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Antioxidant Activity of White (*Morus alba* L.) and Black (*Morus nigra* L.) Berries against CCl4 Hepatotoxic Agent

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Abstract

Background and Aim: Our research investigates black, white and mixture of fresh berries role in liver injury caused by CCl_4 through biochemical parameters and histopathological determinations.

Methods: Using berry as preventing or caring agents in comparing with treating or relieving or might curing agents against CCl_4 hepatotoxicity. Injection with CCl_4 was in the second 10 days, while preventing showed that feeding with berry on the first 10 days and relieving was feeding with berry on the third 10 days.

Results: Preventing infected rats with white berry increased weight (17%), food efficiency ratio FER (6%), decreased aspartate aminotransferase (AST) (81%), urea (25.5%) comparing to infected rat group. Risk factor in lipid profile showed improvement by preventing black, white followed by mixture berries (26-58%) compared with CCl_4 group. Preventing with black, white then mixture berry showed significant improvement for risk factor. Generally, optimistic data has been found for preventing black berry in malondialdehyde (MDA) and fucosidase values in the final stage. In the first stage, preventing white and mixture fresh berry showed significant increase for antioxidant and fucosidase activity, respectively. Histopathological profiles indicated that relieving black berry and protected mixture were much enhanced than protected black and relieving mixture, respectively.

Conclusion: Carbon tetrachloride is showing infection and hepatotoxicity in liver diseases due to its reactive intermediates. White, black and their equal mixture in the diet of Sprague-Dawley rats showed protective and even relieving for CCl_4 toxicity.

Keywords: Black berry; White Berry; Ccl_4; Mda; Fucosidase; Hepatotoxic

Introduction

Liver the largest organ in the vertebrate body is the major site of xenobiotic metabolism. Mostly, research concerned with carbon tetrachloride CCl_4 showed infection and chemical toxic induction for liver damage. Most remarkable pathological characteristics of CCl_4 are fatty liver, cirrhosis and necrosis, which have been resulted from the reactive intermediates as $CCl_3^{\cdot}$ metabolized by the function cytochrome p450 in the endoplasmic reticulum [1]. Blocking or retarding the chain reaction of oxidation is one strategy to prevent or treat oxidative stress induced hepatotoxicity. Intake of oxygen radical scavengers involved in phytochemicals may be a good defense mechanism for hepatoprotection. Whenever ameliorating the antioxidant status, especially improvement of phase II detoxifying and elevation of the antioxidant substance content surely affect on the healthy situation.

Mulberry fruits under family Moraceae are widely regarded as a nutritious food and it can be eaten freshly or widely used in the production of wine, fruit juice, jam and canned food [2]. Berries as well used effectively in medical industries for treatment of sore throat, fever, hypertension and anemia [3]. Moreover, white mulberry fruit is used to protect against liver and kidney damage, strengthen the joints, improve eyesight, and have antiaging effects [4]. In a chemical comparison study, white (*Morus alba* L.), red (*M. rubra* L.) and black (*M. nigra* L.) mulberry fruits investigated to grow in Turkey [5]. They observed that black ones contain 1422 mg gallic acid equivalents and 276 mg quercetin equivalents per 100 g fresh matter. On the other hand, *M. alba* had the highest fat content, and ascorbic acid content.

Moreover, black berry is closely linked to its protective properties against free radical attack induced by fluoride hepatotoxic metal. They found as well that black berry decreased the hepatotoxicity and oxidative stress of sodium fluoride (NaF) [6]. Hepatotoxicity of NaF elevated liver thiobarbituric acid reactive substances (TBARS) and nitric oxide (NO), while reduced superoxide dismutase (SOD), catalase (CAT), total antioxidant capacity and glutathione (GPX). They examined the increased values of plasma transaminase, creatine kinase, lactate dehydrogenase, total lipids, cholesterol, triglycerides, low density lipoprotein-cholesterol, and the decreased value of high density lipoprotein-cholesterol. These effects have been induced by NaF and ameliorated by black berry juice. Common traditional word in the Middle East is saying that care is much better than cure. Sufficient evidence has demonstrated that CCl_4 intermediates deleterious effects in liver, however it is not clear exactly the defense mechanism for the edible plants. The present study tries to discover the actions if fresh white or black berries could perform as care or cure agents against induced hepatotoxicity in rat model.

Material and Methods

Fresh white (*Morus alba* L.) and black (*Morus Nigra* L.) mulberry fruits were purchased from the local market (Monofia, Egypt). All

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berries were cleaned by washing under tap water, and minded minced well in the rat chow diet by 30% ratio for the protective and the treated groups. All chemicals used for blood biological measurements were purchased from Bio-diagnostic chemical company.

Animals and Experimental design

Fifty six male Sprague-Dawley rats, weighing 180-190 g were purchased from Research Institute of Ophthalmology, Giza, Egypt. Animals were given two weeks acclimation period, during which they were fed *ad libitum* a standard rat chow diet, with alternated 12-h dark/light cycle, and the ambient temperature was held between 21-25°C [7]. All studies were performed in accordance with the guide for the care and use of laboratory animals, as adopted and promulgated by research Institute of Ophthalmology, Giza on 2013.

