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Research Article

ASSOCIATION BETWEEN EFFICIENCY OF CERTAIN MEDICINAL PLANTS AND SEVERITY OF RENAL DISORDERS IN RATS

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ABSTRACT

The objective of this study was to evaluate the protective and curative actions of Rosemary and Black mulberry against renal injury induced by CCl_4 in rats. The evaluation was done through measuring certain oxidative stress markers; malondialdehyde (MDA) and nitric oxide (NO) levels. Kidney function indices; creatinine, urea and serum protein were also estimated. Inflammatory mediators; heat shock protein-70 (HSP-70) and transforming growth factor- β_1 (TGF-β_1) as well as cytotoxicity biomarker; cytochrome p450-2E1 (CYP2E1) were also evaluated. The histopathological analysis of kidney was done for results confirmation. The prophylactic and therapeutic effects of Rosemary and Black mulberry were achieved by the observed decrease in MDA, NO, creatinine, urea, HSP-70, TGF-β_1 and significant increase in CYP2E1. In conclusion, both Rosemary and Black mulberry recorded variable degrees of protection and treatment against renal injury induced by CCl_4 in rats. Further studies are needed to identify the molecules responsible for their pharmacological effects.

Keywords: Renal injury, Carbon tetrachloride, Rosemary, Black mulberry.

INTRODUCTION

Carbon tetrachloride (CCl_4), a clear, heavy, and nonflammable liquid is most widely used for experimental induction of hepatic cirrhosis[1]. It is known to be nephrotoxic as well as hepatotoxic to humans[2, 3]. In addition, it has been identified as a probable human carcinogen based on evidence of tumors in animals[4]. Administration of CCl_4 causes an increase in lipid peroxidation products and a decrease in the activity of enzymes protecting lipid peroxidation in the kidney[5]. These detrimental effects of CCl_4 have been attributed to conversion of CCl_4 to highly toxic trichloromethyl and trichloromethyl peroxyl free radicals by cytochrome P450 enzyme, resulting in cell injury[1].

The general strategy for prevention and treatment of organ damage includes reducing the production of reactive metabolites by using antioxidants[6]. Antioxidants appear to act against diseases by raising the levels of endogenous defense [e.g., by up-regulating gene expressions of the antioxidant enzymes, such as superoxide dismutase (SOD), catalase, glutathione peroxidase, and lipid peroxidase[7].

People all over the world are becoming more conscious of the nutrition value, health benefits and safety of their food and its ingredients[8]. In addition, there is a preference for natural functional food ingredients that are believed to be safer, healthier and less subject to hazards than their artificial counterparts. Evaluation of the functional properties of naturally occurring substances, especially those that are present naturally in human diets, has been of interest in recent years[9].

Black mulberry (*Morus nigra* L., Family Moraceae-often called the mulberry family or fig family) contain large amounts of flavonoid pigments (anthocyanins) that give black mulberries their characteristic red to blue color[10]. The total pigment extract which is produced from black mulberry fruits has been widely used as a natural colorant in beverages, baked products, chewing gums, jellies and fruit wine making[11]. Many studies have demonstrated its antioxidant activities[9, 12- 14] as well as its antidiabetic effect[15, 16].

Rosmarinus officinalis L. (Family Lamiaceae), popularly named rosemary, is a common household plant grown in many parts of the world. Rosemary leaves are used for food flavoring and have been used in folk medicine for many conditions; they have antispasmodic, analgesic, antirheumatic, carminative, cholagogue, diuretic, expectorant, and antiepileptic effects[17]. It also recorded anti proliferative effect against human ovarian cancer cell line *in vitro*[18] and anti antiangiogenic effect[19]. In addition, it has antioxidant effects[20], antimicrobial against bacteria, yeasts and filamentous fungi[21] and anti-colitis effect[22].

The aim of the present work is to evaluate the protective and therapeutic effects of the aqueous extract of Rosemary leaves and juice of Black mulberry fruits on renal injury induced by CCl_4 in rats. The evaluation was done through measuring oxidative stress markers, kidney function indices, inflammatory mediators and the cytotoxicity biomarker. Kidney histopathological analysis was also taken into consideration.

