauhati University, Guwahati-781014, Assam.

ABSTRACT

Natural antioxidants present in edible medicinal plants have the ability to induce the expression of various genes which directly or indirectly play vital role in prevention of a particular disease. The present study was undertaken to evaluate the ability of hydroalcoholic extract of *Dillenia indica* fruit in upregulating the expression of hepatic phase II antioxidant enzymes and downregulating lipid peroxidation. Two doses for oral application i.e. 125 mg/kg and 250 mg/kg body weight of mice for 7 days and 14 days have been selected for the study in 6-8 weeks old Swiss albino mice. A standard antioxidant 0.75 % BHA had been used as positive control. The extract of *Dillenia indica* was found to be effective in enhancing the activities of Catalase ($p<0.05$) and Glutathione peroxidise ($p<0.05$) both at low and high dose level. However activities of Glutathione Reductase, Superoxide dismutase, Glutathione S-transferase and Total Glutathione content increased at high dose level only. *Dillenia indica* extract had lowered lipid peroxidation in the liver significantly ($p<0.05$) and thus helps in maintaining the membrane integrity of cells. The study showed the ability of *Dillenia indica* fruit in inducing hepatic phase II antioxidant enzymes and thus enhances the detoxification system in liver.

Keywords: Detoxification, hepatic phase II enzymes, Antioxidant, Glutathione, Lipid Peroxidation, Reactive oxygen species.

Corresponding author

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INTRODUCTION

Liver is the main organ for detoxification and metabolism of toxic compounds. Hepatic phase I enzymes are responsible for conversion of procarcinogens into carcinogen and phase II antioxidant enzymes are mainly responsible for detoxifying carcinogens into water soluble products that can be easily removed from the body [1]. Free radicals such as reactive oxygen species(ROS), reactive nitrogen species(RNS) are generated both by endogenous process in the body and exogenous sources and excess production cause oxidative stress which is responsible for pathology of many diseases [2]. Reactive oxygen species induce almost all forms of DNA damage causing mutation which ultimately leads to gene dysfunction and finally cancer [3].

Under normal physiological condition, antioxidant enzymes such as Catalase (CAT), Glutathione Peroxidase (GPx), Superoxide Dismutase (SOD), Glutathione Reductase (GR), Glutathione S-transferase (GST) as well as non enzymatic antioxidant such as Reduced Glutathione (GSH), Vitamin C, β-carotene etc show protective action against ROS and when the capacity of this antioxidant system decrease, the level of ROS rises [4]. SOD localized in the cytosol and mitochondria is involved in dismutation of superoxide anion formed due to reduction of oxygen to hydrogen peroxide (H_2O_2) [5]. CAT localized in peroxisomes is responsible for removing half of H_2O_2 generated by catalyzing the decomposition of H_2O_2 to water and oxygen [6]. GPx catalyze the detoxification of H_2O_2 by GSH to form oxidized glutathione (GSSG) which is again reduced to sulfhydryl form GSH by GR [7]. GST catalyzes the conjugation of reduced glutathione to a variety of compounds such as drugs, pollutants, pesticides etc. which has a electrophilic centre and detoxify them [8]. Glutathione is found in two redox form- reduced (GSH) and oxidised (GSSG) form. Under normal physiological condition GSH is mainly involved in maintaining apoptosis of cells where protective action is maintained by high GSH/GSSG ratio but upon oxidative stress GSSG converts to GSH which then exits from the cell through specific membrane translocators and induce apoptosis in the cell [9].

Dietary intake of food such cruciferous vegetables, allium vegetables, resveratrol, turmeric, fish oil, grapefruit, quercetin, lycopene, certain beverages etc. at certain dose and duration of time is found to be more effective as modulators of detoxification enzymes in vivo [10]. They contain natural antioxidants and bioactive compounds that have the ability to eliminate free radicals and protect the body from oxidative stress and also prevent lipid and protein oxidation [11]. *Dillenia indica* Linnaeus belonging to Dilleniaceae family is a medium sized evergreen tree, widely distributed in Asian countries and bear fruits (also known as elephant apple) in the month of July-August which ripens in November-December [12]. The plant is found to have good therapeutic values in different diseases and ailments. The fruit is the edible part of the plant and a popular ingredient used in Assamese cuisine and various ethnic communities of Northeast India. It is acidic, sour, bitter, pungent and astringent [13] in taste. Various phytochemicals such as fixed oil, saponin, tannins, sterols, glycosides, free acids are present in *Dillenia indica* [14]. Pharmacologically, *Dillenia indica* has been reported to have antioxidant [15], antidiabetic, antihyperlipidemic [16], antimicrobial [17] and antidiarrheal properties [18].