For the evaluation of chronic carbon tetrachloride toxic effect, CCl_4 mixed first with olive oil by ratio 20%, then injected intra-peritoneal four times (2 ml/kg body weight BW). Chronic CCl_4 treatment was started for groups 2 – 8 beginning from the 11th day and every 3 days. Animals were divided into 8 groups (7 rats each), as followed;

Group (1) Normal control group injected with olive oil on the second 10 days and fed with standard rat chow diet. (2) CCl_4 injected group as described before and fed with standard rat chow diet as previous group. Group 3 (PB): Prevention or care group used the first 10 days, rats were fed by 30% black mulberry fruit mixed well with the chow diet before i.p. CCl_4 injection. group 4 (RB): Treated or cure group used the last 10 days as relieving agent, rats were fed by 30% black mulberry fruit mixed well with the chow diet after the i.p. CCl_4 injection. Group 5 (PW): Prevention group used the first 10 days, rats were fed by 30% white mulberry fruit mixed well with the chow diet before i.p. CCl_4 injection. (RW): Treated group used the last 10 days as relieving agent, rats were fed by 30% white mulberry fruit mixed well with the chow diet after the i.p. CCl_4 injection. Group 7 (PM): Prevention group used the first 10 days, rats were fed by 30% mixture of equal amounts of white and black mulberry fruits mixed well with the chow diet before i.p. CCl_4 injection. Finaly, group 8 (RM): Treated group used the last 10 days as relieving agent, rats were fed by 30% mixture of equal amounts of white and black mulberry fruits mixed well with the chow diet after the i.p. CCl_4 injection. Outside these mentioned preventing or treated periods, rats were fed normal chow diet.

Assessment of activity

Food consumption was monitored daily and body weight was determined once a week. After the experimental period, food was forbidden for 12 hours. The fasting rats were sacrificed and blood samples were collected into clean centrifuge tubes. Blood samples were allowed to coagulate and centrifuge at 3000 rpm for 20 minutes to separate the blood serum. Separated serum was stored at -20°C for subsequent biochemical analyses.

Biochemical analysis

Triglycerides TG, total Cholesterol TC and High density lipoprotein cholesterol HDLc were colorimetrically determined in rat serum using enzymatic colorimetric methods. Low density lipoprotein cholesterol LDL-c and vLDL-c *(TG/5)* were calculatedby (mg/dl) as follows [8].

LDLc = *TC – HDLc – (TG/5)*

Risk factor also has measured by dividing LDL-c by HDL-c values. Liver transaminase enzymes as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured with colorimetric method [9]. Blood urea was determined [10]. The determination of serum creatinine was performed [11]. Plasma lipid peroxide (Malondialdehyde, MDA) was determinedand the tumor marker (α-L-Fucosidase) was measured through the colorimetrical method [12-13].

Histopathological study

Autopsy samples were taken from the rats in different experimental groups. Then, samples were fixed in 10% formal saline solution for twenty four hours. Washing was done in tap water then serial dilutions of absolute ethyl alcohol were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56°C in a hot air oven for twenty four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by slidge microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin and eosin stain for histopathological examination through the light microscope[14].

Statistical analysis

Using SPSS programmeans were calculated among 7 replicates, with their Standard Deviations (± SD) for each group. Analysis of variance was applied to make statistical comparisons (ANOVA) with Dennett's post hoc test in between 5% probability [15].

Results and Discussions

Some natural and synthetic chemical agents are showing suppressing or preventing hepatocellular carcinoma [16,17]. These agents represent chemopreventive as phytochemicals or non-nutritive plant chemicals. Our research has focus on the mechanism and the importance of different colours and mixture for fresh berries to reduce or eleminate CCl_4 intoxication effects.

Changes in body weight and Food Efficiency Ratio (FER):

The body weight gain and FER showed significant depletion in CCl_4 injected group than control one (Table 1). Improvement in weight gain and FER values have been discovered through either preventing or relieving process for white, mixture followed by black berries. In general, prevention with fresh fruits was better enhancer than that for relieving with the berry fruits. Not any treatment surpassed the rat body weight or food efficiency ratio to reach the functions of control rats.

Changes in serum liver-kidney dysfunction indices

The efficacy of white and black mulberry fruits as well as their combination on serum aspartate aminotransferase, alanine

Group	Weight gain (g)	FER %
Control	182.00 ^{a} ± 14.25	149.00 ^{a} ± 15.65
CCl_4	142.50 ^{cd} ± 25.34	113.75 ^{bc} ± 26.37
PB	141.00 ^{d} ± 5.25	96.25 ^{d} ± 7.39
RB	137.00 ^{de} ± 5.25	95.25 ^{d} ± 7.39
PW	167.25 ^{b} ± 5.07	120.75 ^{b} ± 3.31
RW	162.25 ^{b} ± 5.07	118.75 ^{b} ± 3.31
PM	155.25 ^{bc} ± 4.75	113.00 ^{bc} ± 4.60
RM	154.25 ^{bc} ± 4.75	111.00 ^{bcd} ± 4.60
F	7.895	8.735

Values which have different letters differ significantly, while those with similar or partially are non-significant.