MATERIALS AND METHODS

Chemicals

All chemicals used in the present study were of analytical grade, product of Sigma (US), Merck (Germany) and BDH (England).

Plants collection

Rosemary was purchased from a local market; Harraz Market for Medicinal Herbs, Cairo, Egypt. Voucher specimen (RM-2011) was deposited at Therapeutic Chemistry Department, National Research enter, Egypt, as a reference. Dried leaves were ground in a grinder with 2 mm diameter mesh. The dry powder was kept in tightly closed container until needed.

Black mulberry fruits purchased from local district market (Hyper One Market, 6th October City, Cairo). Fresh fruits were mixed with bidistilled water in a blender, and the resulting juice is filtered through natural muslin cloth in a screw press to separate any impurities. The juice was kept in a dark bottle at -20°C until used.

Plants Extraction

The dried leaves of Rosemary were extracted in a Soxhlet apparatus using hot water (40-60°C) for 72 hours. Excess water of Rosemary extract and Black mulberry juice were dried under vacuum at 40°C, producing semisolid residues.

Animals

Male Wistar albino rats (100 to 120 g) were selected for this study. They were obtained from the Animal House, National Research Center, Egypt. All animals were kept in controlled environment of air and temperature with access of water and diet *ad libitum*.

Ethics

Anesthetic procedures and handling with animals complied with the ethical guidelines of Medical Ethical Committee of National Research Centre, Egypt.

Doses of administration

Rats were subcutaneously injected with a dose of 0.15 ml CCl_4/100g body weight three times for one week[23].

Administration regimes for prophylactic and therapeutic effects were trice a week for five consecutive weeks. Black mulberry was orally administered at a dose of 1.5 g/kg body weight[24]. Rosemary was orally administered at a dose of 500mg/kg body weight[17].

Experimental design

60 male rats were used in this study. Animals were divided into 6 groups (10 rats each). Group 1 served as normal healthy control rats. Group 2 was subcutaneously injected with CCl_4.Groups 3 and 4 were received each of plant extract three times/ week for five consecutive weeks before injection with CCl_4 (three times/ one week) (prophylactic groups). Groups 5 and 6 were forced with CCl_4, and then treated with each of plant extract as the same administration regimens described above. These groups served as the therapeutic groups.

Sample preparations

Serum sample: Blood was collected from each animal by puncture of sublingual vein in clean and dry test tubes, left 10 minutes at room temperature to clot and centrifuged at 3000 rpm for serum separation. The separated serum was stored at -80°C for further determinations of kidney function tests, total protein, inflammatory mediators and anti cytotoxicity biomarker.

Tissue sample: kidney tissue was homogenized in cold 0.9% NaCl (1:10 w/v) solution, centrifuged at 3000 rpm for 10 minutes, separated the supernatant and stored at -80°C for further determination of oxidative stress markers.

Biochemical assays

Malondialdehyde (MDA) was estimated as the product of lipid peroxidation process. Its concentration was calculated using the extinction coefficient value $1.56 < 10^5\ M^{-1}\ cm^{-1}$ and read at 535 nm by the method of Buege and Aust[25].

Nitric oxide (NO); as vasodilatory chemokine was assayed by the method of Moshage et al.[26], where Promega's Griess Reagent System is based on the chemical reaction between sulfanilamide and N-1-naphthylethylenediamine dihydrochloride (NED) under acidic phosphoric acid condition to give colored azo-compound which can be measured colorimetrically at 520 nm.

Creatinine was measured by the method of Bartels and Bohmer[27]. Creatinine in the sample reacts with picrates in alkaline medium forming a colored complex at 500 nm.

Urea was determined by the method of Tabacco et al.[28], where the conversion of urea in the sample by urease enzyme provide a colored complex that can be measured by spectrophotometry at 600 nm.

Serum total protein was assayed according to Bradford[29]. Coomassie Brilliant Blue dye reacts with Bradford reagent to give a blue complex which is measured colorimetrically at 595 nm.