The aim of this study is to determine the ability of *Dillenia indica* fruit in induction of hepatic phase II antioxidant enzymes involved in detoxification and removal of free radicals generated in the body either by endogenous or exogenous pathway and also in reduction of lipid peroxidation in liver.

MATERIALS AND METHODS

Chemicals

Bovine Serum Albumin, Pyrogallol, Oxidised Glutathione, Reduced Nicotinamide Adenine Dinucleophosphate (NADPH), 5,5-dithiobis-2-nitrobenzoic acid (DTNB), 1-chloro-2,4-dinitrobenzene (CDNB), Reduced Glutathione, Thiobarbituric acid (TBA), Yeast Glutathione Reductase, Butylated Hydroxy Anisole (BHA) were obtained from Sigma Aldrich USA and rest of the chemicals were purchased from SRL and Merck, India.

Test material

The fruit of *Dillenia indica* was collected from various locations around Guwahati, Assam, India. The fruits were cut into pieces, washed, air and shade dried. It was then powdered by a mechanical grinder, passed through a sieve and stored in air tight container. The powdered plant material was extracted with 80% ethanol

in soxhlet apparatus at 60^{0}c and then filtered in Whatman filter paper no 1. The solvent was then removed from the extract under vacuum rotary evaporator and a semi solid mass was obtained. This extract was stored at 4^{0}c for further use.

Test animals

Healthy Swiss albino mice (*Mus musculus*) of both sexes were obtained from Assam College of Vetenary Sciences, Guwahati, Assam and were maintained under hygienic condition in the animal house, Department of Biotechnology, Gauhati University. The animals were provided with standard pellet diet and with conventional 12 hr light and dark cycle. All the animals were handled as per the guidelines of Animal Ethical Committee of Gauhati University.

Experimental grouping of animals

Healthy mice of 6-8 weeks of age were taken for the study. Two doses of the crude hydroalcoholic extract of *Dillenia indica* fruit – A lower dose i.e. 125 mg/kg body weight of mice and a higher dose i.e. 250 mg/kg body weight of mice (saline used as vehicle) was selected for two time intervals- 7 days and 14 days each. 0.75% Butylated Hydroxy Anisole (BHA) had been taken as positive control. The mice were divided into eight groups with six mice in each group.

Group I (negative control): This group of mice was fed with normal diet and 0.2 ml of vehicle (saline) for 7 days.
Group II (negative control): This group of mice was fed with normal diet and 0.2 ml of vehicle (saline) for 14 days.
Group III: This group of mice was fed with normal diet and administered with *Dillenia indica* extract orally at a dose of 125 mg/kg body weight of mice for 7 days
Group IV: This group of mice was fed with normal diet and administered with *Dillenia indica* extract orally at a dose of 125 mg/kg body weight of mice for 14 days.
Group V: This group of mice was fed with normal diet and administered with *Dillenia indica* extract orally at a dose of 250 mg/kg body weight of mice for 7 days.
Group VI: This group of mice was fed with normal diet and administered with *Dillenia indica* extract orally at a dose of 250 mg/kg body weight of mice for 14 days.
Group VII (positive control): This group of animal was fed with normal diet containing 0.75% BHA for 7 days.
Group VIII (positive control): This group of animal was fed with normal diet containing 0.75% BHA for 14 days.

Body weight of mice was recorded both during start and at the end of the experiment. Diet was withheld from the animals on the night prior to the day of termination of experiment. The animals were then sacrificed by cervical dislocation and assay for the carcinogen metabolizing hepatic phase II antioxidant enzymes- catalase, superoxide dismutase, glutathione reductase, glutathione peroxidase, glutathione s-transferase, non-enzyme reduced glutathione, lipid peroxidation and also total protein estimation was done.

Assay methods

Preparation of homogenates – cytosol and microsomal fractions

After sacrificing the animals by cervical dislocation, the liver was perfuse immediately with 0.9% ice cold NaCl solution and thereafter carefully removed and weighed. It was then cut into two fractions and weighed separately. The bigger fraction was homogenized in ice cold 0.15M Tris-KCl buffer pH7.4 to yield 10% w/v homogenate at 4^{0}c. An aliquot of homogenate was used for assay of acid soluble sulphydryl group i.e. reduced glutathione and protein estimation. The rest were centrifuged at 10,000g for 20 mins at 4^{0}c. The supernatant (cytosol fraction) is obtained after discarding any floating lipid layer. The supernatant was diluted appropriately for assay of antioxidant enzymes.

