Bull. Fac .Agric., Cairo Univ. 71: 129-143 (2020).

STUDY THE BIOLOGICAL ACTIVITY OF *Moringa oleifera* AND COFFEE BEANS ON FUNCTIONS AND HISTOLOGY OF LIVER AND KIDNEY IN DIABETIC RATS

(Received:12.8.2020)

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ABSTRACT

Diabetes mellitus (DM) is associated with hyperglycemia, abnormal lipid profiles and inflammatory disorders. The extracts from leaves of *Moringa oleifera* or beans of green coffee were found to treat metabolic disorders as good sources of polyphenols and flavonoids. Also, coffee is a rich source of dietary antioxidants and chlorogenic acid. The present study aimed to evaluate the protective effect of the ethanolic extract of *Moringa oleifera* (MO) and beans of green coffee (GC) in alloxan induced diabetic rats, the stress oxidant, hepatic-kidney dysfunction and histological changes in liver and kidneys of different experimental groups of rats. The extracts were administered using the gastric tube of rats. Results ascertained that orally administration with ethanolic extract of green coffee beans and *Moringa* leaves significantly reduced serum glucose level ($P < 0.05$), improved serum lipid profiles, liver function enzymes and kidney functions in diabetic rats after 28 days. Histological sections of the liver and kidney tissues showed the protective effects of MO and GC in the treated rats. It is noteworthy that there is a synergistic effect between *Moringa* leaves and green coffee beans.

Key words: *Diabetes mellitus, Moringa oleifera, green coffee beans, glucose, biochemical analysis, phenols, flavonoids, histopathological changes.*

1. INTRODUCTION

Diabetes mellitus (DM) is a disease characterized by hyperglycemia caused by the impairment of insulin secretion, insulin action or both. A chronic increase in glucose levels can lead to macro- and microvascular complications, such as heart disease, hypertriglyceridemia, nephropathy, and neuropathy (Izbéki *et al.*, 2008; Olausson *et al.*, 2008).

Type 2 diabetes is caused by beta-cell dysfunction and declining beta-cell mass in insulin resistant subjects. Apoptosis or "programmed cell death", characterized by DNA fragmentation and cellular shrinkage, is increased in pancreatic beta cells in type 2 diabetes leading to loss of beta-cell mass (Butler *et al.*, 2003).

Diabetes produces disturbances of lipid profiles, especially, an increased susceptibility to lipid peroxidation. In addition, increased oxidative stress has been observed in diabetic patients as indicated by high free radical production (Giugliano *et al.*, 1996). Also, the hyperglycemic state has been reported to negatively affect various organs including brain. It is known that hyperglycemia is associated with decline in cognitive abilities as well as in neurotransmitters such as acetyl choline and glutamate. On the other hand, diabetic neurodegeneration was reported as a result of oxidative stress, advanced glycation end products, and vascular distortion (Al-Brakati *et al.*, 2020). Therefore, the consumption of functional foods and bioactive compounds derived from plants used as food can be used as nutritional tools because of their clinical effects (Olson and Fahey, 2001; Misrha *et al.*, 2011).

Moringa oleifera is an Indian tree and it is referred to as "drum stick tree"or the"horse riding tree. "It belongs to the Family *Moringaceae* ,the order *Brassicales* and the genus *Moringa*, which contains 13 species ranging in height from 5 to 10 m. This tree is important because its flowers, pods, and leaves have medicinal uses. It has been reported that the flower contains a stimulant and is used to treat inflammation; the spots and seeds have liver-protective and antihypertensive properties,

while, the leaves are used to treat microbial infections and to control glucose levels. The leaves are eaten as vegetables of food ingredient because of its high content of vitamins, antioxidants and macronutrients and could improve nutritional deficiencies (Asare *et al.*, 2012). Also, aqueous extract of *Moringa oleifera* leaves shows anti-diabetic activity and controls diabetes and thus exhibits glycemic control (Ndong *et al.*, 2007).