Heat shock protein-70 (HSP-70) was estimated by enzyme-linked immunosorbent assay kit (USCN, Life Science Inc., China). The microtiter plate provided in this kit has been pre-coated with an antibody specific to HSP-70. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for HSP-70, and then avidin conjugated to horseradish peroxidase (HRP) is added to each microplate well and incubated. TMB (3,3′,5,5′-tetramethylbenzidine) substrate solution is also added to each well. Only those wells that contain HSP-70, biotin-conjugated antibody and enzyme-conjugated avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of HSP-70 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Transforming growth factor-β_1 (TGF- β_1) was determined by a sandwich enzyme immunoassay ELISA kit (Kamiya Biomedical Compasny, USA). The microtiter plate provided in this kit has been pre-coated with an antibody specific to TGF-β_1. Test procedures were the same as heat shock protein-70. The color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of TGF-β_1 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Cytochrome P450-2E1 (CYP2E1) was estimated by a sandwich enzyme immunoassay ELISA kit (USCN, Life Science Inc., China). The microtiter plate provided in this kit has been pre-coated with an antibody specific to CYP-450-2E1. Test procedures were the same as heat shock protein-70. The developed color is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of CYP-450-2E1 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Histopathological analysis

Kidney tissues were excised from sacrificed animals, individually weighed, and from them, 5 µm thickness slices were cut, fixed in 10% paraformaldehyde and embedded in paraffin wax blocks. Tissue sections of 5 µm thick were stained with haematoxylin and eosin (H&E) and Masson's trichrome, then examined under light microscope for determination of phathological changes[30].

Statistical analysis & calculations

All data were expressed as mean ± SD of ten rats in each group. Statistical analysis was carried out by one-way analysis of variance (ANOVA), Costat Software Computer Program accompanied with lease significance difference between groups at $p<0.05$.

$$\%\text{change} = \frac{\text{Mean of control} - \text{mean of treated x } 100}{\text{Mean of control}}$$

$$\text{Improvement } (\%) = \frac{\text{Mean of injured group} - \text{mean of treated group x } 100}{\text{Mean of control group}}$$

RESULTS

Potency of Rosemary and Black mulberry on oxidative stress markers of CCl_4 treated rats

Injured kidney by CCl_4 recorded significant increase in malondialdehyde and nitric oxide levels by 45.87 and 68.19%, respectively as compared with control group (Table 1). The prophylactic action of both Rosemary and Black mulberry recorded improvement in malondialdehyde level by 33.42 and 29.79%, respectively, while nitric oxide showed improvement by 36.85 and 54.28%, respectively. The therapeutic action of both plant extracts showed improvement by 33.83 and 26.05% for Rosemary and Black mulberry, respectively, while nitric oxide showed improvement by 40.06 and 48.62% (Fig.1).

Table 1: Effect of Rosemary and Blackberry on oxidative stress markers

Parameters	Control	CCl_4	CCl_4 prophylactic with Rosemary	CCl_4 prophylactic with Blackberry	CCl_4 treated with Rosemary	CCl_4 treated with Blackberry
Malondialdehyde	17.35 ± 0.94^{c}	25.31 ± 0.83^{a}	19.51 ± 1.58^{b}	20.14 ± 0.72^{b}	19.44 ± 0.86^{b}	20.79 ± 0.63^{b}
	----	(+45.87)	(+12.44)	(+16.08)	(+12.04)	(+19.82)
Nitric oxide	6.54 ± 0.27^{c}	11.00 ± 1.16^{a}	8.59 ± 1.75^{b}	7.45 ± 0.66^{bc}	8.38 ± 0.45^{bc}	7.82 ± 0.40^{bc}
	----	(+68.19)	(+31.34)	(+13.91)	(+28.13)	(+19.57)

Values are mean ± SD of five rats in each group. Data are expressed as n mole/g tissue for malondialdehyde and µmole/ g. tissue for nitric oxide. Statistical analysis are carried out using one way analysis of variance (ANOVA) accompanied with least significance difference between groups at $p<0.05$. Unshared super script letters are significant values between each group at $p<0.0001$. Values between brackets are percentages change over control.

Effect of Rosemary and Black mulberry on kidney function indices of CCl_4 treated rats

Rats treated with CCl_4 showed significant increase in creatinine and urea levels by 149.41, 86.01, respectively, while serum protein recorded significant decrease by 43.16% as compared to control group (Table 2). Rosemary and Black mulberry showed prophylactic improvement for creatinine, urea and serum protein by 135.29, 69.32 and 15.78% for Rosemary and 144.70, 35.22 and 14.73%, for Black mulberry, respectively. Treatment with either Rosemary or Black mulberry recorded enhancement in creatinine, urea and serum protein by 145.88, 80.98 and 17.89% for Rosemary and 144.70, 69.54 and 11.57%, for Black mulberry, respectively (Fig.2).