The second fraction of the liver was blotted dry and 500 mg was weighed quickly. It was then homogenized in 2 ml ice cold 1.15% w/v KCl and centrifuged at 10,000g for 20 mins at 4^{0}c. The supernatant was taken and pH was adjusted to 5.4 with acetate buffer pH4.0 and again centrifuged at 10,000 g for 10 mins at 4^{0}c. The pellets were washed by adding 1.15% KCl : glycerol (4:1 v/v) and centrifuging the same at 10,000 g for 10 mins at 4^{0}c and the pellets obtained were resuspended in 0.15 M Tris KCl buffer pH 7.4 : glycerol (4:1

v/v) by one complete stroke to obtain acid microsomal fraction which was then used for assay of lipid peroxidation.

Reduced glutathione

Reduced glutathione was estimated by method described by Moron et al 1979 [19]. 500 µl of homogenates were precipitated by adding 100 µl of 25% TCA and centrifuged at 10,000g for 20 mins at 4^0c. 100 µl of supernatant was taken to which 900 µl of 0.2M sodium phosphate buffer (pH 8.0) and then 2 ml of 0.6 mM DTNB prepared in 0.2 M sodium phosphate buffer was added. The acid soluble sulphydryl group forms a yellow colour complex with DTNB that absorbs at 412 nm and expressed as nmole GSH/gm tissue.

Catalase

Catalase activity was determined by method of Aebi et. al [20] where the rate of decomposition of H_2O_2 is observed. 1 ml of reaction mixture was prepared by adding 0.02 ml of cytosol in 50 mM sodium phosphate buffer pH7.0 to which 0.1 ml of 30 mM H_2O_2 in buffer was added. Absorbance was taken at 240 nm for 3 mins. Specific activity is expressed as µmoles of H_2O_2 consumed /min/mg protein.

Superoxide Dismutase

Superoxide dismutase was assayed by method of Marklund and Marklund ,1974 [21]. 1 ml of reaction mixture was prepared by adding 10 µl of cytosol diluted in 25% triton X-100, 0.1 M sodium phosphate buffer pH8.0, 33 µl of 0.111% EDTA and 60 µl of 0.1mM pyrogallol. Absorbance at 420 nm for 3 mins was taken. One unit of enzyme activity expressed as µmol/mg protein is defined as the quantity of Superoxide dismutase required to produce 50% inhibition of pyrogallol autooxidation.

Glutathione Reductase

Glutathione Reductase was determined by procedure described by Carlberg and Mannervick, 1985 [22]. Reaction mixture of 1 ml was prepared by adding 0.2 M sodium phosphate buffer (pH 7.0), 2mM EDTA, 1mM GSSG and 0.2mM NADPH. 25 µl of cytosol was added and OD was taken at 340 nm for 3 mins. The enzyme activity was measured indirectly by monitoring the oxidation of NADPH followed by decrease in OD/min for 3 mins. One unit of enzyme activity is defined as nmoles NADPH consumed/min/mg protein.

Glutathione Peroxidase

Glutathione Peroxidase activity was measured by coupled assay method described by Paglia and Valentine 1967 [23]. Reaction mixture (1ml) was prepared by adding 50 mM sodium phosphate buffer (pH 7.0) containing 1 mM EDTA, 0.24 U/ml yeast glutathione reductase, 0.3 mM reduced glutathione, 0.2 mM NADPH, 1.5 mM H_2O_2 and 10 µl cytosol sample. Oxidation of NADPH was monitored at 340 nm for 3 mins and 1 unit of enzyme activity is espressed as nmoles of NADPH consumed/min/mg protein.

Glutathione S-transferase

Glutathione S-transferase activity was determined by method of Habig et al 1974 [24]. 1 ml of reaction mixture was prepared by adding 0.334 ml of 100mM phosphate buffer (pH6.5), 0.033 ml of 30 mM CDNB, 0.033 ml of 30 mM reduced glutathione. Incubation for 2 mins was done and then 10 µl of cytosol was added. Absorbance at 340 nm was taken for 3 mins. The specific activity of glutathione S-transferase is expressed as µmoles of GSH-CDNB conjugate formed /min/mg protein using an extinction coefficient of 9.6 $mM^{-1}\,cm^{-1}$.