Aqueous and ethanol extracts of leaves have been used in biological assay in different doses. Meanwhile, leaf powder studies have been most done in clinical research. Thus, in *vivo* models can be used to bring more information about powder leaf consumption effect on different diseases (Yassa and Tohamy, 2014; Stohs and Hartman, 2015). Also, the experimental animal model of diabetes mellitus can be done by chemical induction using streptozotocin or alloxan which diabetogenic action has been employed and proven in different animal species, with different routs of administration or nutritional stratus (Etuk, 2010; Hasanein and Shahidi, 2011).

Administration of *Moringa oleifera* leaves extract seems to prevent oxidative damage caused by high-fat diet (Sharma *et al.*, 2011) also, can be used to improve the body weight and nearly normalize the thyroid hormones and thyroid stimulating hormone levels indicating the inhibitory activity of MO in thyroid hormone synthesis and/or release in mice (Sunhre, *et al* ., 2020).

Due to the high concentrations of antioxidants present in MO leaves (Anwar *et al.*, 2007; Mensah *et al.*, 2012), they can be used in patients with inflammatory conditions, including cancer, antimicrobial hypertension and cardiovascular diseases (Pari and Kumar, 2002; Ferreira *et al.*, 2008; Mishra *et al*., 2011 ; Posmontie, 2011; and Murillo and Fernandez, 2017).

Chlorogenic acid (CGA), an important biologically active dietary polyphenol, is produced by certain plant species and is a major component of green coffee which have purported antioxidant abilities (Nardini *et al.*, 2002). Caffeoylquinic acid, as one of the major coffee polyphenols, is an ester of caffeic acid with quinic acid (Clifford, 2000) and is often referred to as chlorogenic acid. The term chlorogenic acids (CGAs), however, stand for the whole set of hydroxycinnamic esters with quinic acid, including caffeoyl-, feruloyl-, dicaffeoyl-and coumaroylquinic acids.

Although coffee drinking was initially thought to induce negative effects on health, for example, increasing blood pressure and the risk of cardiovascular disease risk in some cohorts (Noordzij *et al.*, 2005 and Cornelis and El-Sohemy, 2007). The main interest in recent years is the potential for positive health effects. These include plausible reductions in risk of type 2 diabetes, Parkinson disease, Alzheimer's disease, and liver and colorectal cancer (Butt and Sultan, 2011; Zhang *et al.*, 2012).

Therefore, the objective of this study was to evaluate the biological activity of the ethanol extract of *Moringa oleifera* leaves and green coffee beans co-administration on functional and histological abnormalities in liver and kidneys in diabetic rats.

2. MATERIALS AND METHODS

2.1. MATERIALS

Leaves of (MO) and (GC) beans were obtained from local market, Cairo, Egypt. Animals were purchased from animal house of Food Technology Research Institute (FTRI), Agricultural Research Center (ARC), Giza, Egypt. Alloxan and other chemicals were obtained from SIGMA Chemical Company, Cairo, Egypt.

2.2. METHODS

2.2.1.Preparation of plant extracts

(MO) plants leaves and green coffee beans were separately powdered by electrical mill. In order to prepare the extract, 150 g of plants powder were mixed with 1000 ml of 95% ethanol (1:10 w/v) and shacked constantly for 48 h. The suspension was filtered through Whatman No. 1 filter paper and the residue was extracted again, and the pooled plants extract was vacuumed and evaporated in a rotary evaporator (IKA, Germany, temperature 50^{o} and 175 mbar pressure). The dried extracts were stored at 4°C until being used.