Effect of Rosemary and Black mulberry on inflammatory mediators and cytotoxicity biomarker

The inflammatory mediators; TGF-β_1 and HSP-70 showed significant increase in CCl_4 injured rats by 81.31 and 175.71%, respectively, while CYP2E1 recorded significant decrease by 44.78% as compared with the control group (Table 3). Improvement in TGF-β_1, HSP-70 and CYP2E1 levels were observed by the prophylactic action of Rosemary and Black mulberry extracts. Rosemary recorded prophylactic improvement by 49.88, 46.66 and 14.46% for TGF-β_1, HSP-70 and CYP2E1, respectively, while Black mulberry showed improvement by 40.32, 62.85 and 19.57%, respectively. Treatment of CCl_4 injured rats with Rosemary showed improvement by 24.33, 67.14 and 13.67% for TGF-β_1, HSP-70 and CYP2E1, respectively, while treatment with Black mulberry enhanced the levels of TGF-β_1, HSP-70 and CYP2E1by 26.55, 36.19 and 15.42%, respectively (Fig.3).

Effect of Rosemary and Black mulberry on kidney histopathology

Kidney histopathological features of normal rats showed normal appearance of tubules, glomeruli and tubulointerstitial cells (Fig. 4 a). Collagen deposition was of normal range in control group (Fig. 4 b).

Kidney section of CCl_4-treated rats showed significant morphological damage especially in the renal cortex. Glomerular and tubular degenerations were observed varying from glomerular basement membrane thickening, mild dilatation or congestion of space of Bowman, interstitial inflammation, tubular cell swelling or congestion, tubular brush border loss and tubular dilatation (Fig. 4c). Marked collagen deposition was recorded (Fig. 4d).

Rats treated with aqueous extract of Rosemary and Black mulberry showed almost normal morphology and normal architecture of the kidney (Fig. 5 a and c). Group of CCl_4 prophylactic with aqueous extract of Rosemary and Black mulberry showed normal morphology with the exception of only few swollen glomeruli and rare vascular congestions that were present in both cortical and cortico-medullar regions (Fig. 5 e and g). In all prophylactic and treated groups, mild collagen deposition was observed (Fig. 5 b, d, f and h).

Table 2: Effect of Rosemary and Blackberry on kidney function indices

Parameters	Control	CCl_4	CCl_4 prophylactic with Rosemary	CCl_4 prophylactic with Blackberry	CCl_4 treated with Rosemary	CCl_4 treated with Blackberry
Creatinine	0.85 ± 0.20^b	2.12 ± 0.15^a	0.97 ± 0.04^b	0.89 ± 0.05^b	0.88 ± 0.06^b	0.89 ± 0.05^b
	----	(+149.41)	(+14.11)	(+4.70)	(+3.53)	(+4.70)
Urea	31.03 ± 2.92^d	57.72 ± 6.20^a	36.21 ± 5.71^b	46.79 ± 7.17^c	32.59 ± 4.47^d	36.14 ± 1.77^d
	----	(+86.01)	(+16.69)	(+50.78)	(+5.02)	(+16.46)
Serum protein	23.75 ± 2.21^a	13.50 ± 1.29^c	17.25 ± 1.25^b	17.00 ± 1.80^b	17.75 ± 1.70^b	16.25 ± 2.06^b
	----	(-43.16)	(-27.36)	(-28.42)	(-25.26)	(-31.57)

Values are mean ± SD of five rats in each group. Data are expressed as mg/dL for creatinine, g/dL for urea and mg/ml for serum protein. Statistical analysis are carried out using one way analysis of variance (ANOVA) accompanied with least significance difference between groups at $p<0.05$. Unshared super script letters are significant values between each group at $p<0.0001$. Values between brackets are percentages change over control.