The values are mean ± SD of 7 rats in each group. Means with different superscripts within a column are significantly different from each other at P<0.05.

Table 1: Effects of white, black and mixture mulberries fruits feeding on body weight gain after 4 weeks and food efficiency ratio (FER).

aminotransferase, blood urea and creatinine is shown in Table (. Administered group with CCl_4 gave dramatic increase in liver and kidney functions as shown in Table 2. Liver enzymes ALT and AST showed significant ($P<0.05$) amelioration for mixture berries and black or white berries, respectively. Generally, slight enhancement had noticed for preventing with fresh fruits than relieving with these fruits in administered groups. Similarly, *Solanum xanthocarpum* ethanolic extract exhibited significant change in marker enzymes as AST by 29 – 51%, and ALT by 25 – 54% (100 – 200 mg/kg) compared to control group in CCl_4 intoxicated animals[17].

Group	***Liver dysfunction indices***		***Kidney toxicity indices***	
	ALT (Iu/d1)	AST (Iu /d1)	Creatinine (mg/d1)	Urea (mg/d1)
control	5.60 ^{bc} ± 3.54	3.50 ^{c} ± 0.77	101.89 ^{c} ± 1.26	46.04 ^{c} ± 17.06
CCl_4	13.00 ^{a} ± 4.43	12.25 ^{a} ± 0.61	114.83 ^{a} ± 0.11	84.82 ^{ab} ± 19.75
PB	10.03 ^{ab} ± 0.57	2.25 ^{c} ± 0.61	100.42 ^{c} ± 3.56	73.45 ^{b} ± 5.55
RB	12.00 ^{ab} ± 3.43	3.25 ^{c} ± 2.4	107.33 ^{b} ± 0.42	80.24 ^{ab} ± 4.23
PW	12.37 ^{a} ± 0.58	2.29 ^{c} ± 0.65	106.33 ^{b} ± 0.32	63.19 ^{c} ± 25.97
RW	13.00 ^{a} ± 4.0	2.95^{c} ± 0.60	107.50 ^{b} ± 1.75	82.02 ^{ab} ± 12.51
PM	2.80 ^{c} ± 1.77	5.25^{b} ± 0.61	106.42 ^{b} ± 0.26	94.58 ^{a} ± 9.82
RM	7.10 ^{b} ± 2.1	6.00 ^{b} ± 0.21	107.50 ^{b} ± 0.32	96.53 ^{a} ± 13.49
F	11.695	93.194	20.039	8.582

Values which have different letters differ significantly, while those with similar or partially are non-significant.

The values are mean ± SD of 7 rats in each group. Means with different superscripts within a column are significantly different from each other at $P<0.05$. F mean degree of freedom.

Table 2: Effects of white and black mulberry fruits feeding on serum GPT (ALT), GOT (AST), Creatinin and Urea.

The most obvious preventing with black berry improved significantly creatinine measure compared to CCl_4 infected. While other administerated fruits showed slight improvement in creatinine (Table 2). White and black berries ameliorated significantly urea values, and that was surpassing mixture fruits effects. General speaking, kidney disfunction showed that preventing samples enhanced significantly in urea and insignificantly in creatinine functions. In addition, increased oxidative stress and reduce antioxidative ability results in renal tubular injury, protein urea and leads to gradual loss of renal function [18].

Changes in serum lipid profile function

Table 3 showed enhancing in HDL-c amounts in the entire administered protective fresh berry comparing to CCl_4 group and these values surpassed than that for relieving treated groups. Triglycerides values have been significantly decreased for preventing samples comparing to relieving groups, except for white berry. Different results have been noticed and varied in total cholesterol values. Risk factor has been dramatically increased for CCl_4 group compared with normal control. For their increase amounts of HDL for preventing groups, enhancement for risk factor has been investigated for black followed by white and equal mixture according to the relieving analogue ones.

Changes in lipid peroxidation (Malondyaldhyd, MDA)

Compared with counterparts of normal group, injected CCl_4 showed significant increase for lipid peroxidation values (Table 4). In the first period, all the administered samples showed antioxidative activity significantly for black and white prevention and insignificantly for mixture relieving. Comparing to CCl_4 intoxicated rats, relieving

