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Research Article

REGULATORY EFFECT OF *BOERHAAVIA DIFFUSA* AND BLACK CARAWAY OIL ON HEPATIC, LUNG AND KIDNEY ANTIOXIDANTS ENZYMES CONTENT IN DMBA-INDUCED HYPERCHOLESTEROLEMIA IN RATS

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ABSTRACT

The root extract of *Boerhaavia diffusa* and Black Caraway Oil have potent regulatory effect on Hepatic, Lung and Kidney antioxidants enzymes and malondialdehyde content in DMBA-Induced rats. The present study was carried out to investigate enzymatic properties of *Boerhaavia diffusa* and Black Caraway Oil using drugs. Enzymatic properties (Hepatic, Lung and Kidney) antioxidants enzymes and malondialdehyde content were evaluated in normal and DMBA-Induced rats. Supplementation of this extract by gavage significantly reduces the enzymatic properties. Moreover this supplementation significantly increases the enzymatic activity level in Hepatic, Lung and Kidney as compare to normal control. Elevated enzymatic properties (Hepatic, Lung and Kidney) antioxidants enzymes and malondialdehyde content were diminished significantly by the treatment of *Boerhaavia diffusa* and Black Caraway Oil in respect to infected group. All the above mentioned parameters were restored to the control level. Thus, it is possible, that supplementation of infected rats with dietary *B. diffusa* for would have been equally effective in the protection of DMBA-Induced oxidative damage in hepatic, pulmonary and renal tissues.

Keywords: Antioxidant enzymes, *Boerhaavia diffusa*, Black Caraway Oil, DMBA.

INTRODUCTION

The term "antioxidant" refers to any molecule capable of stabilizing of deactivation free radicals before they attack cells. These are in particular the primary 'antioxidant'. There are also molecules deserving the "antioxidant" team, because they act as chelating agents binding metal ions (redox activity). To protect the cells and organ systems of the body against reactive oxygen species, humans have evolved a highly complex antioxidant protection system. Oxidative stress has been implicated in various pathological conditions involving cardiovascular disease, cancer, neurological disorders, diabetes, ischemia/reperfusion, other diseases and ageing[1-3] .

Cancer is the second most common cause of death after cardiovascular diseases (CVD) in most developed and in many developing countries, including India. In this country, every year around seven million new cases of cancer are being detected. Cancer is a major health problem worldwide. The global burden of cancer doubled during the last 30 years of the last century. In 2008, it is estimated that there were over 12 million new cases of cancer diagnosed, 7 million deaths from cancer and 25 million persons alive with cancer within five years of diagnosis. The continued growth and ageing of the world's population has immediate consequences on the cancer burden. By 2030, it is estimated that there will be over 26 million incident cases of cancer annually[4]. Deaths from cancer worldwide are projected to continue rising, with an estimated 12 million deaths in 2030[5]. Over 7 million new cases of cancer are diagnosed annually, and about half of the cases are in developing countries[6].

A century ago, cardiovascular disease hypercholesterolemia (CVD) accounted for fewer than 10% of all deaths; today, it accounts for approximately 30% worldwide[7-8] The increase in CVD, through a proliferation of risk factors that are heavily influenced by lifestyle choices, is the new challenge for many developing countries. India contributed to 17 % of the worldwide CVD mortality in 1990 CVD-related deaths in India[9] are expected to rise from about 3 million in 2000 to 4.8 million in 2020[10]. By 2020, about 42 % of the total deaths in India are projected to be due to cardiovascular causes[11]. During the period 2000-2030, about 35 % of all deaths due to CVD in India are projected to occur in the age group of 35-64 years [10]. By 2010, CVD will be the leading cause of death in developing countries. According to World Health Organization (WHO) estimates, in 2005, 17.5 million people died of CVD.

Boerhaavia diffusa (B. diffusa) commonly known as rakta punarnava[12] of family Nyctaginaceae[13]. The plant possesses diuretic and cardio tonic activity[14], hypotensive[15], and anticancerous activity[16]. The preliminary phytochemical studies of ethanolic root extract of *B. diffusa* showed the presence of alkaloids, flavonoids and saponins. Any of these phytoconstituents may be responsible for this pharmacological activity, however detail study yet to be undertaken in order to confirm the clear mode of the pharmalogical action. The purpose of this work was to study of *Boerhaavia Diffusa* and Black Caraway Oil on Oxidative modification of LDL, highly atherogenic Sd-LDL and Lb-LDL in DMBA-Induced Rats. Its English name is Black Cumin or Black Caraway. It should be noted that the latter two names bear no relation to the plants Cumin (*Cuminum cyminum*, Linne) and Caraway (*Caram carvi,* Linne) that belong to the botanical family *Umbelliferae*. It was first identified and described by Linnaeus in 1753. The detailed taxonomy of the plant was described by Muschler[17]. Protective effect of liver damage[18], Chronic cyclosporine nephrotoxicity[19], Oxidative stress [20], Antioxidative and antihistaminic[21], Hepatotoxicity and antioxidant[22], Proliferation and biochemical marker levels of Hep-2 cells [23], Heart rate, some hematological values and pancreatic beta-cell[24], Hepatoprotective effects[25], Antimicrobial activity[26] Antiepileptogenic and antioxidant effect[27], Hypolipidemic and hypoglycemic properties [28], Immunomodulatory[29], diabetic neuropathy [30].

XO exists in plasma, liver, kidney and endothelial cells but not in smooth muscle cells [31]. It has been reported that some flavonoids and structurally related antioxidants inhibit XO activity[32-33]. The tripeptide γ-glutamylcysteinylglycine or GSH is the major nonenzymatic regulator of intracellular redox homeostasis, ubiquitously present in all cell types at millimolar concentration[34]. Glutathione can thus directly scavenge free radicals or act as a substrate for glutathione peroxidase (Gpx) and glutathione-S-transfe*Ras*es (GSTs) during the detoxification of hydrogen peroxide, lipid hydroperoxides and electrophilic compounds. Glutathione peroxidases constitute a family of enzymes, which are capable of reducing a variety of organic and inorganic hydroperoxides to the corresponding hydroxy compounds, utilizing GSH and/or other reducing equivalents. There are several tissue specific Gpx's that exhibit also tissue-specific functions. During catalysis, the oxidation state of the enzyme depends on the relative concentration of the reducing (GSH) and oxidized (hydroperoxides) substrates [35].