Lipid Peroxidation

Lipid peroxidation in microsomes was estimated by thiobarbituric acid reactive substances method (TBARS) described by Varshney and Kale, 1990 [25]. 0.3 ml of microsomal sample was mixed with 1.7 ml of 0.15 M Tris KCl buffer (pH7.4), 0.5 ml of 30% TCA and 0.5 ml of 52mM TBA. It was then put in hot water bath for 45 mins at 80^0c and cooled in ice. It was then centrifuged at room temperature for 10 mins at 4000 rpm

and absorbance was taken at 531.8 nm using distilled water as blank. Lipid peroxidation has been estimated by measuring the formation of malondialdehyde(MDA) per mg protein.

Protein estimation

Protein estimation was done by method of Lowry et.al.1951 [26] using bovine serum albumin as standard and absorbance was taken at 660nm.

Statistical Analysis

All the results were expressed as mean ± standard deviation of 6 animals. Two tailed Student's t-test at $p<0.05$ significance level was used to study the significance of differences between the control groups and *Dillenia indica* extract treated and BHA treated groups of mice by using Microsoft office excel of Windows 7.

RESULTS

It has been observed that oral administration of *Dillenia indica* extract did not produce any side-effects and behavioural changes in the experimental animals. Average body weight and feeding pattern of the experimental animals also remained the same. Findings of the present study had been summarized in **Table 1 and 2** and illustrated from **Fig 1-7.**The oral administration of hydroalcoholic extract of *Dillenia indica* fruit had significant ($p<0.05$ and $p<0.01$) time and dose dependent effect on hepatic phase II antioxidant enzymes. No significant difference in the average protein content of liver was observed.

Table 1: Effect of *Dillenia indica* fruit extract on hepatic antioxidant enzyme of control and experimental group of mice.

Experimental groups	Treatment	Duration	PROTEIN (mg/ml)	GST (µmol CDNB-GSH formed/min/ mg protein)	GSH (nmol/ gm tissue)	LPO (MDA formed/ mg protein)
Group I (negative control)	Only vehicle (saline)	7	6.06±0.30	1.12±0.19	10.38±4.77	0.15±0.03
Group II (negative group)	Only vehicle (saline)	14	6.08±0.38	1.50±0.22	11.96±1.88	0.21±0.04
Group III	*Dillenia indica* extract (125 mg/kg body wt. of mice)	7	6.48±0.34	1.22±0.34	11.4±3.89	0.17±0.23
Group IV	*Dillenia indica* extract (125 mg/kg body wt. of mice	14	5.58±0.37	1.23±0.25	14.3±1.59[b]	0.14±0.03[b]
Group V	*Dillenia indica* extract (250 mg/kg body wt. of mice	7	5.93±0.42	1.89±0.25[a]	12.06±1.13	0.09±0.04[b]
Group VI	*Dillenia indica* extract (250 mg/kg body wt. of mice	14	5.73±0.61	1.9±0.30[b]	26.2±2.51[a]	0.06±0.02[a]
Group VII (positive control)	0.75% BHA	7	5.63±0.53	3.36±1.64[b]	14.16±4.56	0.05±0.01[a]
Group VIII (positive control)	0.75% BHA	14	5.7±0.34	6.06±1.99[a]	20.13±5.42[b]	0.05±0.02[a]

Values are expressed as mean ± S.D of 6 mice.
[a]$p<0.01$, [b]$p<0.05$
Abbreviation: GST- Glutathione S-Transferase, GSH- Reduced Glutathione, LPO- Lipid peroxidation

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Table 2: Effect of *Dillenia indica* fruit extract on hepatic antioxidant enzyme of control and experimental group of mice.

Experimental groups	Treatment	Duration	CAT (µmol H_2O_2/ min/ mg protein)	SOD (µmol/ mg protein)	GR (nmol NADPH consumed/ min/ mg protein)	GPx (nmol NADPH consumed/ min/ mg protein)
Group I (negative control)	Only vehicle (saline)	7	84.38±6.80	2.75±1.67	11.3±1.74	10.99±2.12
Group II (negative group)	Only vehicle (saline)	14	90.11±10.65	2.82±0.4	14.04±2.71	11.1±1.82
Group III	*Dillenia indica* extract (125 mg/kg body wt. of mice)	7	93.04±11.66	3.48±1.73	12.45±2.04	14.26±2.59[b]
Group IV	*Dillenia indica* extract (125 mg/kg body wt. of mice	14	114.7±17.49[b]	3.87±1.41	16.97±2.44	20.09±2.80[a]
Group V	*Dillenia indica* extract (250 mg/kg body wt. of mice	7	108.92±12.14[a]	4.78±0.59[b]	30.44±3.95[a]	18.51±1.23[a]
Group VI	*Dillenia indica* extract (250 mg/kg body wt. of mice	14	147.54±6.87[a]	4.84±0.77[a]	44.67±6.07[a]	27.13±3.49[a]
Group VII (positive control)	0.75% BHA	7	144.86±20.43[a]	3.64±1.65	33.05±4.99[a]	20.17±2.43[a]
Group VIII (positive control)	0.75% BHA	14	177.24±17.30[a]	3.94±1.90	49.68±4.55[a]	25.53±3.21[a]