2.2.2. Total phenolic content

Total phenolic compounds in the extracts were determined as reported previously (Nobosse *et al.*, 2017) using Folin–Ciocalteu's phenol reagent and gallic acid as a standard. In brief, an aliquot (20 µl) of the extract was mixed with 0.2 ml Folin–Ciocalteu reagent (diluted in water 1:16 v/v) and 0.4 ml of 20% sodium carbonate solution. The tubes were vortexed for 15 s and allowed to stand for 40 min at 40°C for color development. Absorbance was recorded against a reagent blank at 760 nm using a UV–

Vis spectrophotometer (Metertech SP8001; Germany). The total phenolic content was expressed as gallic acid equivalent (GAE) in g/100 g dry matters.

2.2.3.Total flavonoid content of the extracts

Flavonoids were determined according to the method described by (Nobosse *et al.,* 2017). Aliquots (100 µl) of *Moringa* and green coffee extracts were mixed successively, with 2.6 ml of deionized water and 0.15 ml of $NaNO_2$ (5%). After incubation at 25°C for 5 min, 0.15 ml $AlCl_3$ (10%) were added and the mixture was re incubated under the same conditions. At last, 1 ml of NaOH 1M was added and the absorbance was measured at 510 nm against a reagent blank. Catechin (0.01%) was used as standard, and the flavonoids content was expressed as catechin equivalent (CE) in g/100 g dry matters.

2.2.4. The total polyphenolic content (TPC) of the extracts

The TPC of the extracts were determined by using Folin – ciocalteu reagent according to the method described by Singleton *et al.,* (1999) and Lee *et al.,* (2002).

2.2.5. Cholorogenic acid content of the extracts

The Cholorogenic acid of the extracts were determined by the method described by Priftis *et al* .,(2015).

2.2.6.Experimental design

2.2.6.1.Animals

Seventy male albino rats weighing 80-120g were maintained at 25°C on a 12 h light/dark cycle with access to food and water available *ad libitum* for two weeks prior to the commencement of the experiment. The animals were distributed into 7 groups (n=10) G1- negative control group: non-diabetic rats received oral administration of saline 0.9% by using stomach tube NaCl for 28 consecutive days. G2- positive control group G3- diabetic group orally injected with 200 mg/kg b. wt. MO leaves extract G4- diabetic group orally injected with 300 mg/kg b.wt. MO extract G5- diabetic group orally injected with 300 mg/kg b.wt. G C beans extract G6- diabetic group orally injected with 400 mg/kg b.wt. GC beans extract G7- diabetic group orally injected ethanolic extract of both MO leaves 300 mg/kg b.wt. and GC beans 300 mg/kg b.wt. The previous extracts were administered using the gastric tube of rats for 28 days (experiment period), rats were fasted overnight before sacrificing. Blood samples were collected, and then centrifuged to separate the serum. Liver and kidneys, were removed from each rat, cleaned and weighted to calculate the organs weight%.

2.2.6.1. Basal diet composition of tested rats

The basal diet was prepared according to Reeves *et al.,* (1993). It consisted of 20% protein (casein), 10% sucrose, 4.7% corn oil, 0.2% choline chloride, 1% vitamin mixture, 3.5% salt mixture, 5% fibers (cellulose) and up to 100g corn starch. All extracts were given by using stomach tube.

Food intake (FI), body weight gain (BWG), food efficiency ratio (FER) and organs weight % (liver and kidney) were calculated.

2.2.6.2.Treatment with alloxan to elevate blood glucose level

Alloxan (150 mg/kg body weight) successfully causes diabetes in rats. Blood glucose level was strongly elevated on the second day after treatment and the average levels of blood glucose in treatment group of rats ranging between 400-500 mg/dl (Desai and Bhide, 1985).

After sacrificing, the body liver and kidneys of rats were dissected, collected and fixed in 10 % neutral buffered formalin. The samples were processed in graded series of alcohol and embedded in paraffin wax, sectioned at 5 µm and stained with hematoxylin and eosin for histological examination.

2.2.6.3. Blood Sampling

Blood samples of rats were centrifuged at 2,000 g for 10 minutes at 4°C and aliquoted for the respective analytical determinations.