SOD catalyzes the conversion of superoxide anion to hydrogen peroxide and oxygen. SOD activity was discovered by McCord and Fridovich in 1969; they later proved that the enzyme is required to sustain life in aerobic conditions [36]. Gpx is a selenium-dependent enzyme (selenoprotein). The substrate of the enzyme is reduced glutathione (GSH), and therefore it depends indirectly on the flavoprotein glutathione reductase (Gred) and cellular NADPH concentration. Glutathione can thus directly scavenge free radicals or act as a substrate for glutathione peroxidase (Gpx) and glutathione-S-transfe*Ras*es (GSTs) during the detoxification of hydrogen peroxide, lipid hydroperoxides and electrophilic compounds. Glutathione S-transfe*Ras*es (GSTS) are three enzymes families-cytosolic, mitochondrial and microsomal-that detoxify noxious electrophilic xenobiotics, such as chemical carcinogens, environmental pollutants and antitumor agents. Glutathione reductase is a flavoenzyme and is represented by a single-copy gene in humans. It has been observed that exposure to agents that lead to increased oxidative stress also leads to an increase in its mRNA content. Further experimental data have shown the importance of GRed activity in GSH metabolism, demonstrating that the enzymatic activity is regulated in response to stress, and that mutations affecting GRed activity would have deleterious consequences. The recycling pathway for GSH formation is thus fundamental in the metabolism of GSH dependent defence reactions. In conclusion, the presence of GSH is essential, but not in itself sufficient, to prevent the cytotoxicity of ROS, being of fundamental importance the functionality of the glutathione-dependent enzymes, which participate in the first and second lines of defence.

This review is mainly intended to provide a comprehensive knowledge regarding our existing knowledge of the pharmacological and toxicological actions of this plant. It is hoped that the provided knowledge will generate a real clinical appraisal and evaluation of the effectiveness of at least the *B. diffusa* and Black Caraway Oil in the treatment of some cardiovascular diseases (hypercholesterolemia), cancer and regulatory effect on Hepatic, Lung and Kidney antioxidants enzymes and malondialdehyde content in DMBA-Induced rats.

MATERIALS AND METHODS

Plant material and extraction procedure

Fresh root of *B. diffusa* were collected from Srinagar (Garhwal) and its adjoining areas. The collected plant was identified by Dr. R. L. Painuly, Taxonomist, Department of Botany and Microbiology, H.N.B. Garhwal University, Srinagar, India and the voucher specimen (GUH-20434) has been preserved in our research laboratory for future reference. The plant root dried in shade, coarsely powdered and subjected to soxhlet extraction using 70% hydro-alcoholic solvent (70% ethanol : 30% distilled water), at 48^{0}C for 24 h. The final extract was allowed to evaporate yielded a 7.78% dark brownish solid residue. Black Caraway Oil was purchased local market from Srinagar (Garhwal).

Animals

White male albino rats weighing 150-180 gm were used for the present study, maintained on animal house under normal condition having natural photoperiod (12 hours light/dark cycle) at temperature 25±1^{0}C and 50-60% humidity. Animal experimentation protocols confirm to the Institutional Animal Ethics Committees guidelines. They were provided with standard feed and tap water ad libitum.

Experimental Design

Animals were divided into four groups and for each group fifteen animals were taken. Group I (Normal control) (0.9% NaCl; 5 ml/kg. body weight orally (b. w. o.)) and hypercholesterolemia was induced to other rats by intraperitoneal injection of 7,12-Dimethylbenz[α]anthracene (DMBA) [Sigma-Aldrich Inc., St. Louis USA] (65 mg/kg. body weight). After 3 weeks, animals showing plasma lipid profile level increase 510.36 ± 2.23 mg/dl were considered hypercholesterolemic. The hypercholesterolemic animals were stabilized for 3 weeks and the next day experiment was started. Group II served as hypercholesterolemic infected control, Group III and IV received *B. diffusa* and Black Caraway Oil 2 ml/kg. b. w. o two equal does) regulatory effect on Hepatic, Lung and Kidney antioxidants enzymes content in DMBA-Induced rats. At the 16 week all the animals were sacrificed and evaluated for the enzymatic activity.

Group I- Normal control (N-C)

Group II- DMBA-Induced infected control (I-C)

Group III- Infected *B. diffusa* treated (I-BdT) [2 ml/Kg b.w]

Group IV- Infected Black Caraway Oil treated (I-BCOT) [2 ml/Kg b.w]

Collection of blood

At the end of the experiment treatment, overnight fasted rats in each group were anaesthetized and blood drawn from cardiac puncture. The blood from each rat in a given group was collected in heparinised tubes, mixed gently by inversion 2-3 times and incubated at 4^{0}C for 2 h. Plasma was separated from blood by centrifugation at 2,500 rpm for 30 min, aliquoted and either stored at 4^{0}C or frozen at –20^{0}C for use in other experiments.

Biochemical lipid profile assays

Lipid profiles (Plasma lipids) biomarker enzyme was evaluated in normal and hypercholesterolemic rats.

Assay of xanthine oxidase activity

Xanthine oxidase activity in plasma, liver, lung and kidney homogenate was assayed by the method described by Noro *et al.* (1983)[37] with suitable modification. The reaction mixture consisted of 70 mM sodium phosphate buffer, pH 7.4, 10 µl of plasma or 100 µl of 10 % liver, lung or kidney homogenate and 8 mM xanthine. After a 5 min pre-incubation, the reaction was started by the addition of xanthine and incubation was carried out for 15 min at 37^{0}C. The reaction was terminated by the addition of 0.5 ml ice-cold 10 % perchloric acid, followed by incubation for 10 min at room temperature. Samples were then centrifuged at 4,000 rpm for 10 min. The absorbance of the supernatant was recorded at 290 nm against a reagent blank in a Beckman DU 640 spectrophotometer. Xanthine oxidase activity was calculated by using a standard uric acid.

