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Original Article

ASSESSMENT EFFECT OF *ALOE VERA, AZADIRACHTA INDICA* AND *MORINGA OLEIFERA* AQUEOUS EXTRACTS ON CARBON TETRACHLORIDE-INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

Objective: This experiment aims to investigate the hepatotherapeutic effect of *Aloe vera* (AV), *Azadirachta indica* (N), and *Moringa oleifera* (MO).

Methods: Eighty albino rats have been divided into ten groups. The first group was fed on a basal diet while the second group was administered paraffin (10 ml/kg body weight) through gavage for four days. The third to the tenth groups received (5 ml/kg body weight) CCl_4: liquid paraffin (2:1) for three days followed by (10 ml/kg body weight) CCl_4: liquid paraffin (2:1) for one day through gavage. Group three kept without any treatment, other groups then received (AV) (60 mg/kg body weight), (MO) (200 mg/kg body weight), (N) (200 mg/kg body weight), bi-extract of (AV+N), bi-extracts of (AV+MO), bi-extract of (MO+N), and tri-extracts of (AV+N+MO) respectively for 36 d. The liver and blood were studied for hepatotoxicity and antioxidant indices.

Results: Biochemical and histopathological analysis revealed that CCl_4 elevated plasma liver enzymes (aspartate transaminase, alanine aminotransferase, and gamma glutamyl transferase). Carbon tetrachloride also caused an elevation in erythrocyte content of glutathione with a concomitant increase in the plasma malondialdehyde content, along with marked atrophy of hepatocytes. However, these effects were ameliorated by the treatment of rats with the different extracts.

Conclusion: Results showed that administration of the aquatic extracts of *Aloe vera*, Neem, and Moringa (separately/mixedly) played a therapeutic role against CCl_4-induced liver damage by improving liver enzyme activities, antioxidant blood parameters, and a liver histopathological picture of intoxicated rats.

Keywords: *Aloe vera, Azadirachta indica*, *Moringa oleifera*, CCl_4 hepatotoxicity, Antioxidant, Rat hepatocytes

INTRODUCTION

The liver is responsible for the metabolism and detoxification of the most components that enter the body. Carbon tetrachloride (CCl_4) has been extensively used to study hepatotoxicity in animal models by initiating lipid peroxidation thereby injurious to kidney, heart, testis, and brain, in addition to liver pathogenesis [1-5]. It is a potent lipid-toxin that has been reported to induce acute and chronic tissue injuries [6,7] through bioactivation of the phase I cytochrome p450 system to form reactive metabolic trichloromethyl radicals (.$OOCCl_3$). These free radicals can covalently bind to macromolecules such as proteins, lipids, and nucleic acids. The double allylic hydrogen bonds of unsaturated fatty acid (PUFA) are susceptible to abstraction by free radicals. CCl_4 exposure induced increasing in lipo peroxide and free peroxide radial concentrations, which are highly reactive and cause injury or necrosis [8, 9].

At the molecular level, CCl_4 activates tumor necrosis factor-alpha (TNF-α), nitric oxide (NO), transforming growth factors alpha (TGF-α), transforming growth factor–beta (TGF-β), and processes that appear to direct the cell primarily toward (self-) destruction or fibrosis. TNF-α pushes toward apoptosis whereas the TGFs appear to direct toward fibrosis. Interleukin (IL)-6, although induced by TNF-α, has a clearly antiapoptotic effect and IL-10 also counteracts TNF-α action. Thus, both interleukins have the potential to initiate recovery of the CCl_4-damaged hepatocyte. Several of the above-mentioned toxication processes can be specifically interrupted with the use of antioxidants and mitogens [8]. Natural antioxidants could prevent the deleterious effects of toxic agents by scavenging free radicals and other reactive oxygen species or by modulating the inflammatory response [10, 11]. Therefore, the use of antioxidants in pharmacology is extensively studied as a treatment for many diseases.

Phenolic compounds are commonly found in both edible and other traditional medicinal plants; they have been reported to have multiple biological activities including free radical scavenging activity [12]. Typical phenolics that possess antioxidant activity are mainly phenolic acids and flavonoids, which commonly accumulate in the epidermal cells of plant organs such as flowers, leaves, stems, roots, seeds and fruits being found in glycosidic form (glycosides) and non-glucosidic form [13]. *Moringa oleifera* Lam is the most widely distributed species of the Moringaceae family throughout the World especially in Asia. It has a remarkable range of pharmacological properties in addition to significant nutritional value [14]. Various plant parts have wide medicinal applicability for the treatment of cardiovascular diseases since it contains nitrile, mustard oil glycosides, and thiocarbamate glycosides. These important bioactive constituents are thought to be responsible for their diuretic, cholesterol lowering, and antiulcer properties [15].

Authors in [16] showed the strong antioxidant properties of MO edible parts *in vitro*. This antioxidant activity of MO extracts is due to the presence of various bioactive compounds such as chlorogenic acid, rutin, quercetin glucoside, and kaempferol rhamnoglucoside. Furthermore, the extract of Moringa leaves and other parts have been shown to have potent antioxidant action *in vivo* [17-20]. Various studies in male rats have demonstrated the hepatoprotective effects of different MO edible part extracts against hepatotoxin-induced acute liver damage [21, 22]. Also, *Azadirachta indica* (Family: Meliaceae) is well known for its various medicinal properties. Different parts of the plant like bark, young fruit, seeds and flowers have been studied for their pharmacological actions, where the leaves of this plant have been found to possess various pharmacological properties [23]. Yet, the *Aloe vera* contains anthraquinone glycosides, polysaccharides, aloeresin, glucomannan and β-sitosterol [24-25]. Antioxidative phenolic was recently

isolated from *Aloe barbadensis* and identified as aloeresin derivatives [26-27].