Group	LDL (mg/d1)	HDL (mg/d1)	TG (mg/d1)	Total Chl (mg/d1)	Risk Factor (mg/d1)
control	39.02 ^{f} ± 12.93	80.16 ^{a} ± 17.55	42.26 ^{b} ± 7.45	127.64 ^{cd} ± 18.29	0.48 ^{c} ± 0.21
CCl_4	107.77 ^{c} ± 2.35	21.05 ^{bcd} ± 14.89	48.20 ^{b} ± 2.12	138.46 ^{bc} ± 16.56	5.12 ^{ab} ± 2.99
PB	70.61 ^{e} ± 10.18	32.64 ^{bc} ± 11.53	22.2 ^{c} ± 1.96	107.69 ^{e} ± 5.41	2.16 ^{b} ± 2.00
RB	95.61 ^{d} ± 15.17	5.24 ^{d} ± 39.97	21.39 ^{c} ± 10.50	105.13 ^{e} ± 7.03	18.25 ^{a} ± 2.67
PW	87.33 ^{d} ± 1.18	23.77 ^{bcd} ± 8.37	43.87 ^{b} ± 7.99	119.87 ^{de} ± 7.45	3.67 ^{b} ± 3.15
RW	125.89 ^{b} ±12.68	19.84 ^{bcd} ± 18.89	20.39 ^{c} ± 2.27	149.57 ^{b} ± 20.92	6.34 ^{ab} ± 5.43
PM	143.07 ^{a} ± 3.53	37.94 ^{b} ± 12.86	13.75 ^{d} ± 3.68	183.76 ^{a} ± 13.51	3.77 ^{b} ± 1.95
RM	64.10 ^{e} ± 1.44	7.92 ^{cd} ± 34.08	63.89 ^{a} ± 1.96	84.62 ^{f} ± 20.64	8.09 ^{ab} ± 5.70
F	80.971	7.23	26.057	25.143	2.298

Values which have different letters differ significantly, while those with similar or partially are non-significant.
The values are mean ± SD of 7 rats in each group. Means with different superscripts within a column are significantly different from each other at $P<0.05$. F mean degree of freedom.

Table 3: Effects of white and black mulberry fruits on serum LDL, HDL, TG, Total Chl and Risk Factor.

Group	15 days **MDA**	30 days **MDA**
Control	4.46 ^{ab} ± 0.03	2.28 ^{e} ± 0.11
CCl_4	8.55 ^{a} ± 0.04	10.88 ^{a} ± 0.22
PB	4.22 ^{ab} ± 10.32	3.96 ^{cd} ± 0.95
RB	7.34 ^{a} ± 0.18	3.62 ^{d} ± 0.84
PW	2.12 ^{b} ± 0.02	4.62 ^{c} ± 1.08
RW	6.36 ^{ab} ± 0.18	6.45 ^{b} ± 0.62
PM	7.59 ^{a} ± 0.06	4.43 ^{cd} ± 0.76
RM	7.10 ^{a} ± 0.04	6.55 ^{b} ± 0.82
F	2.107	75.408

Values which have different letters differ significantly, while those with similar or partially are non-significant.
The values are mean ± SD of 7 rats in each group. Means with different superscripts within a column are significantly different from each other at $P<0.05$. F mean degree of freedom.

Table 4: Effects of white, black and mixture mulberry fruits at 15 days and the end of the experiment on lipid peroxide (MDA).

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black berries showed prospective significant decreasing at the end of experiment. Black berry is an excellent natural antioxidant and free radical scavenger [19]. Moreover, black berry is listed lo be dominant antioxidants rich with vitamin C, vitamin E, anthocyanins, ellagitannins and some proanthocyanidins. In agreement result, relieving black berry showed constituent stable antioxidant activity until the end of the experiment compared with injected rats.

Changes in Fucosidase enzyme

Fucosidase activity which refers to destruction effect resulted from liver carcinoma showed significant (in the first period) and insignificant increase (in the end of experiment) for CCl_4 intoxicated animals (Table 5) comparing to control group. In the first period, significant enzyme values have been shown for preventing mixture followed by black berry comparing to relieving samples. Either preventing or relieving berry mixtures proved obvious results comparing to intoxicated group. Data of Tables 4 and 5 were in agreement with that who investigated that black berry contains the highest amount of gallic acid equivalents and quercetin equivalent per 100 g fresh matter.

Group	15 days Fucosid	30 days Fucosid
control	$1.97^{f} \pm 0.15$	$0.77^{b} \pm 0.49$
CCl_4	$4.14^{b} \pm 0.03$	$1.11^{b} \pm 0.46$
PB	$2.88^{d} \pm 0.04$	$0.12^{c} \pm 0.07$
RB	$3.95^{c} \pm 0.10$	$0.88^{b} \pm 0.01$
PW	$5.24^{a} \pm 0.13$	$0.27^{c} \pm 0.10$
RW	$4.30^{a} \pm 0.03$	$1.94^{a} \pm 0.56$
PM	$0.79^{g} \pm 0.01$	$0.83^{b} \pm 0.11$
RM	$2.12^{e} \pm 0.03$	$0.75^{b} \pm 0.48$
F	19.641	14.833

Values which have different letters differ significantly, while those with similar or partially are non-significant.
The values are mean ± SD of 7 rats in each group. Means with different superscripts within a column are significantly different from each other at P<0.05. F mean degree of freedom.

Table 5: Effects of white, black and mixture mulberry fruits at 15 days and the end of the experiment on Fucosidase activity.