Determination of (antioxidant enzymes) catalase activity in erythrocytes, liver, lung and kidney

Enzymatic activity of catalase in erythrocytes hemolysate and post-mitochondrial supernatant (PMS) of liver, lung and kidney was carried out according to the procedure described by Sinha, 1972[38]. The reaction was carried out in a total volume of 1.0 ml containing 10 mM phosphate buffer, pH 7.0 and erythrocytes hemolysate equivalent to 22-36 µg hemoglobin 1060-1074 µg liver, 600-648 µg lung and 721-657 µg kidney of PMS protein. For zero time control, hydrogen peroxide was added after stopping the reaction. The tubes were heated in a boiling water bath for 15 min, cooled and the optical density was measured at 590 nm against a reagent blank in a Beckman DU 640 spectrophotometer.

Determination of superoxide dismutase activity in erythrocytes, liver, lung and kidney

Enzymatic activity of superoxide dismutase (SOD) in erythrocytes hemolysate and PMS fraction of liver, lung and kidney was determined by the method as described by Kakkar *et al.* 1984[39]. SOD activity was calculated in terms of an arbitrary unit, which is defined as the enzyme concentration required inhibiting the chromogen formation by 50 % in one min under the above assay conditions.

Determination of total, free and protein-bound-sulfhydryl content of glutathione in liver, lung and kidney

The total sulfhydryl content of glutathione in liver, lung and kidney homogenate was determined by the method of Ellman (1959) [40] as modified by Sedlack and Lindsay (1968) [41]. The incubation mixture contained 60 mM tris buffer and 6 mM EDT A, pH 8.2, 3.50-3.75 mg liver, 1.90-2.27 mg lung and 2.20-2.39 mg kidney protein and 0.2 mM DTNB. The reaction mixture was mixed and made up to 5.0 ml by the addition of 3.2 ml absolute methanol. The tubes were

centrifuged at 6,000 rpm for 5 min at 4°C and the absorbance of the clear supernatants were read at 412 nm against a reagent blank in a Beckman DU 640 spectrophotometer.

For the determination of free sulfhydryl group of glutathione content in liver, lung and kidney homogenate, the method of Ellman (1959)[40] as modified by Sedlack and Lindsay (1968) [41] was employed. In order to assay free sulfhydryl content of glutathione, 0.5 ml of 10 % homogenate was precipitated by the addition of 0.5 ml of 4 % sulphosalicylic acid [42]. The absorbance of the sample was read against a reagent blank at 412 nm within 2 minutes of DTNB addition. Free sulfhydryl content in the samples were calculated using a standard calibration curve of reduced glutathione.

Determination of glutathione peroxidase activity in erythrocytes, liver, lung and kidney

Glutathione peroxidase (Gpx) activity in erythrocytes hemolysate and liver lung and kidney homogenate was assayed by a modification of Mill's procedure 2 (1959) as reported by Hafeman *et al.* 1974 [43]. The samples were mixed and optical density was recorded at 412 nm against a reagent blank within 2 min of DTNB addition in a Beckman DU 640 spectrophotometer. The erythrocytes, liver and kidney glutathione peroxidase activity was calculated after deducting the OD value of samples from the zero time control values by utilizing a standard glutathione.

Assay of glutathione reductase activity in erythrocytes, liver, lung and kidney

Glutathione reductase (Gred) activity was determined by the method of Carlberg and Mannervik, 1975[44]. The absorbance of each sample was recorded at 340nm against a reagent blank in a Beckman DU640 spectrophotometer.

Determination of glutathione-S-transferase activity in liver, lung and kidney

The enzymatic activity of glutathione-S-transferase (GST) in liver, lung and kidney PMS fraction was carried out according to the procedure of Habig *et al.* 1974[45]. The assay was carried out in a total volume of 1.0 ml containing 100 mM potassium phosphate buffer, pH 6.5, 1 mM GSH and 265-268µg liver, 180-164µg lung and 150-162 µg kidney PMS protein. The samples were preincubated for 10 min at 30^{0}C, the reaction was started by the addition of 1-chloro 2, 4-dinitrobenzene (CDNB) prepared in absolute ethanol (1 mM) followed by an incubation for 2 and 4 min at 30^{0}C. At the end of incubation the tubes were placed in an ice-bath for 15 min, after which the optical density was recorded at 340 nm against a reagent blank in a Beckman DU 640 spectrophotometer.

Assay of arylesterase activity in plasma and HDL

Plasma and HDL arylesterase activity was determined by the method of Ayub *et al.* (1999) [46] by using phenyl acetate as the substrate. The reaction mixture included 100 mM tris buffer, pH 8.0, 1 mM $CaCl_2$, suitable aliquots of plasma and 1 mM phenyl acetate. The initial rates of hydrolysis (within linear range) were determined spectrophotometrically at 270 nm against a reagent blank. Nonenzymatic hydrolysis of phenyl acetate was substracted from the total rate of hydrolysis. Arylesterase activity in the above samples was calculated by using the molar extinction coefficient of $1.31 \times 10^3\ M^{-1}\ cm^{-1}$ [47].

RESULTS

Average Body Weight and Diet Consumption in each Group of Rats before and after 16 Weeks of *B. diffusa* and Black Caraway Oil Treatment

The average body weight (g) of DMBA-Induced control rats (I-C), *B. diffusa* treated (I-BdT) and Black Caraway Oil treated (I-BCOT) rats was 165, 182 and 180(g), respectively, whereas for normal control (N-C) rats the average body weight was 155 g, whereas, the average body weight of N-C, I-BdT and I-BCOT rats showed a significant gain of 38 %, 48 % and 24 % respectively after treatment. On the other hand, average diet consumption/group/day (g) of I-C, I-BdT and I-BCOT was 175, 191 and 191 (g), respectively, whereas, the average diet consumption of N-C, I-BdT and I-BCOT rats showed a significant gain of 3 %,5 % and 5 % respectively after 16 weeks of treatment. In addition, there was no significant change on average diet consumption of I-C rats (Table 1), in comparison to their initial diet consumption (before treatment). These results demonstrate that in DMBA-Induced *B. diffusa* treated rats the average diet consumption and the gain in body weight after 16 weeks was significantly higher than rats in N-C and I-BCOT groups.