The present study was accordingly designed to investigate the potential of the extract of *Moringa oleifera* edible parts, *Azadirachta indica* (Neem) leaves and *Aloe vera* gel against CCl_4 induced hepatotoxicity in rats by assaying biochemical and histopathology of liver tissues.

MATERIALS AND METHODS

Plant materials

Fresh specimens of *Aloe vera* (L.) BURM. Fil. were collected from the greenhouse of the National Research Center in Egypt. Fresh leaves of this plant were used in this study. *Aloe vera* leaves were weighed, washed, and cut in the middle then the gel was separated by scratching with a spoon. The gel (400g = 2.5g dried matter) [28] was homogenized in a blender then diluted with an equal volume of distilled water and homogenized for the second time. This stock was kept at 4 °C and used to prepare AV extract (60 mg/kg body weight).

Shaded dried leaves of *Moringa oleifera* were collected from the greenhouse of the Agricultural Research Center in Egypt. 500 gm of dried grounded leaves of Moringa added with 6liters of tap water and boiled then the residual sieved by mesh sieve. Aqueous Moringa stock (2400 ml) kept at 4 °C and used to prepare MO extract (200 mg/kg body weight).

Sun dried leaves of *Azadirachta indica* were collected from the nursery of the Ministry of Agricultural and Land Reclamation in Egypt. 350 gm of dried grounded leaves of Neem added with 5liters of tap water and boiled then the residual sieved by mesh sieve. Aqueous Neem stock (1450 ml) kept at 4 °C and used to prepare N extracts (200 mg/kg body weight).

Animals and treatment [29, 30]

Eighty male albino rats weighing (100-120g) were provided by the animal house of the National cancer Institute in Egypt. The rats were kept in ordinary cages at room temperature of 25±4 °C with a 12 h dark/light cycles. They had free access to standard laboratory feed and water according to the study protocol. After acclimation, the rats were randomly divided into ten groups of eight animals each. The animal ethics committee, Ain Shams University, Cairo, Egypt, approved the study protocol.

Group 1: Served as normal healthy control, was fed basal diet and tap water.

Group 2: Received paraffin (10 ml/kg body weight) through gavage for four days and tap water.

Group 3: Disease control-Treated with Carbon tetrachloride for four days.

Group 4: Diseased animals received *Aloe vera* extract (AV) (60 mg/kg body weight) as the drinking water.

Group 5: Diseased animals received *Moringa oleifera* extract (MO) (200 mg leaves extract/kg body weight) as the drinking water.

Group 6: Diseased animals received *Azadirachta indica* (N) (200 mg leaves extract/kg body weight) as the drinking water.

Group 7: Diseased animals received the extracts of (AV+N) in equal volumes (1:1) as the drinking water.

Group 8: Diseased animals received the extracts of (AV+MO) in equal volumes (1:1) as the drinking water.

Group 9: Diseased animals received the extracts of (MO+N) in equal volumes (1:1) as the drinking water.

Group 10: Diseased animals received the extracts of (AV+N+MO) in equal volumes (1:1:1) as the drinking water.

Carbon tetrachloride diluted with paraffin CCl_4: liquid paraffin (2:1) was administered in a dose of (5 ml/kg body weight) for 3 d followed by (10 ml/kg body weight) CCl_4: liquid paraffin (2:1) for one day through gavage to all animal groups except for control animals in groups 1 and 2. Animals of the diseased group received only CCl_4, to assist assessing the severity of toxicity produced by carbon tetrachloride administration. Diseased animals from the treated groups received different mixtures of aqueous extracts as the drinking water for 36 d. Four rats from each group were weighed then killed by cutting the jugular vein after 18 and 36 d of treatments to evaluate the treatment effect of each extract during this period. The blood was collected while the liver was removed. Liver samples for the histological examination were kept at 10% formaldehyde and those for analytical procedure kept at-4 °C.

Biochemical investigations

Blood samples have been taken from the jugular vein. Heparin was used as an anticoagulant.

Peripheral blood processing

The collected blood was centrifuged at 4000 rpm for 15 min and plasma was separated.

Erythrocyte processing

The erythrocyte pellet was washed thrice with saline, and the cell suspension was diluted with 4 times its volume ice-cold distilled water. Then it was centrifuged at 4000 rpm for 15 min. Finally, the supernatant (erythrocyte lysate) was separated.

Liver samples processing

Liver samples were fixed in 10% phosphate-buffered formalin, and paraffin sections (3–5 mm) were prepared. Liver sections were used for hematoxylin and eosin (HE) staining. The sections were examined using a light microscope Olympus, CX31, Tokyo, Japan.

Assessment of hepatotoxicity

In order to estimate hepatotoxicity, plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, total protein and gamma glutamyl transferase (GGT) were evaluated using commercially available kits. Moreover, Histopathologic examination of liver tissue was done to assess the degree of congestion, vacuolar degeneration of hepatocytes and hepatocyte atrophy.