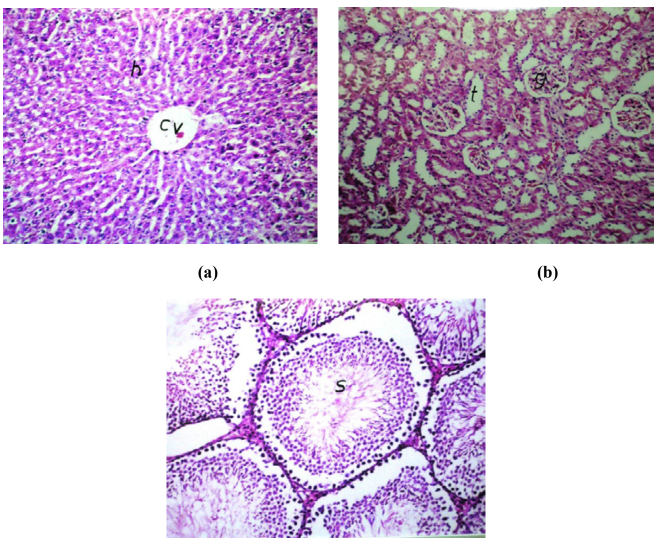


Figure 1: Histograms of control rat group (a) liver section, (b) kidney section, (c) testes section (HX - E×40).

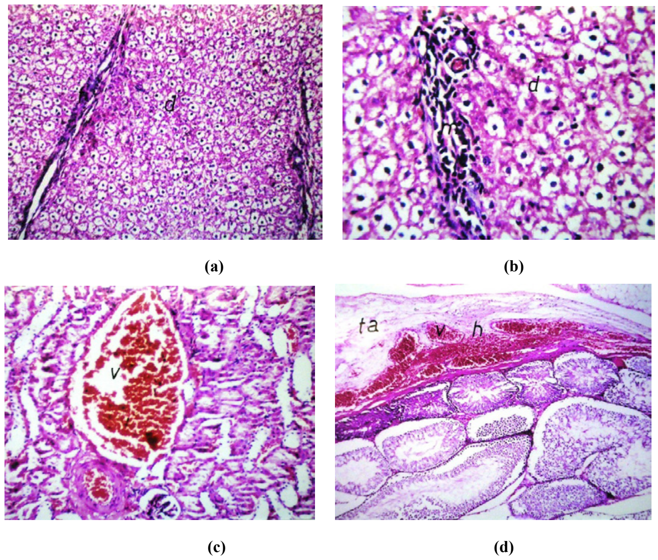


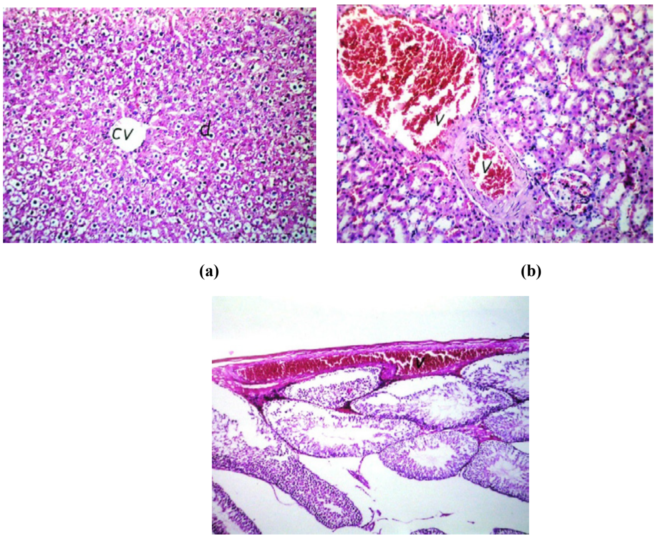
Figure 2: Histograms of rat group injected with CCl_4 (a, b) liver section, (c) kidney section, and (d) testes section (HX - E×40 (a,c) x8 (b) x16 (d)).

Histopathological of organs

In our research, we followed the pathology of histograms in liver, kidney, and testes through the prevention and relieving roles of white, black and equal mixing amounts for berries. Control group histograms showed normal histological structure of the central vein liver and surrounding hepatocytes (Figure 1a). Normal structure of the glomeruli and tubules at the cortex kidney was observed (Figure 1b). As well, mature active seminiferous tubules with complete spermatogenesis (Figure 1c) were observed. While, toxic CCl_4 injected group, liver sections showed fibrosis with inflammatory cells infiltration in the portal area associated with ballooning degeneration in the hepatocytes (Figure 2a-b). Kidney sections showed congestion in the cortical blood vessels with impaction by blood cells (Figure 2c). Moreover, testes histograms showed focal haemorrhage with sever congestion of the blood vessels in the tunica albuginea (Figure 2d).