Xanthine oxidase activity

We have investigated the possible inhibitory effect of *B. Diffusa* and Black Caraway Oil on plasma, hepatic, lung and kidney xanthine oxidase activity in DMBA-Induced rats. As seen in **Table 2**, plasma, hepatic, lung and kidney xanthine oxidase activity was significantly increased from 6.32, 0.612, 0.392 and 0.179 units in N-C to 10.04 (59 %), 0.850 (39%), 0.575 (47 %) and 0.456 (155 %) units in DMBA-Induced rats (I-C). *B. diffusa* had a significant effect in preventing this increase in xanthine oxidase activity of plasma, hepatic, lung and kidney and reduced them by 29 %, 25 %, 8 % and 36 %, respectively, when compared to the corresponding values in I-C. Similarly, treatment of DMBA-Induced rats with Black Caraway Oil was associated with a significant decline of 24 %, 16 %, 9 % and 36 %, respectively, in plasma, hepatic, lung and kidney xanthine oxidase activity, when compared to the corresponding values of I-C rats. These results demonstrate that both *B. Diffusa* and Black Caraway Oil, in addition to their potent antioxidant activity also significantly inhibited the elevated levels of xanthine oxidase associated with plasma, liver, lung and kidney of DMBA-Induced rats, indicating a dual therapeutic benefit in the treatment of these disorders.

Table 1: Average body weight and diet consumption in each group of rats before and after 16 weeks of *b. Diffusa* (i-bdt) and black caraway oil (i-bcot) treatment

Group	Average body weight/rat (g)		Average diet consumption/group/day (g)	
	Before treatment	After Treatment	Before treatment	After treatment
N-C	155.24±2.66*	215.16±12.61 (+38.59%)[a]	185±4.00	191.64±9.40 (+3.58%)
I-C	165.85±3.91*	196.11±16.12 (+18.24%)[a]	175±5.12	170±4.12 (-2.85%)
I-BdT	182.26±3.22*	232.21±12.16 (+48.25%)[a]	191.23±6.62	220±10.11 (+15.04%)[a]
I-BCOT	180.21±5.06*	242.32±11.46 (+23.92%)[a]	191.23±6.62	201.64±3.66 (+5.45%)[a]

*Values are mean ± SD from 15 rats in each group.

N-C, normal control; I-C, Infected control; I-BdT, through orally in two equal doses of 2ml/Kg b.w.orally and I-BCOT, given through orally in two equal doses of 2ml/Kg b.w.orally for 16 weeks.

Significantly different from N-C at [a]p<0.001.

Significantly different from I-C at [a]p<0.001.

Table 2: *B. diffusa* (i-bdt) and black caraway oil (i-bcot) on plasma, liver, lung and kidney xanthine oxidase activity in dmba-induced rats after 16 weeks of treatment

Group	Xanthine oxidase activity			
	Plasma (U/ml)†	Liver (U/mg protein)‡	Lung (U/mg protein)‡	Kidney (U/mg protein)‡
N-C	6.32±0.021*	0.612±0.0021	0.392±0.0011	0.179±0.0011
I-C	10.04±0.126* (+58.86%)[a]	0.850±0.0025 (+38.89%)[a]	0.575±0.0010 (+46.68%)[a]	0.456±0.0011 (+154.75%)[a]
I-BdT	7.11±0.116* (-29.18%)[a]	0.636±0.0016 (-25.18%)[a]	0.526±0.0012 (-8.52%)[a]	0.290±0.0042 (-36.40%)[a]
I-BCOT	7.66±0.152* (-23.70%)[a]	0.712±0.0012 (-16.23%)[a]	0.522±0.0021 (-9.22%)[a]	0.288±0.0060 (-36.84%)[a]

†One unit of enzyme activity is defined as nmole of uric acid formed/min/ml.

‡One unit of enzyme activity is defined as nmole of uric acid formed/min/mg protein.

*Values are mean ± SD homogenate of pooled liver, pooled lung or pooled kidney of 15 rats in each group. N-C, normal control; I-C, Infected control; I-BdT, through orally in two equal doses of 2ml/Kg b.w.orally and I-BCOT, given through orally in two equal doses of 2ml/Kg b.w.orally for 16 weeks.

Significantly different from N-C at [a]p<0.001.

Significantly different from I-C at [a]p<0.001.

Table 3: Impact of *B. diffusa* (i-bdt) and black caraway oil (i-bcot) on liver, lung and kidney catalase and superoxide dismutase activities in dmba-induced rats after treatment

Group	Liver		Lung		Kidney	
	Catalase (U/mg protein)†	Superoxide dismutase (U/mg protein)‡	Catalase (U/mg protein)†	Superoxide dismutase (U/mg protein)‡	Catalase (U/mg protein)†	Superoxide dismutase (U/mg protein)‡
N-C	4.68±0.142*	0.854±0.002	2.51±0.062	2.11±0.006	3.45±0.154	0.932±0.004
I-C	3.32±0.223* (-29.06 %)[a]	0.654±0.002 (-23.42 %)[a]	2.27±0.033 (-9.56 %)[b]	1.883±0.004 (-10.76 %)[a]	2.23±0.052 (-35.36 %)[a]	0.802±0.002 (-13.95 %)[b]
I-BdT	4.22±0.121* (+27.11 %)[a]	0.787±0.003 (+20.34 %)[a]	2.40±0.054 (+5.73 %)[b]	2.140±0.004 (+13.65 %)[a]	3.50±0.042 (+56.95 %)[a]	0.924±0.002 (+15.21 %)[a]
I-BCOT	4.12±0.022* (+24.10 %)[a]	0.770±0.005 (+17.74 %)[a]	2.54±0.022 (+11.89 %)[a]	2.156±0.004 (+14.50%)[a]	3.56±0.166 (+59.64 %)[a]	0.935±0.005 (+16.58 %)[a]

†One unit of enzyme activity is defined as the μmoles of H_2O_2 decomposed/min/mg protein.