2.2.6.4. Blood Sugar Determination

Fasting blood sugar level of samples was estimated using glucose kit according to Hebi *et al.,* (2017).

2.2.6.4. Determination of Lipid profile

Colorimetric method for cholesterol was determined according to Richmond (1973). Enzymatic colorimetric method used to determine triglycerides (TG) according to Fossati and Principe (1982). HDL-cholesterol was determined according to Fnedewaid (1972) and Gordon and Amer (1977) methods. Determination of LDL cholesterol and VLDL cholesterol was by Lee and Nieman (1996) method.

Very low density lipoprotein (vLDL cholesterol) is calculated as TG/5.

LDL cholesterol = Total cholesterol – (HDL cholesterol + vLDL cholesterol).

Determination of atherognic index (AI): This index was calculated as the vLDL + LDL cholesterol / HDL ratio according to the formula of Kikuchi –Hayakawa *et al*., (1998).

2.2.6.5. Determination of superoxide dismutase (SOD)

Superoxide dismutase activity was assayed spectrophotometrically by inhibition of epinephrine autoxidation as previously described by Misra and Fridovish (1972).

2.2.6.6. Determination of Malondialdehyde (MDA)

Malondialdehyde activity was assayed spectrophotometrically as described by Ohkawa *et al.*, (1979).

2.2.6.7. Determination of glutathione (GSH)

Glutathione was determined according to Mohammed *et al*., (2018).

2.2.6.8. Liver Enzymes activities

Serum alanine aminotransferase (ALT) activity was estimated using the modified kinetic method of Wilson and Islam (2012) using a kit supplied by Human company, Germany, according to the instructions of the supplier. Serum aspartate aminotransferase (AST) activity was estimated using the modified kinetic method of Schumann and Klauke (2003) using a kit supplied by Human company, Germany, according to the instructions of the supplier. Serum alkaline phosphatase (ALP) activity was estimated using the modified kinetic method of Tietz and Shuey (1986) using a kit supplied by Human company, according to the instructions of the supplier.

2.2.6.9. Renal Functions

These functions were determined by using commercial kits (Biomed Company, Germany). Urea was determined according to the method described by Chaney and Marbach (1962), Uric acid was determined according to the method described by Trinder (1969) and creatinine was determined according to the method of Jaffé (1986).

2.2.6.10. Histopathological examination

For the histopathological changes, liver and kidney tissues from diabetic control rats treated with physiological and ethanolic solution were fixed in 10% neutral buffered formalin for 24 hours, dehydrated in a graded alcohol series and after chloroform treatment embedded in paraplast. Deparaplasted 5–6 µm thick sections were stained with hematoxylin and eosin (HE) following standard protocol. Stained slides were examined under a light microscope. Liver sections were examined for vacuolization, lymphocyte infiltrations, necrosis and apoptosis.

Kidney sections were examined for lymphocyte infiltrations, reduction of Bowman's spaces and changes in renal tubules.

2.2.6.11. Statistical analysis

The results are presented as means ±S.D. The obtained data were statistically analyzed according to the SPSS-PC (statistical package software, version, 11.0). One way analysis of variance (ANOVA) was used to test the differences between groups (Marsman, *et al*., 2019).

3. RESULTS

3.1. Bioactive components of the tested materials

Results in Table (1) showed that the total phenolic, polyphenols, total flavonoids and chlorogenic acid of green coffee beans extract were 15, 35, 29 and 11 Mg/g, respectively, and *Moringa oleifera* leaves extract were 32, 28, 9.30, and 3.60 Mg/g, respectively.

Table (1):Phenolic, polyphenols, flavonoids and chlorogenic acid contents of *Moringa oleifera* leaves and green coffee beans extracts.