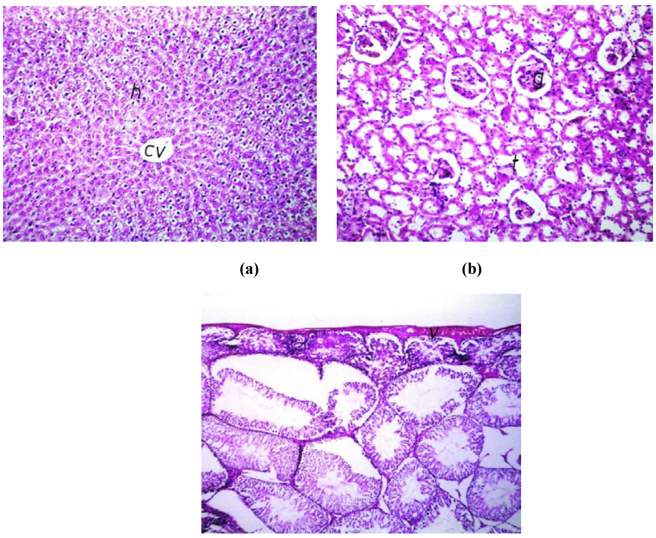
Lipid peroxidation is a marker of cellular oxidative damage initiated by reactive oxygen species [20]. Antioxidants are known to reduce the development of chemically induced liver damage [21]. Group prevented or protected by black berry before injection showed ballooning degeneration in hepatocytes liver section (Figure 3a) and congestion in the kidney cortical blood vessels (Figure 3b). Also, congestion in the blood vessels of the testes tunica albuginea has been found (Figure 3c). Group treated with black berry after injection as relieve showed mild ballooning degeneration in hepatocytes liver blood vessels (Figure 4a) and normal kidney histological structure (Figure 4b). Mild congestion was noticed in the testes tunica albuginea for the blood vessels (Figure 4c). Treatment with black berry after CCl_4 as relieve agent showed better histological results than protecting with the fresh fruits.

Group prevented or protected by white berry before injection showed ballooning degeneration in hepatocytes liver section with fibrosis in the portal area (Figure 5a) and sever congestion in the kidney cortical blood vessels (Figure 5b). Also, mild congestion in the blood vessels of the testes tunica albuginea with mild eosinophilic transudation has been found (Figure 5c). Group relieved with white berry after injection showed ballooning degeneration in some of the



(c)

Figure 3: Histograms of injected rats post-prevented by black berry (a) liver section, (b) kidney section, and (c) testes section (HX - E×40 (a,b) x16 (c)).



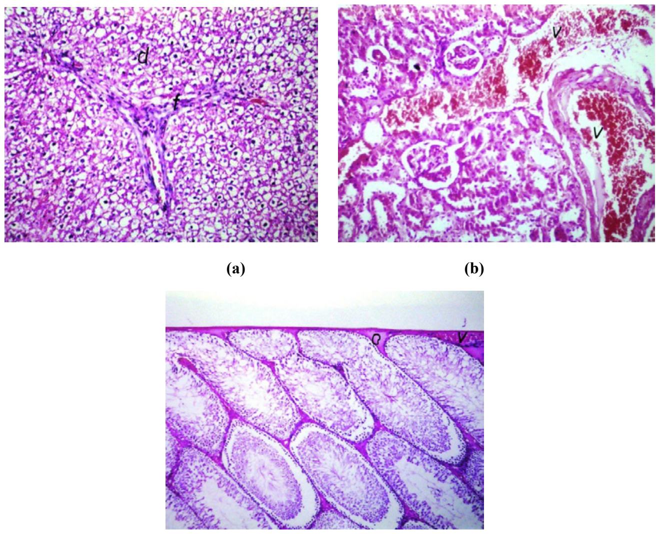
(c)

Figure 4: Histograms of injected rats treated with black berry (a) liver section, (b) kidney section, and (c) testes section (HX - E×40 (a,b) x16 (c)).

hepatocytes liver sections (Figure 6a). Sever congestion in the kidney cortical portion was noticed for the blood vessels associated with focal haemorrhage (Figure 6b). Mild eosinophilic transudation was noticed in the testes subcapsular area of tunica albuginea (Figure 6c). Not obvious amelioration has been found in the administration of the fresh white berry before or after CCl_4 injection.

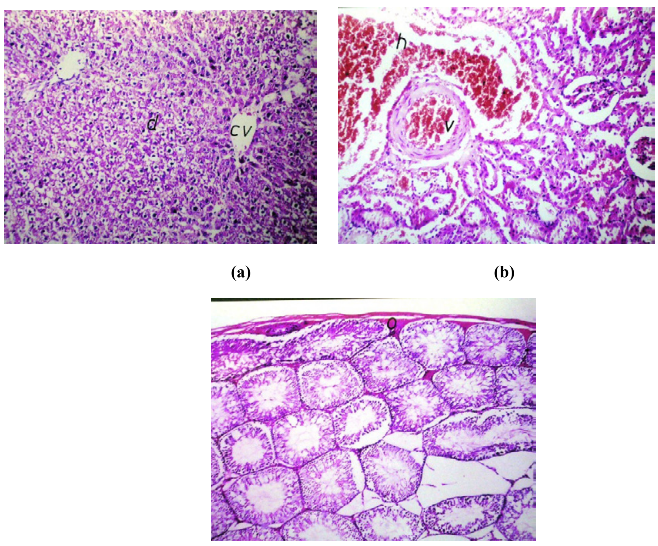
Group prevented or protected by equal mixed berry before injection showed ballooning degeneration in the hepatocytes liver section (Figure 7a). There was no histological alteration was recorded in kidney sections (Figure 7b) or testes histological patterns (Figure 7c). Group treated with mixed berry after injection showed dilatation in the central vein associated with ballooning degeneration in the hepatocytes liver sections (Figure 8a). Focal haemorrhage with congestion in the blood vessels were detected in the kidney cortical portion (Figure 8b) and mild eosinophilic transudation was noticed underneath the tunica albuginea (Figure 8c). Protecting by fresh mixed berries before injection showed better structures than that for relieving way with fresh mixed group.