Assessment of oxidative stress

For determination of oxidative stress, plasma lipid peroxide malondialdehyde (MDA) was determined, and reduced glutathione (GSH) was assessed in erythrocyte lysate by using commercially available kits.

RESULTS

Biochemical evaluation

Administration of CCl_4 markedly increased the activity of liver marker enzymes such as AST, ALT, and GGT as compared with the control group ($p<0.001$). In addition, it increased ($p<0.01$) total protein content but no significant difference on albumin. Elevation in the secretion of these enzymes was potentially decreased by 18 d of treatment with mono, bi, and tri aqueous extracts of *Aloe vera* (AV), *Azadirachta indica* (N), and *Moringa oleifera* (MO) (table 1). Prolonged treatment for 36 d significantly restored them to normal (table 2).

CCl_4 treatment in rats significantly increased ($p<0.001$) plasma lipid peroxide malondialdehyde (MDA) content while decreased the activity of ($p<0.05$) reduced glutathione GSH in the erythrocyte lysate. These alterations were remarkably attenuated by administration of separately/mixedly *Aloe vera* (AV), *Azadirachta indica* (N) and *Moringa oleifera* (MO) aqueous extracts after 18 and 36 d (table 3 and 4).

Histopathological evaluation (table 5)

Liver sections from the control groups 1 and 2 showed normal liver histology with no atrophy of hepatocytes or congestion (fig. 1. A and B). CCl_4 treated control animals at group 3 showed marked atrophy of hepatocytes around central veins and vacuolar degeneration of the rest of hepatocytes with inflammation of the portal tract (fig. 1. C). Sections examined of the animals treated with (AV) group 4 for

18 d revealed a congested central vein and vacuolar degeneration of hepatocytes in the centrilobular zone while those treated for 36 d revealed mild congestion of central veins without atrophy or degeneration of hepatocytes (fig. 1. D and E).

Table 1: Effect of aqueous extracts on biochemical parameters after 18 d of treatment

Group	Extract	AST (IU/l)	ALT (IU/l)	GGT (IU/l)	Albumin (g/dl)	Total protein (g/dl)
Normal	-	35.8±2.6	17.28±1.5	1474±407	3.4±0.2	4.9±1.1
Paraffin	-	36.7±8.4	18.7±3	1713.8±72	3.6±0.1	5.7±1.1
CCl_4	-	90±12.4***	52.3±1.8***	1933.3±32***	3.5±0.3	6.21±0.6**
Group 4	AV	44.9±4***	17.28±1.5***	1882.9±18***	3.2±0.2	5.2±0.1***
Group 5	MO	46±2***	21.1±2.5***	1914.2±17	3.7±0.1	5.2±0.2***
Group 6	N	47±1.4***	14.2±2***	2038.4±210	4.7±0.3	5.9±0.1
Group 7	AV+N	80±20.4*	16.32±3.4***	1937.7±34.8	3.7±0.7	5.4±0.4**
Group 8	AV+MO	98.5±13.4	20.2±10***	1889.9±19**	3±0.7	4.55±0.5***
Group 9	MO+N	81±4.3	35.5±9.5***	1907.8±9.3*	3.5±0.2	4.8±0.14***
Group 10	AV+N+MO	75.3±16.5**	20.4±3.7***	1934.5±14	3.25±0.4	4.75±0.5***

AV: mono-aqueous extracts of *Aloe vera,* MO: mono-aqueous extract of Moringa, N: mono-aqueous extract of Neem, AV+N: bi-aqueous extract of *Aloe vera* and Neem, AV+MO: bi-aqueous extract of *Aloe vera* and Moringa, MO+N: bi-aqueous extract of Moringa and Neem, and AV+N+MO: tri-aqueous extract of *Aloe vera*, Neem and Moringa, Values are expressed as mean±SD (n=4), p*<0.05, p**<0.01, and p***<0.001

Table 2: Effect of aqueous extracts on biochemical parameters after 36 d of treatment

Group	Extract	AST (IU/l)	ALT (IU/l)	GGT (IU/l)	Albumin (g/dl)	Total protein (g/dl)
Normal	-	35.8±2.6	17.28±1.5	1474±407	3.4±0.2	4.9±1.1
Paraffin	-	36.7±8.4	18.7±3.3	1713.8±72	3.6±0.1	5.7±1.1
CCl_4	-	90±12.4***	52.3±1.8***	1933±31.9***	3.5±0.3	6.2±0.6**
Group 4	AV	35±0.5***	34.6±2.7***	1395±72***	3.5±0.1	6±0.4
Group 5	MO	38±0.2***	29.3±12***	1364±30***	2.9±0.5	5.6±1
Group 6	N	35±0.2***	31.2±2.7***	1426±82***	2.6±0.4	5.4±0.6**
Group 7	AV+N	49.5±6.3***	29.7±7***	1366±27***	3.4±0.4	6±0.3
Group 8	AV+MO	52.3±0.7***	31.7±2***	1382±60***	2.6±0.3	5.6±0.1
Group 9	MO+N	58±0.4***	41.8±1***	1391±10***	2.6±0.1	5.3±0.1**
Group 10	AV+N+MO	58±0.2***	35.3±5***	1390±5***	2.5±0.1	6±0.3