	***Moringa oleifera* leaves**	**Green coffee beans**
Total phenolic (Mg/g)	32.00	15.00
Polyphenols (Mg/g)	28.00	35.00
Total flavonoids (Mg/g)	9.30	29.00
Chlorogenic acid(Mg/g)	3.6	110.00

3.2. Effect of *Moringa oleifera* and/or green coffee extracts on body weight gain, food intake and food efficiency ratio of rats

The obtained data in Table (2) illustrated the body weight gain (BWG), feed intake (FI), and feed efficiency ratio (FER) of all hyperglycemic rats. It is clear that, the best (BWG) was recorded for group 5 (hyperglycemic rats orally injected with 300 mg/kg b.wt. Green coffee), the best (FI) was recorded for group 3 (hyperglycemic rats orally injected with 200 mg/kg b.wt. MO leaves), and the best (FER) was recorded for group 6 (hyperglycemic rats orally injected with 400 mg/kg b.wt. green coffee) when compared to control group.

Table (2): Effects of *Moringa oleifera* and/or green coffee extracts on body weight gain, food intake and food efficiency ratio in rats.

Parameters	Groups of rats						
	G1	G2	G3	G4	G5	G6	G7
Body weight gain(g)	1.35^{a} ± 0.24	0.96^{e} ± 0.01	1.04^{d} ± 0.02	1.05^{c} ±0.020	1.12^{b} ± 0.07	1.05^{c} ± 0.02	1.02^{d} ± 0.03
Food intake (g)	19.70^{a} ±1.22	17.53^{d} ±1.45	19.69^{a} ±1.77	19.11^{ab} ±2.13	19.38^{ab} ± 1.73	18.39^{c} ±0.95	19.14^{ab} ± 1.28
Food efficiency ratio	0.069^{a} ± 0.004	0.039^{d} ±0.004	0.053^{bc} ±0.001	0.055^{bc} ±0.009	0.0560^{ab} ± 0.001	0.057^{b} ±0.004	0.054^{bc} ± 0.0035

Each value is the mean of n=10 animals ± standard deviation.
Lowercase letters indicate significant differences between treatments when compared at P≤ 0.05.
G1- negative control group. G2- positive control group. G3- diabetic group with 200 mg/kg b. wt. *Moringa olifera* leaves extract. G4- diabetic group with 300 mg/kg b.wt. MO extract.
G5- diabetic group with 300 mg/kg b.wt. green coffee beans extract. G6- diabetic group with 400 mg/kg b.wt. GC beans extract. G7- diabetic group with 300 mg/kg b.wt. MO leaves extract and 300 mg/kg b.wt GC beans extract.

3.3. Effect of *Moringa oleifera* and/or green coffee beans extracts on liver and kidneys weight (%)

Results in Table (3) show that the mean value of the relative liver and kidneys weight was significantly high ($P < 0.05$), increasing as a result of alloxane induced diabetes in G2 compared to the negative group. Treating these diabetic rats with 300 mg/kg body weight GC beans and 300 mg/kg of MO leaves extracts in G4 and G3 or 100 mg/kg GC beans and 100 mg/kg MO,200 mg/kg GC beans and 200 mg/kg MO,300 mg/kg GC beans and 300 mg/kg MO extracts in G5,G6 and G7 respectively, significantly ($P < 0.05$) decreased the weight of these organs approaching the normal relative weight of G1. Also in Table (3) the kidneys weight showed significant difference compared to the control group or with diabetic group.

3.4. Effects of *Moringa oleifera* and/or green coffee extracts on blood glucose level

Results in Table (4) exhibited blood glucose levels of normal and diabetic rats treated with *Moringa* leaves and green coffee beans dietary supplements. Mean values are significantly different ($p < 0.05$) from other groups. It can be observed that in the control group G1 and in diabetes group G2 treated with normal saline, there was highly significant difference between the levels of glucose on the first week of dosing day (439.67 and 95.58) in G2 and G1. When the animals were treated with MO extract or GC beans with different concentration, there was a significant reduction in glucose levels, comparing with the first week. However, in the second week of treatments, the glucose levels in groups G3, G4, G5, G6 and G7 decreased compared with diabetic group but showed significant in comparison with control group. In the third and fourth weeks the glucose levels showed more ($p < 0.05$) significant decrement compared with the diabetic group.