‡One unit of enzyme activity is defined as the amount of enzyme required to inhibit O.D. at 560 nm of chromogen production by 50 % in one minute.

*Values are mean ± SD from PMS fraction of pooled liver, pooled lung or pooled kidney of 15 rats in each group.

Significantly different from N-C at [a]p<0.001 and [b]p<0.01.

Significantly different from I-C at [a]p<0.001 and [b]p<0.05.

Antioxidant enzymes activity in erythrocytes, liver, lung and kidney

The status of antioxidant enzymes, such as catalase, superoxide dismutase (SOD), glutathione peroxidase (Gpx), glutathione-S-transferase (GST) and glutathione reductase (Gred) including reduced glutathione (GSH) concentrations in liver, lung and kidney of experimental DMBA-Induced rats are highly important. Catalase activity in liver, lung and kidney were significantly decreased from a value of 4.68, 2.51 and 3.45 units in N-C to 3.32 (29 %), 2.27 (19 %) and 2.23 (35 %) units, in I-C **(Table 3)** respectively. Administration of *B. diffusa* to DMBA-Induced rats (I-BdT) resulted in a significant increase in liver and kidney catalase activities by 4.22 (27 %) and 3.50 (56 %) units, respectively, whereas, catalase activity in lung was increased by only 6 %, when compared to corresponding tissue values of I-C rats. In Black Caraway Oil treated (I-BCOT) group, liver catalase activity was significantly increased by 24 %, whereas, lung and kidney catalase activity were significantly increased and fully restored to normal level. However, in comparison to corresponding tissue values of normal control rats (N-C), the decline in hepatic, lung and kidney SOD activity of DMBA-Induced rats was 23 %, 11 % and 14 %, respectively. Treatment of *B. diffusa* and Black Caraway Oil DMBA-Induced rats resulted in a significant increase in hepatic SOD activity by 20 % and 18 %, respectively, whereas, SOD activities in lung and kidney was fully restored to respective normal values.

Impact on hepatic, lung and kidney total, free and protein-bound –sulfhydryl contents of glutathione

Reduced glutathione, a major non-protein thiol plays a crucial role in coordinating the antioxidant defence processes in the body. Perturbation of GSH status of a biological system can lead to serious consequences. Hepatic total, free and protein-bound-SH contents of glutathione were significantly reduced from a value of 77, 14 and 64 units in N-C to 37 (51 %), 12 (9 %) and 26 (59 %) units in DMBA-Induced rats, respectively. Feeding of *B. diffusa* and Black Caraway Oil to DMBA-Induced rats significantly increased the hepatic total and protein-bound-SH levels of glutathione by 93 % and 129 % in I-BdT and by 89 % and 126 %, in I-BCOT respectively, in comparison to the corresponding values in I-C **(Table 4).** Whereas, free-SH content in *B. diffusa* and Black Caraway Oil treated group was increased and restored to a value similar to N-C rats. Total, free and protein-bound-SH contents of glutathione in lung were significantly decreased in DMBA-Induced rats by 53 %, 17 % and 60 %, respectively, in comparison to the corresponding values in N-C. Administration of *B. diffusa* to DMBA-Induced rats prevented this decrease and increased the lung total and protein-bound-SH levels of glutathione by 103 % and 118 %, respectively, in comparison to the corresponding values in I-C, whereas, free-SH level was fully normalized. Similarly, in I-BCOT rats, an increase of 67 % and 132 %, respectively, in total and protein-bound-SH levels of glutathione was

seen, when compared to the corresponding values in I-C, while no effect was seen on free glutathione. Whereas, kidney total, free and protein-bound-SH contents of glutathione were significantly reduced by 61 %, 12 % and 68 %, respectively, in DMBA-Induced rats, when compared to the corresponding values in N-C. *B. diffusa* treatment to DMBA-Induced rats substantially increased the kidney total and protein-bound-SH levels of glutathione by 146 % and 186 %, respectively, whereas, in I-BCOT rats, a significant increase of 126 % and 160 %, respectively, was observed in both treated groups, which were restored similar to normal value in N-C rats. These results indicate that in general decline in liver, lung and kidney GSH contents of DMBA-Induced rats was significantly blocked after *B. diffusa* and Black Caraway Oil treatment, indicating an antioxidant effect of dietary *B. diffusa* and Black Caraway Oil.

Enzymes activity (Gpx, Gred and GST)

In DMBA-Induced rats, Gpx activity in liver, lung and kidney were significantly increased from a value of 58, 105 and 62 units in N-C to 73 (24 %), 117(12%) and 93 (49 %) units, respectively in I-C rats **(Table 5).** As evident, after treatment with *B. diffusa*, Gpx activity in liver and lung was significantly decreased by 24 % and 21 %, whereas, kidney Gpx activity was fully normalized after *B. diffusa* and Black Caraway Oil treatment. In I-BCOT rats, the Gpx activity in liver, lung and kidney were decreased by 25 % and 21 %, respectively, when compared to corresponding tissue values in I-C group. On the other hand, in DMBA-Induced rats, the enzymatic activities of hepatic Gred and GST were decreased significantly by 23% and 24%, respectively, when compared to corresponding values of N-C rats. Feeding of *B. diffusa* and Black Caraway Oil to DMBA-Induced rats significantly blocked the decrease in hepatic Gred and GST activities and increased them to a similar value of 27 % and 20 %, respectively, Compared to corresponding values of lung Gred and GST activities in N-C, the enzymatic activities of Gred and GST in I-C group were significantly decreased by 22 % and 39 %, respectively, whereas, the enzyme activities in kidney were reduced by 9 % and 40 %, respectively, when compared to corresponding values of N-C. Administration of *B. diffusa* and Black Caraway Oil to DMBA-Induced rats significantly prevented the decrease in Gred activity and increased to a level, which is similar to normal value, whereas, GST activity in kidney was increased by only 50 % and 39 %, respectively, when compared to respective values in I-C rats. In summary, hepatic, lung and kidney catalase, SOD, Gpx, Gred and GST enzymes, which constitute a mutually supportive team of defence against ROS, are significantly decreased in DMBA-Induced rats. However, feeding of *B. diffusa* and Black Caraway Oil substantially quenches these free radicals (ROS), thus positively normalizing the above enzyme levels.