Hepatotoxicity induced by CCl_4 as mentioned in many research work is due to metabolite $CCl_3^{\cdot}$ a free radical that alkylated cellular proteins and macromolecules as polyunsaturated fatty acids in the presence of oxygen. Hepatocellular necrosis leads to elevation of the serum marker enzymes released from the liver into blood [22]. The balance between ROS production and antioxidant defences may be lost for oxidative stress resulted, which through biological series leading to hepatic necrosis.



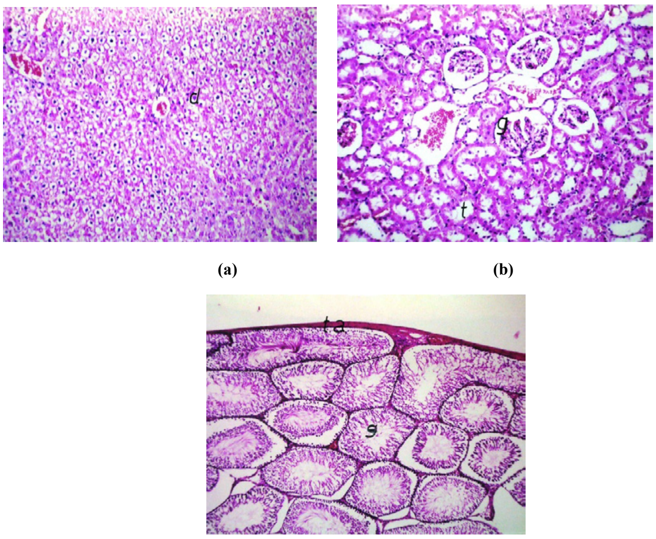
(c)

Figure 5: Histograms of injected rats post-prevented by white berry (a) liver section, (b) kidney section, and (c) testes section (HX - E×40 (a,b) x16 (c)).



(c)

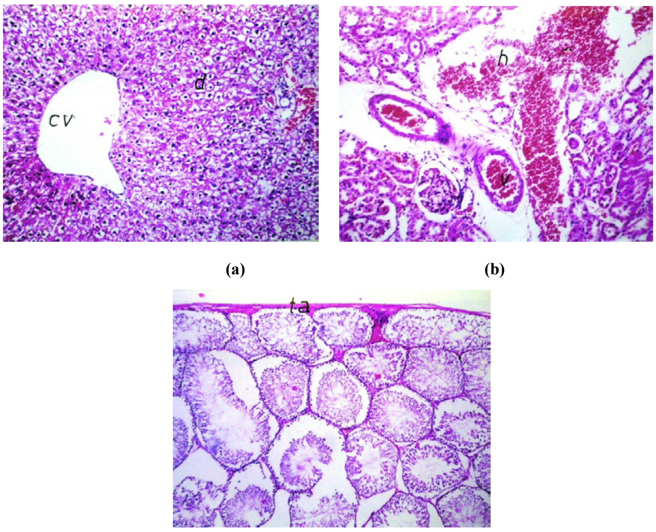
Figure 6: Histograms of injected rats treated with white berry (a) liver section, (b) kidney section, and (c) testes section (HX - E×40 (a,b) x16 (c)).



(a) (b)

(c)

Figure 7: Histograms of injected rats post-prevented by mixture berries (a) liver section, (b) kidney section, and (c) testes section (HX - E×40 (a,b) x16 (c)).



(a) (b)

(c)

Figure 8: Histograms of injected rats treated with mixture berries (a) liver section, (b) kidney section, and (c) testes section (HX - E×40 (a,b) x16 (c)). [liver CV; central vein – h; surrounding hepatocytes –d; degenerated hepatocytes; -f; fibrosis; -m; inflammatory cells infiltiration and kidney g; glomeruli – t; surrounding cortex tubules –v; cortical blood vessels congestion; -h; focal haemorrhage and testes s; spermatogenic series; -v; congestion in blood vessels; -h; focal haemorrhage; -ta; tunica albuginea; o; eosinophilic transudation].

The histological observations support the obtained results from serum enzyme assays. Increasing lipid peroxidation in serum or liver increased tissue damage and failure of antioxidant defence mechanisms to prevent the formation of excessive free radicals [23]. Moreover, destruction MDA and fucosidease activity values (Tables 3 and 4) are in constituent trends with that of histopathological results.