Table (3): Effects of *Moringa oleifera* and/or green coffee beans extracts on the liver and kidneys weight (%).

Organs%	Groups of rats						
	G1	G2	G3	G4	G5	G6	G7
Liver	15.26^{e} ±1.23	21.34^{a} ± 0.15	16.87^{d} ± 0.08	16.87^{d} ± 0.12	17.95^{c} ± 0.09	17.59^{c} ± 1.07	19.69^{b} ± 0.10
Kidney	3.37^{d} ±0.06	5.21^{a} ± 0.05	5.14^{ab} ±0.02	4.73^{c} ± 0.08	4.59^{c} ± 0.30	4.49^{c} ± 0.21	5.07^{b} ± 0.01

Each value is the mean of n=10 animals ± standard deviation.
Lowercase letters indicate significant differences between treatments when compared at P≤ 0.05.
G1- negative control group. G2- positive control group. G3- diabetic group with 200 mg/kg b. wt. *Moringa olifera* leaves extract. G4- diabetic group with 300 mg/kg b.wt. MO extract.
G5- diabetic group with 300 mg/kg b.wt. green coffee beans extract. G6- diabetic group with 400 mg/kg b.wt. GC beans extract. G7- diabetic group with 300 mg/kg b.wt. MO leaves extract and 300 mg/kg b.wt GC beans extract.

Table (4):Effects of *Moringa oleifera* and/or green coffee extracts on blood glucose level (mg/dL).

Time (weeks)	Groups of rats						
	G1	G2	G3	G4	G5	G6	G7
First	95.58^{d} ± 5.89	439.67^{a} ±20.54	183.66^{c} ±11.37	181.33^{cd} ±12.95	273.34^{b} ±31.85	245.81^{bc} ±19.67	97.08^{d} ± 23.46
Second	112.65^{e} ±17.65	536.18^{a} ± 44.67	177.83^{d} ±15.76	166.26^{d} ±12.65	232.65^{c} ±20.32	220.45^{cd} ±20.32	255.65^{b} ±24.65
Third	108.34^{f} ±5.67	520.54^{a} ± 56.34	164.23^{e} ±13.76	160.34^{e} ±13.87	199.34^{c} ±21.34	178.65^{d} ±19.54	232.19^{b} ±26.65
Fourth	122.23^{e} ±8.13	499.63^{a} ± 30.56	140.83^{d} ±12.56	138.56^{de} ±11.45	179.95^{c} ±12.72	160.23^{cd} ±13.54	212.76^{b} ±21.43

Each value is the mean of n=10 animals ± standard deviation.

Lowercase letters indicate significant differences between treatments when compared at P≤ 0.05.

G1- negative control group. G2- positive control group. G3- diabetic group with 200 mg/kg b. wt. MO leaves extract. G4- diabetic group with 300 mg/kg b.wt. MO extracts. G5- diabetic group with 300 mg/kg b.wt. GC beans extract. G6- diabetic group with 400 mg/kg b.wt. GC beans extract. G7- diabetic group with 300 mg/kg b.wt. MO leaves extract and 300 mg/kg b.wt GC beans extract.

3.5. Effects of *Moringa olifera* and/or green coffee extracts on lipids profile and arteriosclerosis index

Results in Table (5) show the effects of orally injected MO and GC on total cholesterol, triglycerides, HDL- and LDL-cholesterol levels. The diabetic rats showed a significant increase in the total serum cholesterol during the experimental periods. The HDL-cholesterol level significantly decreased ($p < 0.05$) in the diabetic rats compared to control rats. After administration with GC and MO extracts, HDL-cholesterol level increased significantly ($p < 0.05$) in treated groups, compared with the untreated rats group.