AV: mono-aqueous extracts of *Aloe vera,* MO: mono-aqueous extract of Moringa, N: mono-aqueous extract of Neem, AV+N: bi-aqueous extract of *Aloe vera* and Neem, AV+MO: bi-aqueous extract of *Aloe vera* and Moringa, MO+N: bi-aqueous extract of Moringa and Neem, and AV+N+MO: tri-aqueous extract of *Aloe vera*, Neem and Moringa, Values are expressed as mean±SD (n=4), p*<0.05, p**<0.01, andp***<0.001

Table 3: Effect of aqueous extracts on plasma MDA content and erythrocyte GSH content after 18 d of treatment

Group	Extract	MDA nmol/ml	GSH mg/dl
Normal	-	3.5±2	3.25±2
Paraffin	-	3.6±2	3.6±2
CCl_4	-	11.23±3***	1.5±0.9*
Group 4	AV	2.8±1***	5.7±1.3***
Group 5	MO	4.4±0.7***	8.6±0***
Group 6	N	6.7±0.5***	8.6±3.8***
Group 7	AV+N	4±0.5***	7.7±0.7***
Group 8	AV+MO	4±1***	8.5±3.2***
Group 9	MO+N	3.5±1.5***	7.1±2.7***
Group	AV+N+MO	2.9±2***	5.8±0.4***

AV: mono-aqueous extracts of *Aloe vera,* MO: mono-aqueous extract of Moringa, N: mono-aqueous extract of Neem, AV+N: bi-aqueous extract of *Aloe vera* and Neem, AV+MO: bi-aqueous extract of *Aloe vera* and Moringa, MO+N: bi-aqueous extract of Moringa and Neem, and AV+N+MO: tri-aqueous extract of *Aloe vera*, Neem and Moringa, Values are expressed as mean±SD (n=4), p*<0.05, p**<0.01, and p***<0.001

Table 4: Effect of aqueous extracts on plasma MDA content and erythrocyte GSH content after 36 d of treatment

Group	Extract	MDA nmol/ml	GSH mg/dl
Normal	-	3.5±2	3.25±2
Paraffin	-	3.6±2	3.6±2
CCl_4	-	11.2±2.5***	1.5±0.9*
Group 4	AV	3.8±2***	6.8±1.6***
Group 5	MO	2.1±0.4***	5.4±0.3***
Group 6	N	3.1±0.3***	5.85±0.6***
Group 7	AV+N	1.8±0.4***	5±0.8***
Group 8	AV+MO	1.8±1***	5.9±1.8***
Group 9	MO+N	1.7±0.5***	5.5±0.4***
Group 10	AV+N+MO	2.3±0.7***	4.5±0.5***

AV: mono-aqueous extracts of *Aloe vera,* MO: mono-aqueous extract of Moringa, N: mono-aqueous extract of Neem, AV+N: bi-aqueous extract of *Aloe vera* and Neem, AV+MO: bi-aqueous extract of *Aloe vera* and Moringa, MO+N: bi-aqueous extract of Moringa and Neem, and AV+N+MO: tri-aqueous extract of *Aloe vera*, Neem and Moringa, Values are expressed as mean±SD (n=4), p*<0.05, p**<0.01, and p***<0.001

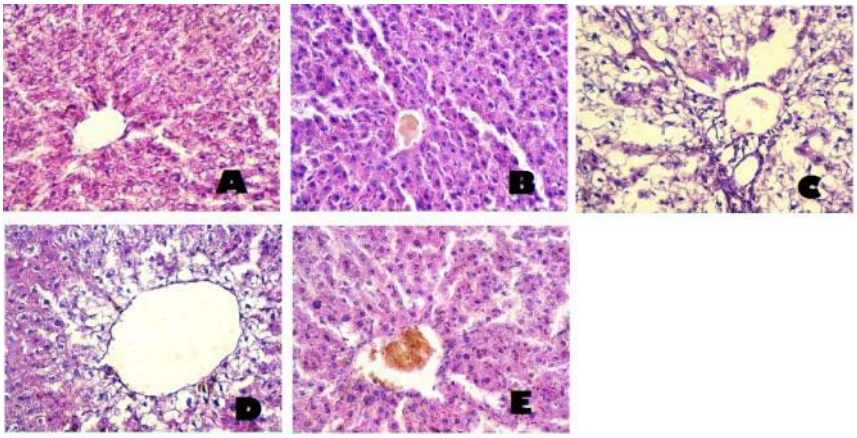

Fig. 1: Hepatic histology of rats (light microscope, × 400), A: normal control, B: paraffin control, C: CCl_4 control, D: treated with *Aloe vera* for 18 d, and E: treated with AV for 36 d

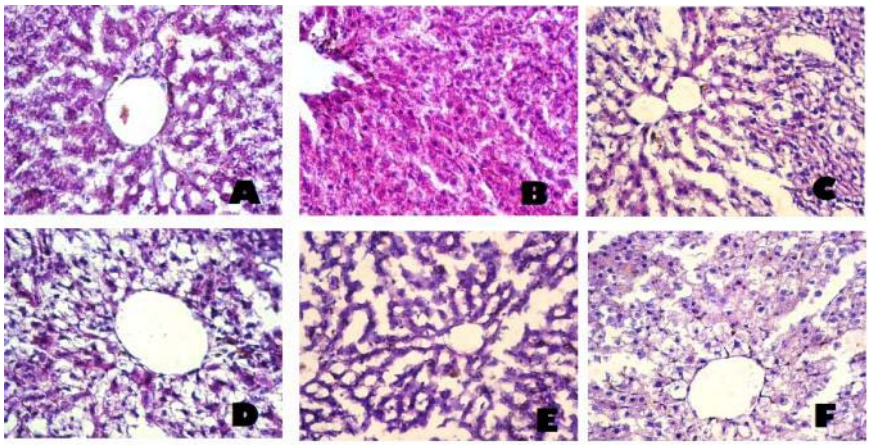

Fig. 2: Hepatic histology of rats (light microscope, × 400), A: treated with Moringa for 18 d, B: treated with Moringa for 36 d, C: treated with Neem for 18 d, D: treated with Neem for 36 d, E: treated with *Aloe vera* and Neem for 18 d, and F: treated with *Aloe vera* and Neem for 36 d