As recorded in many research, the presence of bioactive compounds, especially polyphenols, flavonoid as anthocyanins and pigments responsible for most the colors in berry fruits [24]. The consumption of colored berries brings a positive impact on several chronic conditions, such as obesity, diabetes, cancer, cardiovascular and neurodegenerative diseases. In a recent study, antioxidant activity, phenolic compounds and mineral composition were assessed and found to be higher in black than white berry. Research proved that black, white and equal mixture showed protective and relieving roles in hepatotoxic effect for CCl_4 biological injures [25]. These results are keys for the design of future dietary intervention studies examining the role of mulberry fruits in disease risk reduction.

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References

1. Recknagel , RGlende, E Jr Dolak J Waller, R. (1989) Mechanisms of carbon tetrachloride toxicity. Pharmacology and Therapeutics 43: 139-154.
2. Wattanathorn J, Muchimapura S, Thukhammee W, Tong-un T, Wannanon P, et al. (2012) Mulberry fruits protects against age-Related cognitive decline. American Journal of Applied Sciences 9: 1503-1511.
3. Gong, S.X, Zhu JP (2008) Mulberry relieving nutritional anemi. J Zhejiang Univ Traditional Chinese Med 32: 350-352.
4. Shizhen L (2008) Compendium of Materia Medica. Foreign Language Press 1st edition ISBN-10: 7119032607 4400.
5. Ercisli S, Orhan E (2007) Chemical composition of white (*Morus alba*), red (*Morus rubra*) and black (*Morus nigra*) mulberry fruits. Food Chem 103: 1380-1384.
6. Hassan H, Yousef M (2009) Mitigating effects of antioxidant properties of black berry juice on sodium fluoride induced hepatotoxicity and oxidative stress in rats. Food Chem. Tox 47: 2332-2337.
7. Compbell, J (1961) Methodology of protein evaluation (PAG), *June Meeting, New York. USA*. Nutr. Document A101 odd. 37.
8. Friedewald W, Levy R, Fredrickson D (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem 18: 499-502.
9. Reitman S, Frankel S (1957) A colourimetric method for the determination of serum oxaloacetic and glutamic pyruvic transaminases. Am. J. Clin. Path 28: 56-63.
10. Fawectt JK, Scott JE (1960) A rapid and precise method for the determination of urea. Journal of Clinical Pathology 13: 156-159.
11. Larsen K (1972) Creatinine assay by a reaction-kinetic principle. Clin. Chim. Acta 41:209-217.
12. Ohkawa H, Ohishi W, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem 95: 351-358.
13. Zielke K, Okada S, O'Brien J (1972) Fucosidosis; diagnosis by serum assay of alpha-L-fucosidase. J. Lab. Clin. Med. 79: 164-169.
14. Banchroft J, Stevens A, Turner D (1996) Theory and practice of histological techniques. 4th Ed. Churchil Livingstone, New York, London, San Francisco, Tokyo.
15. SPSS (1998) Statistical Package for Social Science. Computer Software, Ver. 10. SPSS Company, London, UK.
16. Stagos D, Amoutzias G, Matakos A, Spyrou A, Tsatsakis A, et al. (2012) Chemoprevention of liver cancer by plant polyphenols. Food Chem. Tox 50: 2155-2170.
17. Gupta R, Hussain T, Panigrahi G, Das A, Singh G, et al. (2011) Hepatoprotective effect of *Solanum xanthocarpum* fruit extract against CCl_4 induced acute liver toxicity in experimental animals. Asian Pacific J, Tropic Med 4:964-968.
18. Shah SV, Baliga R, Rajapurkar M, Fonseca VA (2007) Oxidants in chronic kidney disease. J. Am. Soc. Nephrol 18: 16-28.
19. Hassan H, Abdel Aziz A (2010) Evaluation of free radical-scavenging and anti-

oxidant properties of black berry against fluoride toxicity in rats. Food Chem. Tox 48: 1999-2004.

20. Farber JL, Kyle ME, Coleman JB (1990) Biology of disease: mechanisms of cell injury by activated oxygen species. Laboratory Investigation 62: 670-679.

21. Hui-Yin C, Gow-Chin Y (2007) Antioxidant activity and free radical scavenging capacity of extracts from guava (*P. guajava* L.) leaves. Food Chem 101: 686-694.

22. Shenoy K,Somayaji S, Bairy K (2001) Hepatoprotective effects of *Ginkgo biloba* against carbon tetrachloride induced hepatic injury in rats. *Indian J. Pharmacol* 33: 260-266.

23. Amresh G, Kant R, Zeashan H, Gupta R, Rao Ch, et al. (2007) Gastroprotective effects of ethanolic extract from *Cissampelos pareira* in experimental animals. J. Nat. Med 6:323-328.

24. Costa A, Garcia-Diaz D, Jimenez P, Silva P (2013) Bioactive compounds and health benefits of exotic tropical red-black berries. *J. Functional Foods* 5: 539-549.

25. Sanchez-SalcedoE, Mena P, Garcia-Viguera C, Martinez J, Hernandez F (2015) Phytochemical evaluation of white (*Morus alba* L.) and black (*Morus nigra* L.) mulberry fruits, a starting point for the assessment of their beneficial properties. *J. Functional Foods* 12: 399-408.