In the diabetic rats, the LDL-cholesterol level was significantly increased compared to the control group (88.02 *vs.* 18.85 mg/dl). However, after treatment with GC and/or MO extract, LDL-cholesterol levels reduced significantly in treated groups (51.54, 57.72, 43.96, 45.83 and 24.57 mg/dl) compared to the untreated diabetic group (88.02 mg/dl).

On the other hand, triglycerides level significantly (111.49) increased in the diabetic rats compared to the control rats group (65.55 mg/dl) at the end of the experimental period. After administration of GC and / or MO extracts treatment, triglycerides level was significantly ($p < 0.05$) decreased in the treated rats as shown in Table (5).

3.6. Effects of *Moringa olifera* and/or green Coffee extracts on liver function and kidney function

Data in Table (6) reveal the level of ALT, AST and ALP measured as a marker of fatty

Table (5):Effects of *Moringa olifera* and/or green coffee extracts on lipids profiles and arteriosclerosis index.

Parameters	Groups of rats						
	G1	G2	G3	G4	G5	G6	G7
CHL(mg/dl)	70.67^{e} ±0.34	134.66^{a} ±2.51	95.67^{cd} ± 0.45	108.33^{b} ±1.28	95.14^{cd} ±0.62	98.24^{c} ±1.95	77.34^{e} ± 1.53
T.G. (mg/dl)	65.55^{e} ±1.17	111.49^{a} ±1.67	88.65^{c} ± 1.97	95.67^{b} ± 1.88	90.34^{bc} ±1.11	84.68^{c} ±1.76	74.53^{d} ± 1.85
HDL(mg/dl)	38.71^{a} ±0.23	24.23^{d} ±0.47	26.40^{c} ±0.19	31.48^{b} ± 0.16	33.11^{b} ±0.24	35.47^{ab} ±0.72	37.87^{a} ± 0.42
LDL(mg/dl)	18.85^{e} ±0.12	88.02^{a} ±0.70	51.54^{b} ± 0.65	57.72^{b} ± 0.94	43.96^{c} ±0.15	45.83^{c} ±0.75	24.57^{d} ± 0.94
vLDL (mg/dl)	13.11^{c} ±0.23	22.30^{a} ± 0.33	17.73^{b} ± 0.19	19.13^{ab} ± 0.17	18.07^{b} ±0.22	16.94^{b} ±0.15	14.91^{bc} ± 0.17
AI (mg/dl)	0.83^{d} ± 0.02	4.53^{a} ± 0.04	2.62^{b} ± 0.05	2.43^{b} ± 0.04	1.87^{c} ± 0.01	1.77^{c} ± 0.03	1.04^{d} ± 0.01

Each value is the mean of n=10 animals ± standard deviation.

Lowercase letters indicate significant differences between treatments when compared at P≤ 0.05.

G1- negative control group. G2- positive control group. G3- diabetic group with 200 mg/kg b. wt. MO leaves extract G4- diabetic group with 300 mg/kg b.wt. MO extract G5- diabetic group with 300 mg/kg b.wt. GC beans extract G6- diabetic group with 400 mg/kg b.wt. GC beans extract G7- diabetic group with 300 mg/kg b.wt. MO leaves extract and 300 mg/kg b.wt GC beans extract.

Table (6): Effects of *Moringa olifera* and/or green Coffee extracts on liver function and kidney function.