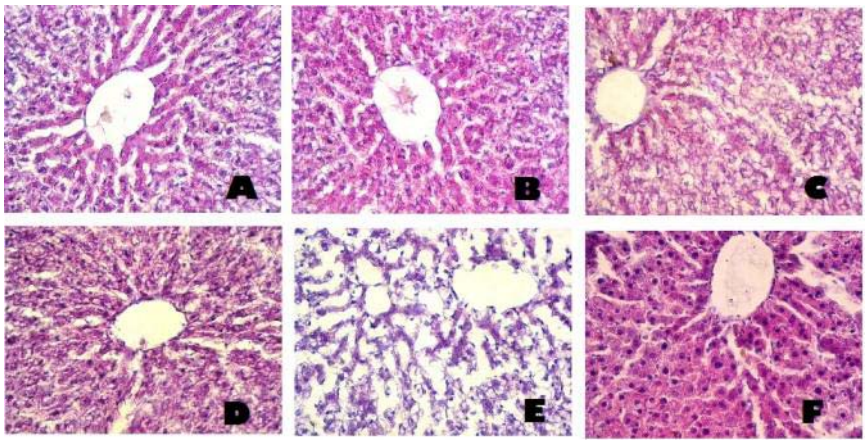

Fig. 3: Hepatic histology of rats (light microscope, × 400), A: treated with *Aloe vera* and Moringa for 18 d, B: treated with *Aloe vera* and Moringa for 36 d, C: treated with Moringa and Neem for 18 d, D: treated with Moringa and Neem for 36 d, E: treated with *Aloe vera*, Neem and Moringa for 18 d, and F: treated with *Aloe vera*, Neem and Moringa for 36 d

The sections of animals treated with (MO) group 5 for 18 d showed congestion of central vein and sinusoids of centrilobular zone with no degeneration or atrophy of hepatocytes and those treated for 36 d showed normal liver like control with preserved liver architecture, without congestion, vacuolar degeneration, or atrophy of hepatocytes (fig. 2. A and B). Liver sections from the animals treated with (N) group 6 for 18 d showed marked congestion of centrilobular zone with consequent pressure atrophy of hepatocytes, and vacuolar degeneration of hepatocytes in mid zone and periportal zone whereas those treated for 36 d showed

improvement of congestion and atrophy with remaining vacuolar degeneration of hepatocytes (fig. 2. C and D). Sections of animals treated with (AV+N) group 7 for 18 d and 36 d showed same histopathologic features as in group 6 (fig. 2. E and F). Animals sections treated with (AV+MO) group, 8 for 18 d, showed congestion of central veins and centrilobular zones with mild mid zonal vacuolar degeneration of hepatocytes while extended treatment for 36 d showed improvement of congestion and vacuolar degeneration (fig. 3. A and B). Animals sections treated with (MO+N) group, 9 for 18 d, showed marked vacuolar degeneration while extended treatment for 36 d showed restoration of normal liver histology with the disappearance of vacuolar degeneration (fig. 3. C and D). Sections of animals treated with (AV+N+MO) group 10 for 18 d showed marked congestion and pressure atrophy of centrilobular and mid zonal hepatocytes and vacuolar degeneration at the periphery while extended treatment for 36 d revealed complete restoration of normal liver histology (fig. 3. E and F).

Table 5: Histopathological evaluation of liver after 18 and 36 d of treatment

Group	Extract	Congestion of central vein and sinusoids		Vacuolar degeneration of hepatocytes		Pressure atrophy of hepatocytes	
		After 18 d	After 36 d	After 18 d	After 36 d	After 18 d	After 36 d
Normal	-	-	-	-	-	-	-
Paraffin	-	-	-	-	-	-	-
CCl_4	-	+	+	+	+	+	+
Group 4	AV	+	+	+	_	_	_
Group 5	MO	+	_	_	_	_	_
Group 6	N	+	_	+	+	+	_
Group 7	AV+N	+	_	+	+	+	_
Group 8	AV+MO	+	_	_	_	_	_
Group 9	MO+N	_	_	+	_	_	_
Group 10	AV+N+MO	+	_	+	_	+	_

AV: mono-aqueous extracts of *Aloe vera,* MO: mono-aqueous extract of Moringa, N: mono-aqueous extract of Neem, AV and N: bi-aqueous extract of *Aloe vera* and Neem, AV and MO: bi-aqueous extract of *Aloe vera* and Moringa, MO and N: bi-aqueous extract of Moringa and Neem, and AV, N and MO: tri-aqueous extract of *Aloe vera*, Neem and Moringa