Values are expressed as mean ± S.D of 6 mice.

[a]p<0.01, [b]p<0.05

Abbreviation: - CAT- Catalase, SOD- Superoxide Dismutase, GR- Glutathione Reductase, GPx- Glutathione Peroxidase

Catalase activity was found to be both time and dose dependent when compared with the negative control group. There was significant increase (p<0.01) in CAT activity by 1.29 fold in Group V and 1.63 fold in Group VI when treated with higher dose for 7 and 14 days respectively. With the low dose there was increase (p<0.05) by 1.27 fold in Group IV at 14 days. In the BHA treated positive control group, significant increase (p<0.01) by 1.71 fold in Group VII and 1.90 fold in Group VIII was observed respectively (Fig 1).

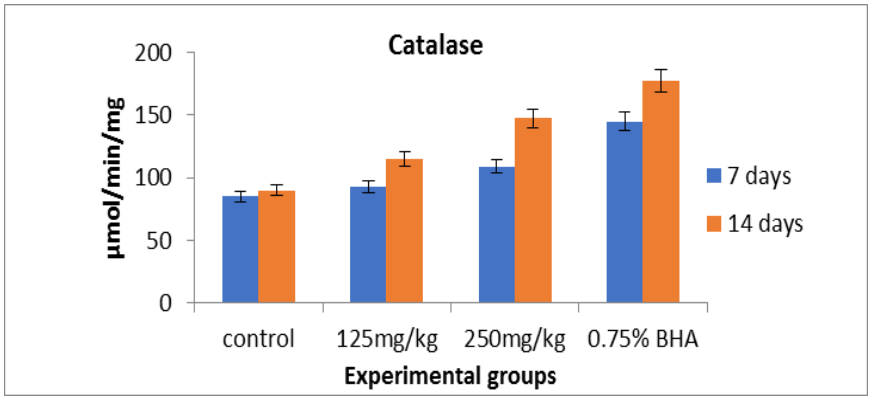

Fig1: Effect of *Dillenia indica* extract (125mg/kg and 250 mg/kg) and BHA on catalase activity of different experimental groups of mice

The activity of SOD increased only at high dose level i.e. at 250mg/kg body wt. of mice by 1.73 fold (p<0.05) in Group V and 1.72 fold (p<0.01) in Group VI at 7 and 14 days respectively when compared with negative control. SOD activity in the BHA treated positive control group was comparable to the negative control group (Fig 2).

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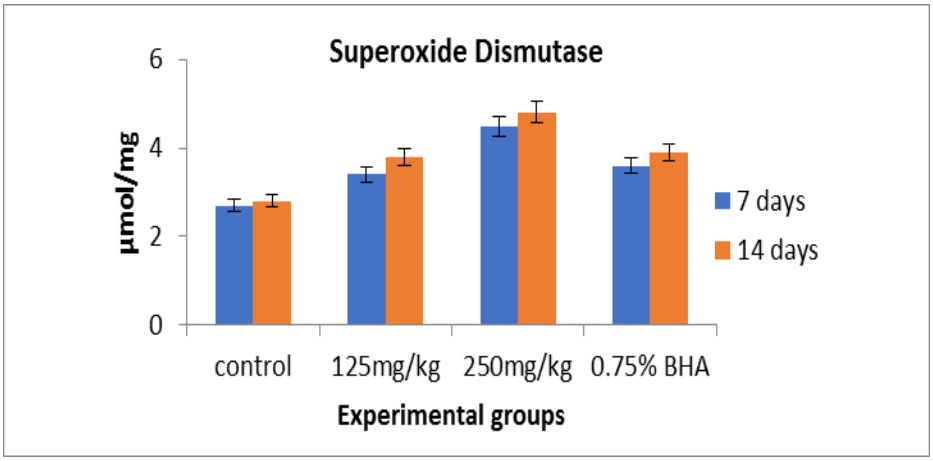

Fig2: Effect of Dillenia indica extract (125mg/kg and 250 mg/kg) and BHA on superoxide dismutase activity of different experimental groups of mice

Glutathione Reductase enzyme showed dose dependent activity with significant induction ($p<0.01$) in the high dose by 2.69 fold in Group V at 7 days and 3.18 fold in Group VI at 14 days in comparison to negative control groups. In the BHA treated Groups VII and VIII there was increase by 2.92 and 3.53 fold respectively (Fig 3).