Table 3: Effect of Rosemary and Blackberry on inflammatory mediators and cytotoxicity biomarker

Parameters	Control	CCl_4	CCl_4 prophylactic with Rosemary	CCl_4 prophylactic with Blackberry	CCl_4 treated with Rosemary	CCl_4 treated with Blackberry
TGF-β_1	39.65 ± 2.51^d	71.89 ± 2.84^a	52.11 ± 4.34^c	55.90 ± 2.55^c	62.24 ± 2.07^b	61.36 ± 2.30^b
	----	(+81.31)	(+31.42)	(+40.89)	(+56.97)	(+54.75)
HSP-70	2.10 ± 0.91^c	5.79 ± 0.44^a	4.81 ± 0.44^b	4.47 ± 0.48^b	4.38 ± 0.28^b	5.03 ± 0.17^b
	----	(+175.71)	(+129.04)	(+112.85)	(+108.57)	(+139.52)
GYP2E1	18.80 ± 0.90^a	10.38 ± 0.62^c	13.10 ± 1.16^b	14.06 ± 1.11^b	12.95 ± 0.75^b	13.28 ± 0.75^b
	----	(-44.78)	(-30.31)	(-25.21)	(-31.11)	(-29.36)

Values are mean ± SD of five rats in each group. Data are expressed as Pg/ml for TGF-β_1 and ng/ml for HSP-70 and GYP2E1. Statistical analysis are carried out using one way analysis of variance (ANOVA) accompanied with least significance difference between groups at $p<0.05$. Unshared super script letters are significant values between each group at $p<0.0001$. Values between brackets are percentages change over control.

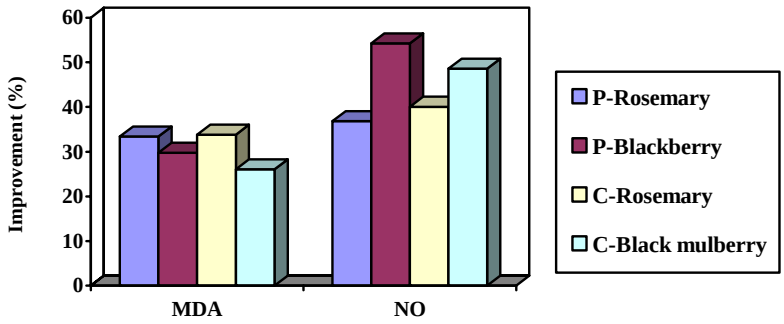

Fig. 1: Improvement percentages of malondialdehyde (MDA) and nitric oxide (NO).

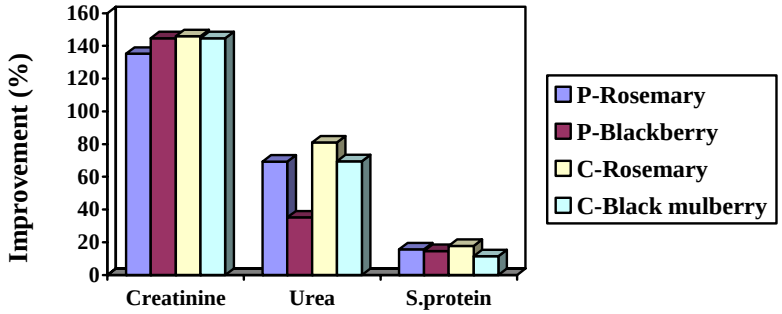

Fig. 2: Improvement percentages of creatinine, urea and serum protein.

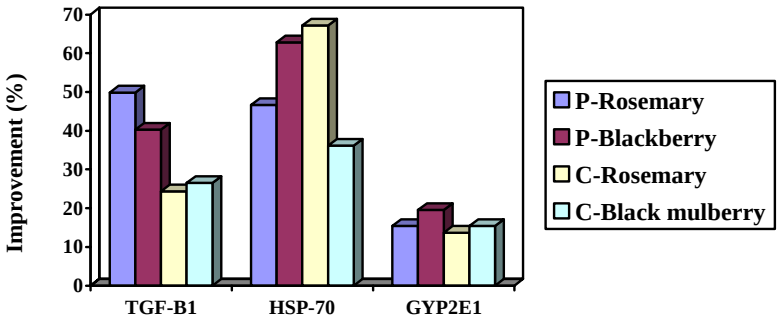

Fig. 3: Improvement percentages of heat shock protein-70, transforming growth factor-$\beta_1$and cytochrome P450-2E1.