DISCUSSION

Liver disorders were expressed in several forms i.e. Jaundice, acute and chronic hepatitis, hepatotoxicity, and degenerative disorders resulting in fibrosis of the liver are still without appropriate therapies. Many chemicals and drugs can injure the liver [31]. Wide varieties of herbal formulations are in use to counter the hepatotoxicity due to such insults. Drugs from the herbal origin are the only treatment available for liver damage. However, there has always been a need for more studies that can provide a clear picture for the prolonged effect of these herbs, and better agents that can provide faster recovery. In the present study, the mono-aqueous extracts of *Aloe vera* (AV), *Azadirachta indica* (N), and *Moringa oleifera* (MO), bi-aqueous extracts of AV+N, AV+MO and MO+N, and tri-aqueous extract AV+N+MO were investigated for hepato-therapeutic activity against liver damage caused by CCl_4. Each plant has demonstrated liver-protecting potentials but has not been combined in any study. Carbon tetrachloride is commonly used the toxin to induce liver damage in experimental studies.

CCl_4 administration significantly elevated ($p<0.001$) the plasma levels of AST, ALT and GGT although it moderately ($p<0.01$) increased the total protein content while had no significant effect on albumin (table. 1 and 2). CCl_4 causes chronic hepatocyte injuries, as well as alters membrane integrity, which cause hepatic enzymes, leak out [32]. After 18 d of aqueous extracts AST level was significantly ($p<0.001$) decreased by mono aqueous extracts of *Aloe vera*, mono aqueous extract of Moringa, and mono aqueous extract of Neem but less with the tri-aqueous extract *Aloe vera*, Neem, and Moringa ($p<0.01$) and bi aqueous extract of *Aloe vera* and Neem ($p<0.05$). While it significantly ($p<0.001$) restored to normal after 36 d of treatment with the mono, bi, and tri aqueous extracts of *Aloe vera*, Neem, and Moringa. On the other hand, ALT was significantly ($p<0.001$) decreased after 18 d of treatment by all mono, bi, and tri-aqueous extracts of *Aloe vera*, Neem, and Moringa.

Moreover, after 18 d of treatment GGT level was significantly decreased by *Aloe vera* mono aqueous extract and less with *Aloe vera* and Moringa bi aqueous extract ($p<0.01$) and Moringa and Neem bi aqueous extract ($p<0.05$). However, after 36 d of treatment, it was significantly ($p<0.001$) restored to the normal level by all mono, bi, and tri-aqueous extracts of *Aloe vera*, Neem, and Moringa. It is noted that group 6 treated with Neem mono aqueous extract has the least effect on lowering GGT assuming that *Azadirachta indica* (N) had a minimum protective effect against liver cell damage. These results indicate that *Aloe vera*, Neem, and Moringa had the ability to treat CCl_4 hepatotoxicity, which is in agreement with previous studies [33-35] that reports their protective consequence against CCl_4 induced hepatotoxicity.

Malondialdehyde (MDA) is one of the products of polyunsaturated fatty acid peroxidation and is a good indicator of the degree of lipid peroxidation [36], which is related to CCl_4 induced tissue damage. In the present study (Table. 3 and 4), the significant increase ($p<0.001$) in the plasma MDA level of CCl_4- intoxicated rats was significantly reduced ($p<0.001$) by treatment with the mono-aqueous extracts of *Aloe vera* (AV), *Azadirachta indica* (N) and *Moringa oleifera* (MO); bi-aqueous extracts of AV+N, AV+MO and MO+N, and tri-aqueous extract AV+N+MO after 18 d indicating their ability to break the chain reaction of lipid peroxidation.

According to these results, we may assume that the therapeutic potential of these different aqueous extracts is dependent on an antioxidant mechanism. These results concluded that *Aloe Vera*, Neem, and Moringa extracts effectively inhibit CCl_4-induced tissue damage due to the presence of various antioxidant bioactive compounds. They also are in line with previous studies [33,37,38] investigating the hepatoprotective nature of these plants.

Glutathione is an important non-enzymatic antioxidant that protects the cell. This study showed that administration of CCl_4 caused depletion ($p<0.05$) of GSH level observed in erythrocytes lysate which can be an important factor in the CCl_4 induced toxicity. The level of GSH was significantly restored ($p<0.001$) following the treatment with the mono-aqueous extracts of *Aloe vera,* Neem, and Moringa, bi-aqueous extracts of AV+N, AV+MO and MO+N, and tri-aqueous extract AV+N+MO. The mechanism of the therapeutic action of these extracts against CCl_4 toxicity might be due to restoration of GSH level and presence of bioactive compounds. These results are in agreement with previous findings [33,35,38] on the hepatoprotective nature of these plants.

Regarding histopathological results (table. 5), various histopathologic changes in the form of congestion, vacuolar degeneration, and pressure atrophy of hepatocytes are seen due to CCl_4 administration.

Group 4 (administered *Aloe vera* mono aqueous extract) showed a protective effect against hepatocyte atrophy and vacuolar degeneration when the treatment was extended from 18 to 36 d indicating that AV aqueous has a protective effect on hepatocyte integrity. It is noted that vacuolar degeneration is present in group 4 (AV) treated for 18 d while it is absent in groups 5 (administered Moringa mono aqueous extract) and 8 (administered the bi-aqueous extract AV+MO) treated for 18 d indicating that MO is more powerful than AV for improving liver cell injury.