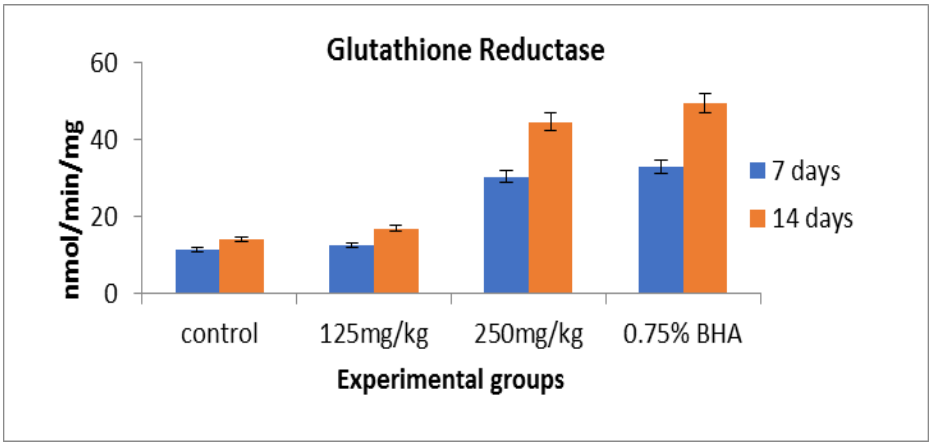

Fig3: Effect of Dillenia indica extract (125mg/kg and 250 mg/kg) and BHA on Glutathione Reductase activity of different experimental groups of mice

As compared to negative control group, Glutathione peroxidase activity showed both time and dose dependent activity with significant increase by 1.2 fold ($p<0.05$) in Group III at 7 days and 1.80 fold ($p<0.01$)in Group IV at 14 days when treated with low dose. With the high dose level GPx activity increased by 1.68 fold ($p<0.01$) in Group V at 7 days and 2.44 fold ($p<0.01$) in Group VI at 14 days. An increase ($p<0.01$) of 1.83 fold in Group VII and 2.30 fold in Group VIII at 7 and 14 days respectively was found when the animals were fed with the BHA treated diet (Fig 4).

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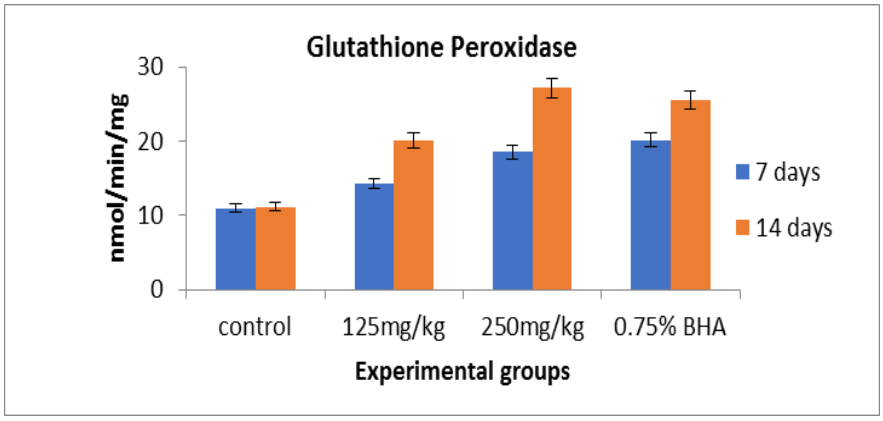

Fig4: Effect of Dillenia indica extract (125mg/kg and 250 mg/kg) and BHA on Glutathione Peroxidase activity of different experimental groups of mice