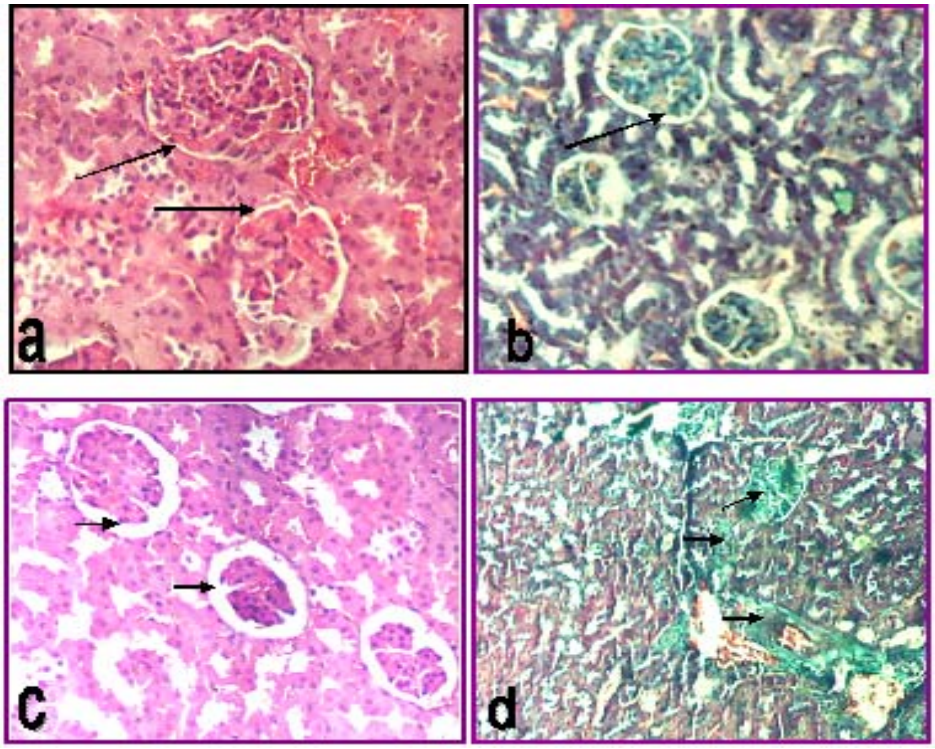

Fig. 4: Photomicrography sections (100X) of control (a,b) and CCl_4 injured kidney (c,d) stained with haemtoxyline & eosin and Masson's trichrome. Arrows indicate normal glomeruli and normal interstitial spaces with normal collagen deposition. Small arrows indicated dilatation in glomeruli and its interstitial spaces and marked collagen deposition.