In the liver section of groups administered Neem mono aqueous extract and bi-aqueous extract of AV+N, there was marked congestion with consequent pressure atrophy of hepatocytes and marked vacuolar degeneration at the peripheral zone after 18 d of treatment. Congestion and hepatocyte atrophy were relieved when the treatment was extended for 36 d. However, there was still vacuolar degeneration of hepatocytes. These results indicate that Neem had the least protective effect on liver cell injury in comparison to other groups where there was no hepatocyte atrophy and vacuolar degeneration that has been improved when treatment was extended for 36 d. These results are consistent with biochemical results, concluded that group 6 (N) has the least effect on GGT assuming that *Azadirachta indica* (N) had the minimum protective effect against liver cell damage.

In all groups, there was an improvement in the histopathologic features of liver cell injury (table 5) when the treatment was extended for 18 to 36 d except group 6 and 7 (where there was no improvement of vacuolar degeneration with long-term treatment). This observation is consistent with biochemical results indicating that liver enzymes are elevated in rats treated for 18 d then lowered in rats treated for 36 d.

CONCLUSION

In the present study, the aquatic extracts of *Aloe vera*, *Azadirachta indica*, and *Moringa oleifera* separately/mixedly ameliorated the hepatotoxic effect of carbon tetrachloride and restored the antioxidant status after 36 d of treatment. Administration of extracts orally substantially restored liver enzymes, erythrocyte glutathione content, and plasma malondialdehyde to the normal level along with the improvement of the histopathological features of the injured liver cells. Treatment with Moringa mono extract, *Aloe vera* mono extract, (*Aloe vera* and Moringa) bi extract, (Moringa and Neem) bi extract, Neem mono extract, (*Aloe vera* and neem) bi extract, and (*Aloe vera*, Moringa, and Neem) tri extract for 36 d were more effective in restoring liver enzymes and improving the histopathological features of liver cells than the 18 d duration. The Neem mono extract and the (*Aloe vera* and Neem) bi extract had the least protective effect on hepatotoxicity as they relieved the congestion and hepatocyte atrophy. However, there was still vacuolar degeneration of hepatocytes after 36 d of treatment, which could be enhanced if the treatment is extended than 36 d. These results emphasize that administration of *Aloe vera* gel, Moringa and Neem leaves aquatic extracts could protect against carbon tetrachloride-induced hepatotoxicity. Further studies are recommended to decrease the treatment duration by increasing the concentration of the used extracts and investigate any side effects.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Tirkey N, Pilkhwal S, Kuhad A, Chopra K. Hesperidin, a citrus bioflavonoid, decreases the oxidative stress produced by carbon tetrachloride in rat liver and kidney. BMC Pharmacol 2005;5:2.
2. Preethi KC, Kuttan R. Hepato and renoprotective action of *Calendula offcinalis* L. flower extract. Indian J Exp Biol 2009;47:163.
3. Khan RA, Khan MR, Sahreen S. Evaluation of *Launaea procumbens* use in renal disorders: a rat model. J Ethnopharmacol 2010;128:452-61.
4. Murugesan GS, Sathishkumar M, Jayabalan R, Binupriya AR, Swaminathan K, Yun SE. Hepatoprotective and curative properties of Kombucha tea against carbon tetrachloride-induced toxicity. Microbiol Biotechnol 2009;19:397-402.
5. Usmani S, Kushwaha P. A study on hepatoprotective activity of *Calotropis gigantea* leaves extract. Int J Pharm Pharm Sci 2010;2:101-3.
6. Cabre M, Camps J, Paternain JL, Ferre N, Joven J. Time-course of changes in hepatic lipid peroxidation and glutathione metabolism in rats with carbon tetrachloride-induced cirrhosis. Clin Exp Pharmacol Physiol 2000;27:694-9.
7. Jaramillo-Juarez F, Rodriguez-Vazquez ML, Rincon-Sanchez AR, Consolacion M, Martinez GG, Ortiz J, *et al.* Caffeic acid phenethyl ester against carbon tetrachloride toxicity in rats. Ann Hepatol 2008;7:331-8.
8. Weber LW, Boll M, Stampfl A. Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. Crit Rev Toxicol 2003;33:105-36.
9. Miyazaki T, Bouscarel B, Ikegami T, Honda A, Matsuzaki Y. The protective effect of taurine against hepatic damage in a model of liver disease and hepatic stellate cells. In: Junichi A, Stephen WS, Takashi I. Taurine 7. 1st ed. New York: Springer-Verlag; 2009. p. 293-303.
10. Grimble RF. Nutritional antioxidants and the modulation of inflammation: theory and practice. New Horiz (Baltimore) 1994;2:175-85.
11. Domitrović R, Jakovac H, Milin Č, Radošević-Stašić B. Dose and time-dependent effects of luteolin on carbon tetrachloride-induced hepatotoxicity in mice. Exp Toxicol Pathol 2009;61:581-9.
12. Kähkönen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, *et al.* Antioxidant activity of plant extracts containing phenolic compounds. J Agric Food Chem 1999;47:3954-62.
13. Pietta PG. Flavonoids as antioxidants. J Nat Prod 2000; 63:1035-42.
14. Khalafalla MM, Abdellatef E, Dafalla HM, Nassrallah AA, Aboul-Enein KM, Lightfoot DA, *et al.* Active principle from *Moringa oleifera* Lam leaves effective against two leukemias and a hepatocarcinoma. Afr J Biotechnol 2015;9:8467-71.
15. Anwar F, Latif S, Ashraf M, Gilani AH. *Moringa oleifera*: a food plant with multiple medicinal uses. Phytother Res 2007; 21:17-25.
16. Atawodi SE, Atawodi JC, Idakwo GA, Pfundstein B, Haubner R, Wurtele G, *et al*. Evaluation of the polyphenol content and antioxidant properties of methanol extracts of the leaves, stem, and root barks of *Moringa oleifera* Lam. J Med Food 2010;13:710-6.
17. Ashok KN, Pari L. Antioxidant action of *Moringa oleifera* Lam (drumstick) against antitubercular drugs induced lipid peroxidation in rats. J Med Food 2003;6:255-9.
18. Arabshahi DS, Devi DV, Urooj A. Evaluation of antioxidant activity of some plant extracts and their heat, pH and storage stability. Food Chem 2007;100:1100-5.
19. Verma AR, Vijayakumar M, Mathela CS, Rao CV. *In vitro* and *in vivo* antioxidant properties of different fractions of *Moringa oleifera* leaves. Food Chem Toxicol 2009;47:2196-201.
20. Sreelatha S, Padma PR. Modulatory effects of *Moringa oleifera* extracts against hydrogen peroxide-induced cytotoxicity and oxidative damage. Hum Exp Toxicol 2010;30:1359-68.
21. Hamza AA. *Curcuma longa*, *Glycyrrhiza glabra,* and *Moringa oleifera* ameliorate diclofenac-induced hepatoxicity in rats. Am J Pharmacol Toxicol 2007;2:80-8.
22. Sreelatha S, Padma PR. Protective mechanisms of *Moringa oleifera* against CCl_4-induced oxidative stress in precision-cut liver slices. Forschende Komplementärmedizin 2010;17:189-94.
23. Asolkar LV, Kakkar KK, Chakre OJ. Second supplement to a glossary of Indian medicinal plants with active principles Part-I (AK). New Delhi: Publications and Information Directorate (CSIR); 1992.
24. Fleming T. PDR for herbal medicines. 2nd ed. Montvale: Medical Economics Company; 2000.
25. Reynolds T, Dweck AC. *Aloe vera* leaf gel: a review update. J Ethnopharmacol 1999;68:33-7.
26. Lee KY, Weintraub ST, Yu BP. Isolation and identification of a phenolic antioxidant from *Aloe barbadensis*. Free Radicals Biol Med 2000;28:261-5.