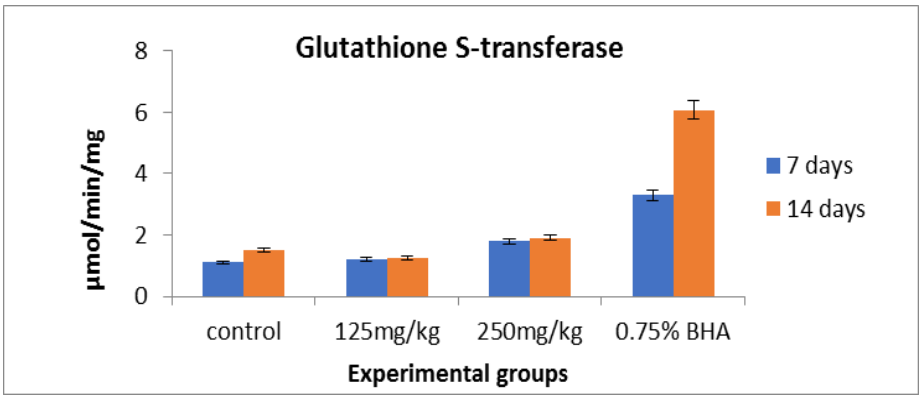

Fig5: Effect of Dillenia indica extract (125mg/kg and 250 mg/kg) and BHA on Glutathione S-transferase activity of different experimental groups of mice

Significant alteration ($p<0.05$) in the GST activity was found in the high dose treated groups by 1.6 fold ($p<0.01$) in Group V and 1.2 folds ($p<0.05$) in Group VI at 7 and 14 days respectively. An increase by 3.0 fold in Group VII at 7 days and 4.04 fold in Group VIII at 14 days has been found in the BHA treated group of animals (Fig 5).

The total reduced glutathione level elevated significantly in a time dependent manner by 1.3 fold in Group IV ($p<0.05$) and 2.19 fold in Group VI ($p<0.01$) at low and high dose respectively for 14 days. The BHA treated group also recorded an increase by 1.68 fold in Group VIII ($p<0.05$) at 14 days only.

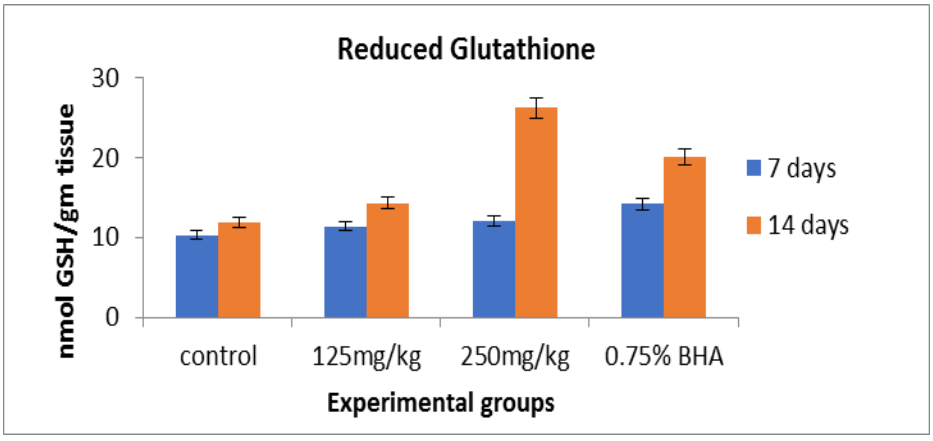

Fig6: Effect of Dillenia indica extract (125mg/kg and 250 mg/kg) and BHA on Glutathione level of different experimental groups of mice

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Compared to the negative control mice Group I and Group II, the lipid peroxidation activity measured as the formation of malondialdehyde (MDA) decreased in a time and dose dependent manner in the experimental groups. Significant decrease by 1.50 fold in Group IV ($p<0.05$) at 14 days has been observed in low dose level whereas in the high dose level decrease upto 1.57 fold in Group V ($p<0.05$) at 7 days and 3.5 fold in Group VI ($p<0.01$) at 14 days was observed. A decrease by 3.0 fold ($p<0.01$) has been observed in both the BHA treated groups (Group VII and Group VIII) (Fig 7).