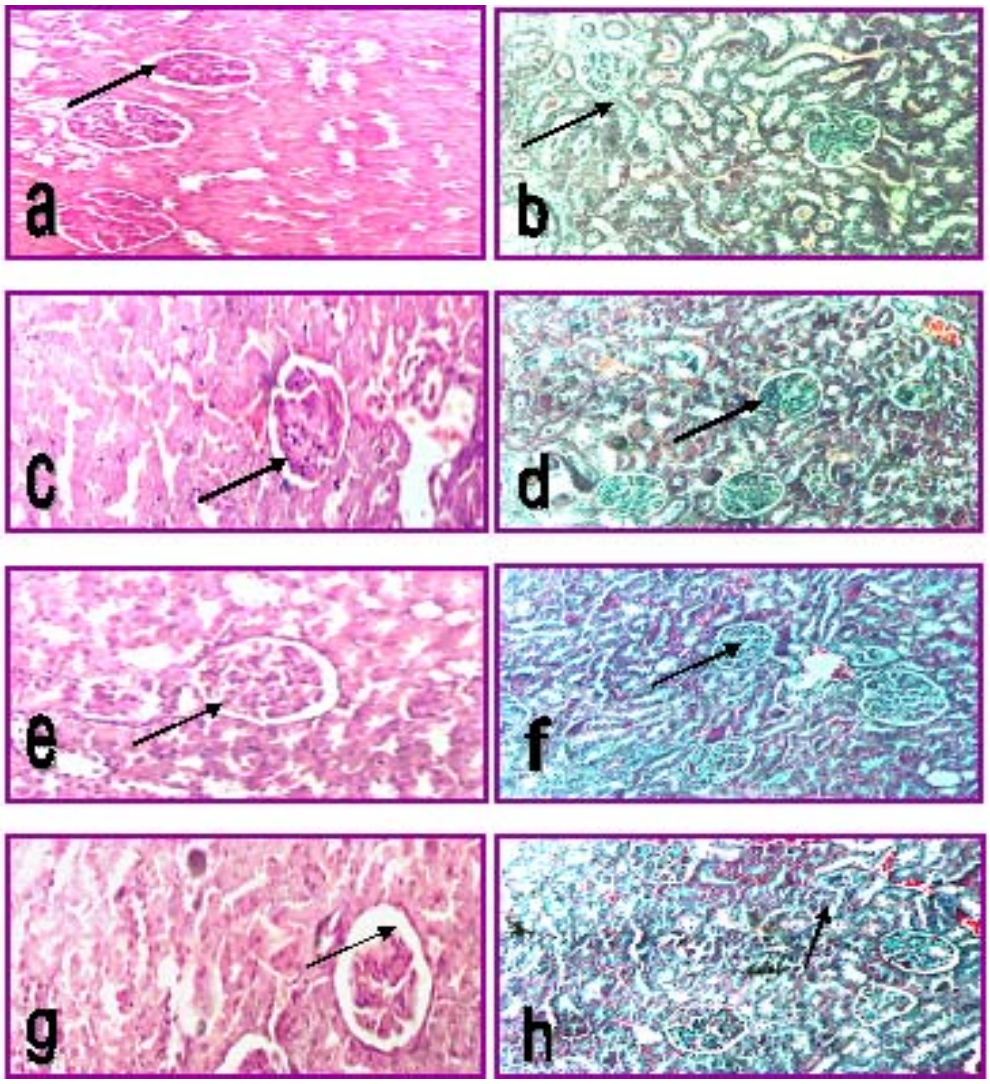

Fig. 5: Photomicrography sections (100X) stained with haematoxylin & eosin and Masson's trichrome of kidney treated with Rosemary, (a,b) and Black mulberry (c, d), prophylactic with Rosemary (e, f) and Black mulberry (g, h). Arrows indicate normal glomeruli, normal interstitial spaces and mild collagen deposition.

DISCUSSION

The mechanism of CCl_4 hepatotoxicity is well documented in the rat model[31, 32]. According to these reports, CCl_4-induced liver injury is due to the conversion of CCl_4 to CCl_3 and CCl_3O_2 by the cytochrome P450 enzyme. These highly reactive free radicals cause cell damage. However, the pathogenesis of CCl_4-induced renal injury has not been clearly clarified. Rincon et al.[33] showed that effects of CCl_4 on kidney structure and function depended on the functional state of the liver. Ogawa et al.[34] suggested etiologic independence of the renal and hepatic events. The oxidative damage to the lipids and proteins of the rat kidney resulting from chronic toxicity of CCl_4 inhalation was reported by Abraham et al[2]. It has also been reported that systemically administered CCl_4 in rats was distributed at higher concentrations in the kidney than in the liver[35]. Since the kidney has an affinity for CCl_4[2] and contains cytochrome P450 predominantly in the cortex[36], therefore the mechanism of CCl_4 nephrotoxicity is probably the same as that of the liver and also independent from the diminished functionality of the liver.

In the present study, CCl_4 injured kidney recorded significant increase in NO and lipid peroxidation process. The reductive dehalogenation of CCl_4 by the P450 enzyme system to the highly reactive trichloromethyl radical initiates the process of lipid peroxidation which is considered to be the most important mechanism in the pathogenesis of renal damage[37]. Although NO was described initially as a vasodilatory chemokine, it plays a major role as antioxidant[38]. The observed increase of NO level permitted vasodilatation in the kidney which contributed by disturbance in Na^+-K^+- ATPase. The activity of renal Na^+-K^+-ATPase varies in parallel with sustained changes in Na^+ or K^+ transport, indicating the participation of this enzyme in the chronic adaptation of the kidney to altered Na^+ reabsorption or K^+ secretory load[39]. Not only these hemodynamic effects, but also alterations in membrane lipid composition that influence membrane fluidity, cation transport and Na^+-K^+- ATPase activity can predispose renal tubular cells to injury[40].