27. Yagi A, Kabash A, Okamura N, Haraguchi H, Moustafa SM, Khalifa TI. Antioxidant, free radical scavenging and anti-inflammatory effects of aloesin derivatives in *Aloe vera*. Planta Med 2002;68:957-60.
28. Bolkent S, Akev N, Ozsoy N, Sengezer-Inceli M, Can A, Okyar A, *et al.* Effect of *Aloe vera* (L.) Burm fil leaf gel and pulp extracts on kidney in type-II diabetic rat models. Indian J Exp Biol 2004;42:48-52.
29. Adzet T, Camarasa J, Laguna JC. Hepatoprotective activity of polyphenolic compounds *Cynara scolymus* against CCl_4 toxicity in isolated rat hepatocytes. J Nat Prod 1987;50:612-7.
30. Hayes JR, Condie LW, Borzelleca JF. Acute, 14-day repeated dosing and 90-day subchronic toxicity studies of carbon tetrachloride in CD-1 mice. Toxicol Sci 1986;7:454-63.
31. Leo MA, Arai M, Sato M, Lieber CS. Hepatotoxicity of vitamin A and ethanol in the rat. Gastroenterology 1982;82:194-205.
32. Hu YY, Liu CH, Wang RP, Liu C, Liu P, Zhu DY. Protective actions of salvianolic acid A on hepatocyte injured by peroxidation *in vitro*. World J Gastroenterol 2000;6:402-4.
33. Parmar SR, Dave GS, Patel HV, Kalia K. Hepato-protective value of some plants extract against carbon tetrachloride toxicity in male rats. J Cell Tissue Res 2009;9:1737-43.
34. Kumar CS, Balamurugan B, Murugeswaran S, Natarajan P, Saravanan SP, Petchimuthu S, *et al.* Hepatoprotective activity of leaves and roots extracts of *Moringa oleifera* Lam. Int J Medicobiol Res 2010;1:90-3.
35. Pandy S, Devmurari V. Bio-evaluation of antioxidant-hepatoprotective activity of methanolic and aqueous extracts of *Azadirchata indica* leaves. Ann Biol Res 2010;1:147-52.
36. Nakbi A, Tayeb W, Grissa A, Issaoui M, Dabbou S, Chargui I, *et al.* Effects of olive oil and its fractions on oxidative stress and the liver's fatty acid composition in 2, 4-Dichlorophenoxyacetic acid-treated rats. Nut Metab 2010;7:80-9.
37. Mohammed A, Abubakar SA. Potency of aqueous extract of *Azadirachta indica a. juss* against lipid peroxidation and liver damage in rats. Int J Mod Biochem 2012;1:27-35.
38. Fakurazi S, Sharifudin SA, Arulselvan P. *Moringa oleifera* hydroethanolic extracts effectively alleviate acetaminophen-induced hepatotoxicity in experimental rats through their antioxidant nature. Molecules 2012;17:8334-50.
39. Núnez M. Hepatotoxicity of antiretrovirals: incidence, mechanisms, and management. J Hepatol 2006;44:132-9.