Table 4: Impact of *B. Diffusa* (i-bdt) and black caraway oil (i-bcot) on liver, lung and kidney total, free and protein-bound -sulfhydryl contents of glutathione in dmba-induced rats after treatment

Group	Liver			Lung			Kidney		
	Total-SH	Free-SH	Protein-bound-SH	Total-SH	Free-SH	Protein-bound-SH	Total-SH	Free-SH	Protein-bound-SH
N-C	76.82±1.21*	13.80±0.314	64.52±1.10	66.86±0.561	12.73±0.104	56.11±0.558	58.70±0.406	10.31±0.082	50.30±0.401
I-C	37.51±2.46* (-51.17%)[a]	12.52±0.116 (-9.27%)[a]	26.20±0.462 (-59.39%)[a]	31.16±0.604 (-53.39%)[a]	10.61±0.071 (-16.65 %)[a]	22.31±0.627 (-60.26%)[a]	23.02±0.448 (-60.78%)[a]	9.04±0.026 (-12.32%)[a]	15.87±0.442 (-68.45%)[a]
I-BdT	72.42±1.62* (+93.07%)[a]	14.18±0.146 (+13.26%)[b]	60.08±0.564 (+129.31%)[a]	63.18±0.438 (+102.76%)[a]	16.13±0.104 (+52.03%)[a]	48.65±0.520 (+118.06)[a]	56.75±0.603 (+146.52%)[a]	13.18±0.054 (+45.80%)[a]	45.46±0.611 (+186.45%)[a]
I-BCOT	70.90±1.40* (+88.75%)[a]	13.60±0.262 (+8.63%)[a]	59.11±0.228 (+125.61%)[a]	52.18±1.45 (+67.46%)[a]	10.82±0.050 (+1.98%)[b]	51.67±0.142 (+131.60%)[a]	52.06±0.102 (+126.67%)[a]	12.58±0.064 (+39.16%)[a]	41.38±0.114 (+160.74%)[a]

*Values are mean (nmole SH group/mg protein)± SD homogenate of pooled liver, pooled lung or pooled kidney of 15 rats in each group.

N-C, normal control; I-C, Infected control; I-BdT, through orally in two equal doses of 2ml/Kg b.w.orally and I-BCOT, given through orally in two equal doses of 2ml/Kg b.w.orally for 16 weeks.

Significantly different from N-C at [a]p<0.001.; Significantly different from I-C at [a]p<0.001 and [b]p<0.05.

Table 5: *B. Diffusa* (i-bdt) and black caraway oil (i-bcot) mediated effect on liver, lung and kidney glutathione peroxidase, glutathione reductase and glutathione-s-transferase activities in dmba-induced rats after 16 weeks of treatment

Group	Liver			Lung			Kidney		
	Glutathione peroxidase (U/ mg protein)†	Glutathione reductase (U/ mg protein)‡	Glutathione-S-transferase (U/mg protein)#	Glutathione peroxidase (U/ mg protein)†	Glutathione reductase (U/ mg protein)‡	Glutathione-S-transferase (U/mg protein)#	Glutathione peroxidase (U/ mg protein)†	Glutathione reductase (U/ mg protein)‡	Glutathione-S-transferase (U/mg protein)#
N-C	58.46±1.06*	10.68±0.213	135.22±1.01	104.63±1.66	10.16±0.081	104.26±1.55	62.36±0.641	13.67±0.140	93.62±1.06
I-C	72.61±1.42* (+24.20%)[a]	8.22±0.218 (-23.03%)[a]	102.96±1.11 (-23.86%)[a]	117.48±1.59 (+12.28%)[a]	7.88±0.112 (-22.44 %)[a]	63.31±1.36 (-39.27 %)[a]	93.24±0.465 (+49.52 %)[a]	12.36±0.121 (-9.58%)[a]	56.24±1.11 (-39.93%)[a]
I-BdT	55.22±1.08* (-23.95%)[a]	10.43±0.151 (+26.88%)[a]	123.48±1.22 (+19.93%)[a]	92.86±0.620 (-20.96 %)[a]	11.69±0.081 (+48.35)[a]	87.52±1.02 (+38.24 %)[a]	63.14±0.251 (-32.28%)[a]	15.23±0.169 (+23.22%)[a]	84.26±1.41 (+49.82%)[a]
I-BCOT	54.62±1.81* (-24.78%)[a]	10.63±0.616 (+29.32%)[a]	123.96±1.06 (+20.40%)[a]	92.96±0.864 (-21.87%)[a]	10.88±0.022 (+38.07%)[a]	86.21±1.63 (+36.17%)[a]	62.98±0.452 (-32.45%)[a]	14.99±0.08 (+21.28%)	78.32±1.26 (+39.26%)[a]

†One unit of enzyme activity is defined as nmole oxidized glutathione formed/min/mg homogenate protein. ‡One unit of enzyme activity is defined as nmole NADPH oxidized/min/mg PMS protein.

*Values are mean ± SD from homogenate of pooled liver, pooled lung or pooled kidney and PMS fraction of pooled liver, pooled lung or pooled kidney of 15 rats in each group. Significantly different from N-C at [a]p<0.001.

Significantly different from N-C at [a]p<0.001; Significantly different from I-C at [a]p<0.001.