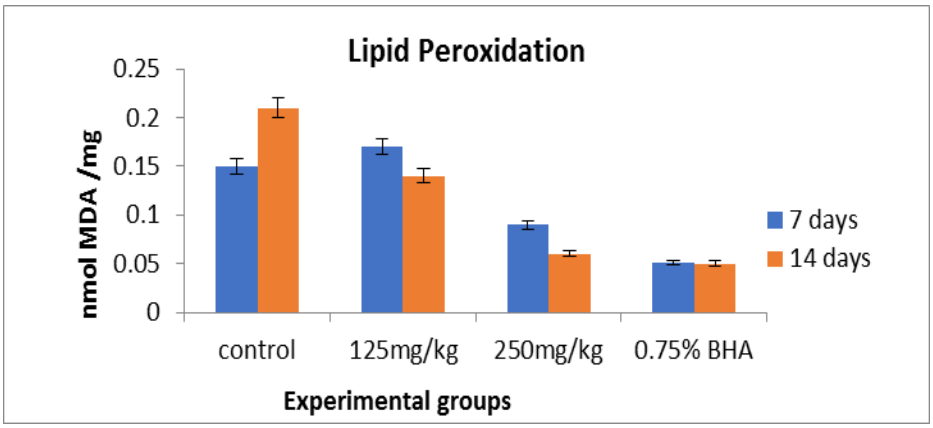

Fig7: Effect of Dillenia indica extract (125mg/kg and 250 mg/kg) and BHA on Lipid peroxidation of different experimental groups of mice

DISCUSSION

Oxidative stress plays a major role in induction of DNA damage and affects on intracellular signal transduction pathways [27]. Imbalance between reactive oxygen species concentration and antioxidant defence mechanism in the body induce oxidative stress and lipid peroxidation which causes damage to tissue and membrane proteins, enzymes and carbohydrates [28]. In the present study *Dillenia indica* fruit has been found to be effective in increasing the activity of hepatic phase II antioxidant enzymes and decreasing lipid peroxidation in a dose and time dependent manner. It has been observed that CAT and GPx activity increased significantly in a time and dose dependent manner whereas GR, SOD and GST activity increased at higher dose only. The total GSH content increased only after 14 days of treatment. The study showed that oral administration of *Dillenia indica* extract has the potential to up regulate the expression of various antioxidant and detoxifying enzymes in the hepatic system. The herbal antioxidants can be used as supplementary medication in drug induced liver- toxicity due to their easy availability and dietary nature [29].

Lipid peroxidation is a process under which oxidants such as free radicals attack lipids containing carbon-carbon double bond especially polyunsaturated fatty acid (PUFA). High level of lipid peroxidation causes oxidative damage to the cell which overwhelms repair capacity and induce apoptosis and necrosis leading to molecular cell damage, ultimately causing various pathological conditions [30,31]. Mice treated orally with *Dillenia indica* fruit extract showed decrease in lipid peroxidation level upto 3.5 fold when high dose was given for 14 days. This indicates that *Dillenia indica* fruit has the ability to decrease cell damage or necrosis caused by lipid peroxidation.

Previous studies have reported that betulinic acid, a pentacyclic lupane-type triterpene is isolated and quantified by HPLC method in *Dillenia indica* fruit (97.9 mg betulinic acid/g of ethyl acetate fraction)[32]. The compound betulinic acid has antioxidant properties which might be responsible for improving tissue redox system and maintaining hepatic antioxidant system by remarkably increasing the levels of GSH,SOD,CAT,GPx and decreasing lipid peroxidation in liver [33]. Triterpenoids are reported to induce nuclear factor erythroid 2-related factor (Nrf2) which is a transcription factor belonging to leucine zipper family and regulates the expression of many detoxification and antioxidant enzymes, molecular chaperones and also stress response proteins [34]. Under normal physiological condition Nrf2 binds to kelch-like ECH-associated protein-1(Keap1) in the cytoplasm but under oxidative stress Nrf2 dissociates from Keap1 and gets activated. The activated Nrf2 translocates into the nucleus, interact with antioxidant response element (ARE) and promotes the expression of antioxidant and detoxifying enzyme [35,36] The increased level of antioxidant enzymes and decreased level

of lipid peroxidation in liver observed in the present study may be due to the presence of the betulinic acid, the phytochemical found in high content in the *Dillenia indica* or may also be due to the synergistic effects of various phytochemicals such as phenols, tannins, sterols, saponins etc present in the fruit together with this triterpenoid. However further study regarding separation, identification and characterization of the novel biomolecule/molecules responsible for the antioxidant activity is necessary. Thus the study indicates that *Dillenia indica* fruit is a natural source with high antioxidant value.

CONCLUSION

Dillenia indica appears to be a promising plant in reducing oxidative stress caused by free radicals. It has the potential to induce the expression of hepatic phase II antioxidant enzymes and lower lipid peroxidation in liver as studied in mice model. But the diverse mechanism and expression level of antioxidant enzyme varies with various diseases and so further studies regarding the expression level of enzymes with respect to its absorption and bioavailability is necessary.

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