CCl_4 treated rats recorded also significant increase in urea and creatinine levels. This was in agreement with Khan et al.[37] who reported that chronic renal injuries by CCl_4 intoxication was associated with urea and creatinine elevation and considered as indicators of kidney injury, where the serum creatinine level does not rise until at least half of the kidney nephrons are destroyed. Renal injuries may contribute to low level of serum protein that might have resulted from remarkable leakage into urine due to injuries in glomeruli and tubules[41].

We also observed significant increase in heat shock protein-70, TGF-β_1, and significant decrease in GYP2E1 in CCl_4 treated rats. This was in accordance with Fink[42] Kim et al.[43,44] who postulated that thermal, oxidative, hemodynamic, osmotic, and hypoxic stresses induce HSP. The same authors added that this stress response results in cytoprotection. Specifically, HSP prevent nonspecific protein assembly, assist in denatured protein refolding, and interfere with proapoptotic pathways. Glomerular capillary hypertension imposes cellular stresses on renal target cells and they are thus potential inducers of a stress response that may counterbalance the deleterious effects of these insults [45].

The transforming growth factor β_1 (TGF- β_1) family of cell signaling active polypeptides have attracted much attention because of their ability, from nematodes to mammals, to control cellular functions that regulate embryo development and tissue homeostasis[46].

On the basis of sequence similarities, TGF- β_1 molecules can be subdivided into TGF- β_1 sensu stricto, bone morphogenetic proteins (BMP) and activins, all of which appear to share common features in their downstream signaling mechanisms[47]. TGF has recently been suggested to be involved in the mechanism of compensatory renal growth. Thus, using immunochemical staining, TGF-β_1 in rat proximal tubule cells appears to increase and act as a modulator of compensatory renal hyperplasia[48].

Microsomal cytochrome P450 monooxygenases play important roles in the biotransformation of numerous endogenous and xenobiotic compounds[49]. While the liver is regarded as the richest source of P450s and other drug-metabolizing enzymes, the P450s expressed in various extra-hepatic tissues can also contribute to target tissue toxicity induced by tissue-selective toxicants[50]. The kidney is a major target organ for chemical-induced toxicity. A number of P450 isoforms are expressed in the kidney of rodents, including members of the CYP1A, 2B, 2C, 2E, 2J, 3A, 4A and 4F subfamilies[51]. The P450 content in kidney microsomes is 10% of the concentration in liver microsomes in rats[51]. Within the kidney, the renal proximal tubule has the highest concentrations of P450s and cytochrome P450 reductase (CPR),[52] and it is also the primary target for xenobiotic-induced renal toxicity[53]. Chemical-induced nephrotoxicity can be caused either by the parent compounds or by their reactive metabolites generated through biotransformation. The nephrotoxic metabolites can be produced by local P450s in the kidney, or else they can be generated in the liver or other organs and then transported into the kidney through systemic circulation [54].

Numerous experimental studies have demonstrated the beneficial effects of antioxidant treatment on CCl_4-induced tissue injury. In the present study, we hypothesized that Rosemary and Black mulberry would effectively protect kidneys by their antioxidant, anti-inflammatory and anti-cytotoxic effects against CCl_4-induced injury. Our results demonstrate that Rosemary and Black mulberry will be able to reduce the damage to the rat kidney induced by chronic CCl_4 poisoning. This was verified by the improvement occur in MDA, NO, urea, creatinine, HSP-70, TGF- β_1, CYP2E1 and the histopathological observations. Carnosic acid and carnosol found in Rosemary as well as phenolic compounds, including flavonoids, anthocyanins and carotenoids in Black mulberry recorded highly antioxidant activity against free radical species and oxidative stress[13,18], which give an additional support of the observed protection.

CONCLUSIONS

Rosemary and Black mulberry succeeded to protect the kidney against injury induced by CCl_4. Therapy with either Rosemary or Black mulberry showed more potent effect than the prophylactic action through the observed improvement in kidney architecture. Further studies are needed to identify the molecules responsible for these pharmacological effects.

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