Arylesterase activity in plasma and HDL

We have also investigated the impact of *B. diffusa* and Black Caraway Oil on plasma, HDL and its subfractions HDL_2 and HDL_3, isolated from plasma of each group by dextran sulfate precipitation method. As shown in **Table 6**, arylesterase activity in plasma, HDL and its subtractions HDL_2 and HDL_3 of DMBA-Induced rats was significantly reduced from 642, 553, 162 and 372 units in N-C rats to 546 (15 %), 466 (16 %), 141 (13 %) and 318 (14 %) units in I-C rats. Treatment of DMBA-Induced rats with *B. diffusa* significantly blocked the decrease in arylesterase activity and increased it by 27 %, 11 %, 11 % and 13 %, respectively, in I-BdT, whereas, in I-BCOT rats, arylesterase activity was increased only by 6 %, 7 %, 5 %, and 8 %, respectively, when compared to corresponding values in I-C group. The distribution of arylesterase activity in HDL and its subfractions, HDL_2 and HDL_3, was 86 %, 29 % and 67 %, respectively, of plasma arylesterase activity in normal control. These results indicate that feeding of 2 ml/Kg.b.w.o. *B. diffusa* was more efficient in terms of increase in HDL-associated arylesterase activity in comparison to I-BCOT rats, which were fed 2 ml/Kg.b.w.o. Black Caraway Oil. Consistent with these results, the ratio of LDL-C to arylesterase activity in I-C rats was significantly increased from 0.907 x 10^{-3} in N-C to 3.02 x 10^{-3} (233 %) in I-C rats. This increase in the ratio of LDL-C to arylesterase activity in I-C rats was significantly reduced to a value of 1.886 x 10^{-3} (37 %), after *B. Diffusa* treatment (I-BdT). Similarly, in I-BCOT rats, ratio value was significantly reduced by1.848 x 10^{-3} (39 %) after treatment. These results suggest that treatment of DMBA-Induced rats with dietary BD and BCO significantly prevented the increase in LDL-C/arylesterase activity ratio and decreased it to a level, which is 81 % or 83 %, respectively, of ratio value of normal control rats indicating a potent antioxidant effect of *B. Diffusa* and Black Caraway Oil.

Table 6: Effect of *B. Diffusa* (i-bdt) and black caraway oil (i-bcot) on arylesterase activity in plasma, hdl, hdl_2, hdl_3, on the ratio of ldl-c: arylesterase in dmba-induced rats after treatment

Group	Arylesterase activity and ratio†				
	Plasma (nmole/min/ml)	HDL (nmole/min/ml)	HDL_2 (nmole/min/ml)	HDL_3 (nmole/min/ml)	LDL-C†: arylesterase activity
N-C	642.11±4.40*	552.92±6.20	162.41±1.36	372.21±2.62	0.9068x10^{-3} ±0.620x10^{-5}
I-C	546.15±5.96* (-14.94 %)[a]	466.22±8.12 (-15.68 %)[a]	141.15±2.62 (-13.09 %)[a]	318.82±2.42 (-14.34 %)[a]	3.02 x10^{-3} ±1.12 x10^{-5} (+233.04 %)[a]
I-BdT	696.70±4.66* (+27.56 %)[a]	518.62±10.21 (+11.24 %)[a]	156.26±2.17 (+10.70 %)[a]	360.46±2.60 (+13.06 %)[a]	1.8861 x10^{-3} ±3.1 x10^{-5} (-37.55 %)[a]
I-BCOT	580.20±6.41* (+6.23 %)[b]	497.31±4.36 (+6.67 %)[b]	148.61±1.46 (+5.28 %)[c]	345.16±2.16 (+8.26 %)[b]	1.8481 x10^{-3} ±0.711 x10^{-5} (-38.80 %)[a]

*Values are mean ± SD from pooled plasma of 15 rats in each group.

N-C, normal control; I-C, Infected control; I-BdT, through orally in two equal doses of 2ml/Kg b.w.orally and I-BCOT, given through orally in two equal doses of 2ml/Kg b.w. orally for 16 weeks.

Significantly different from N-C at [a]p<0.001; Significantly different from I-C at [a]p<0.001, [b]p<0.05 and [c]p<0.02.

DISCUSSION

Several epidemiologic studies have established a strong link DMBA-Inducing and increased cardiac morbidity and mortality. In 16 weeks treatment on the DMBA induced hypercholesterolemic rats significantly reduced the elevated plasma enzymes levels. This indicates that the extract may two equal doses and by continuous treatment it acts effectively.

The average body weight gain of 18 % in DMBA-Induced infected control rats (I-C) rats was significantly lower than a gain of 38 % in normal (N-C) rats (Table 1). This reduction in growth rate in I-C rats did not correlate with the reduction in the amount of average food consumption per day (- 3 % vs. + 4 % in N-C). Therefore, reduced average weight gain in the I-C group may be due to overall adverse effect of massive amount of free radicals and toxic compounds present in the DMBA-Induced. These results are consistent with the previous report showing a lower body weight gain in rats after 3 weeks of DMBA-Induced[48]. Administration of I-C rats with both 2ml/kg.b.w.o *B. diffusa* (I-BdT) and Black Caraway Oil (I-BCOT) treatment was associated with a body weight gain of 48 % and 24 %, as compared to their respective entry point values. Similarly, in *B. diffusa* treated (I-BdT) rats food consumption per day was increased by 15 %, as compared to an increase of only 5 % in Black Caraway Oil treated group (I-BCOT). In contrast, dietary *B. diffusa* (I-BdT) and Black Caraway Oil (I-BCOT) is naturally occurring having no toxicity.

This finding provides a perfect correlation between lipid peroxidation products and decreased activities of CAT and SOD, which play an important role in scavenging the toxic intermediate products of incomplete lipid peroxidation. A decrease in the activity of these enzymes, as seen in liver, lung and kidney of I-C rats (Table 3), can lead to the excessive availability of superoxide's and peroxyl radicals, which in turn generate hydroxyl radicals, resulting in the initiation and propagation of more lipid peroxidation products [49-68]. A similar decrease in hepatic CAT and SOD activities *B. diffusa* and Black Caraway Oil feeding of I-C rats significantly reduced the FFA and lipid peroxidation products, and increased the CAT and SOD activities in liver, lung and kidney, and reversed these parameters to near normal levels.

It is well known that glutathione (GSH) acts as a reducing agent and plays a vital role in detoxification. It provides antioxidant protection in the aqueous phase of cellular systems [50]; its antioxidant activity is through the thiol group of its cysteine residue. Like ascorbic acid, another important water soluble antioxidant, GSH can directly reduce a number of ROS and is oxidized to GSSG in the process. Liver is viewed as a glutathione-generating site, which supplies the kidney and intestine with other constituents for glutathione resynthesis [50]. Intrahepatic glutathione is reported to afford protection against liver dysfunction by at least two ways: (i) as a substrate for glutathione peroxidase (Gpx), GSH serves to reduce large variety of hydroperoxide before they attack unsaturated lipids or convert already formed lipid hydroperoxide to the corresponding hydroxyl compounds; (ii) As a substrate of glutathione-S-transferase (GST), it enables the liver to detoxify foreign compounds or other metabolites and to excrete the products, preferably in to bile. Our results show a decline in GSH (Table 4), Gred and GST activities and an increased GPx activity in liver, lung and kidney of I-C rats (Table 5). In addition, since GSH also acts as substrate and co-substrate in essential enzymatic reactions of Gpx and GST, reduction of GST activity may also be due to decreased levels of GSH in tissues. Gpx is also a scavenging enzyme, but an increase in its activity in tissues of I-C rats may further reduce the GSH content. In addition, an increased Gpx activity represents a compensatory mechanism to degrade H_2O_2. Thus, during oxidative stress, depletion of GSH, which is of clinical importance in tissue injury, mediated a significant

impact on the antioxidant poise of liver cells. However, in their study hepatic GSH content was significantly increased, in DMBA-Induced rats, which is in contradiction to our results.

Treatment of DMBA-Induced hyperlipidemic rats with *B. diffusa* (I-BdT) and Black Caraway Oil (I-BCOT) for 16 weeks, significantly restored the altered tissue activities of SOD, catalase, Gpx, Gred and GST including total, free and protein bound-SH contents of glutathione, to near normal control values, indicating an almost total alleviation of oxidative damage by these antioxidants. Elevated lipid peroxidation products formed by DMBA-Induced may generate a tissue antioxidant/oxidant imbalance that could represent a crucial link between DMBA-Induced and atherosclerosis. Administration of 2ml/Kg.b.w.o *B. diffusa* and Black Caraway Oil to I-C rats significantly rectifies this imbalance, even though the exact mechanism(s) remain obscure. Both *B. diffusa* (I-BdT) and Black Caraway Oil (I-BCOT) mediated a near normalization of peroxide levels and scavenging enzyme activities as well as GSH in liver, lung and kidney of infected rats, indicating a strong antilipid/lipoprotein peroxidative effect of these hypolipidemic agents.

Increasing evidence support an antioxidant role of serum HDL-complexed paraoxonase (PON) or arylesterase enzyme in the protection of LDL as well as HDL from oxidative modification. In addition, depletion of PON results in the loss of the antioxidant function of HDL, and addition of PON to HDL restores the protective function of HDL [51]. In contrast to DMBA-Induced infected rats with severe hyperlipidemia, there was a little decline in cholesterol content of HDL (-14 %) and its subfractions, HDL_2 and HDL_3 and hence a decrease of only 29 % to 24 % in plasma, HDL, HDL_2 and HDL_3-associated arylesterase activity (Table 2). Results suggest that induction of normal control, as seen in infected control rats, is closely associated with the decline of both antiatherogenic HDLs and arylesterase enzyme, which is consistent with a very mild dyslipidemia in I-C rats, and hence only a marginal decline was seen in both HDL associated cholesterol content (-14 %) as well as arylesterase activity (-16 %).

Based on these results in infected rat hyperlipidemic and carcinogenic rats, that a similar *B. diffusa* (I-BdT) and Black Caraway Oil (I-BCOT) mediated amelioration of overall antioxidant defence system, as seen in different tissues of I-C rats, may be operative in infected rats (I-C). Thus, it is possible, that supplementation of normal control with dietary *B. diffusa* for treatment would have been equally effective in the protection of DMBA-Induced oxidative damage in hepatic, pulmonary and renal tissues. These results indicate a potent free radical scavenging property of both *B. diffusa* (I-BdT) and Black Caraway Oil (I-BCOT).

CONCLUSION

It is well known that DMBA-Inducing is associated with a substantial increase in oxidative stress, oxidant stress may be increased owing to a higher production of ROS, which are controlled by antioxidant enzymes, SOD, catalase, Gpx, Gred and GSH. An impaired radical scavenger function has been linked to decreased/increased activity of enzymatic and nonenzymatic antioxidants. Our results show a significant decrease in the activities of antiperoxidative enzymes, CAT, SOD, Gred, GST as well as GSH, in liver, lung and kidney of DMBA-Induced rats, except Gpx activity which was significantly increased. Treatment of DMBA-Induced hyperlipidemic rats with *B. diffusa* and Black Caraway Oil, significantly reversed/restored the altered tissue activities of SOD, catalase, Gpx, Gred and GST including total, to near normal control values, indicating an almost total alleviation of oxidative damage by these antioxidants. Both *B. diffusa* and Black Caraway Oil mediated a near normalization of peroxide levels and scavenging enzyme activities as well as GSH in liver, lung and kidney of infected rats, indicating a strong antilipid/lipoprotein peroxidative effect of these hypolipidemic and anticarcinogenic agents. Based on these results in DMBA-Induced hyperlipidemic and carcinogenic rats in conjunction with the data in infected rats showing that supplementation of *B. diffusa* for significantly improved the integrity of erythrocytes membrane as seen by improved protection against lipid peroxidation as well as reversal of enzymatic activities of CAT, SOD, Gpx and Gred to near normal levels, it is likely, that a similar *B. diffusa* and Black Caraway Oil mediated amelioration of overall antioxidant defence systems, as seen in different tissues of I-C rats, may be operative in infected rats. Thus, it is possible, that supplementation of infected rats with dietary *B. diffusa* for would have been equally effective in the protection of DMBA-Induced oxidative damage in hepatic, pulmonary and renal tissues.

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