Parameters	Groups of rats						
	G1	G2	G3	G4	G5	G6	G7
AST (IU/L)	122.45^{d} ±0.67	165.91^{a} ±1.65	146.51^{b} ±0.98	148.11^{b} ±0.77	123.41^{d} ±0.24	129.33^{c} ±0.65	124.34^{d} ±0.87
ALT(IU/L)	31.35^{d} ±0.82	49.51^{a} ±0.57	48.03^{a} ±0.19	46.65^{b} ± 0.86	31.39^{d} ±0.12	40.87^{c} ± 1.64	36.77^{d} ± 0.65
ALP(IU/L)	102.34^{e} ±1.34	265.35^{a} ±2.13	264.35^{a} ±1.45	148.76^{c} ±1.77	113.71^{d} ±1.33	193.56^{b} ±2.46	112.34^{d} ±2.11
Urea(mg/dl)	51.36^{d} ±1.17	98.67^{a} ±1.52	81.67^{b} ±1.68	78.47^{b} ±1.95	55.28^{d} ±1.44	71.46^{c} ± 1.32	52.16^{d} ± 1.95
Uric acid(mg/dl)	3.55^{c} ±0.13	5.20^{a} ±1.11	3.73^{c} ±0.89	4.15^{b} ± 0.17	4.18^{b} ±0.21	3.64^{c} ± 0.31	3.59^{c} ± 0.5
Creatinine (mg/dl)	0.71^{bc} ±0.02	0.96^{a} ±0.01	0.74^{bc} ±0.02	0.67^{c} ± 0.01	0.75^{b} ±0.02	0.75^{b} ± 0.01	0.73^{bc} ± 0.02

Each value is the mean of n=10 animals ± standard deviation.
Lowercase letters indicate significant differences between treatments when compared at $P \leq 0.05$.
G1- negative control group. G2- positive control group. G3- diabetic group with 200 mg/kg b. wt. MO leaves extract. G4- diabetic group with 300 mg/kg b.wt. MO extract. G5- diabetic group with 300 mg/kg b.wt. GC beans extract. G6- diabetic group with 400 mg/kg b.wt. GC beans extract. G7- diabetic group with 300 mg/kg b.wt. MO leaves extract and 300 mg/kg b.wt GC beans extract.

liver induced liver cell inflammation and damage. While circulating ALT levels were not affected by both interventions.

It is noteworthy that serum urea, uric acid and creatinine levels were significantly ($p<0.05$) elevated in diabetic control group while these elevated levels remarkably decreased in diabetic treated groups with 300 mg/kg b.wt. GC extract and 300 mg/kg MO extract in G3 and G4 or 100 mg/kg green coffee and 100 mg/kg MO extract ,200 mg/kg GC extract and 200 mg/kg MO extract , 300 mg/kg GC extract and 300 mg/kg MO extract in G5, G6 and G7, respectively. So, the diabetic rats treated with GC beans extract in combination with MO leaves showed no further significant effects ($P<0.05$) on serum urea, uric acid and creatinine levels when compared with the diabetic groups.

3.7. Effects of *Moringa oleifera* and/or green coffee extracts on antioxidant enzymes

Results in Table (7) show that the green coffee beans or *Moringa oleifera* leaves extract consumption was associated with decreasing malondialdehyde (MDA) in the treated groups compared with diabetic group. Superoxide dismutase (SOD) in diabetic group was significantly different in comparison with the control group but the treated groups showed no significant differences ($P < 0.05$) compared with control group except in G7 (diabetic group with 300 mg/kg b.wt. MO leaves extract and 300 mg/kg b.wt. GC beans extract) showed significant difference in comparison with control group Glutathione (GSH) was decreased in positive control group but the combination of green coffee beans or *Moringa oleife*ra leaves extract increased GSH amount and lowered the MDA level ratio back to normal level in diabetic rats.

3.8. Effects of *Moringa oleifera* and/or green coffee extracts on kidneys histopathological changes

The kidney of the negative control rats shows normal renal histological structure of renal parenchyma and glomeruli as shown in Fig. (1a). Fig. (1b) shows kidney of rat from the positive control group with thickened glomerular basement membrane, vacuolated endothelial lining glomerular tuft, and vacuolated epithelial lining renal tubules. Treating diabetic rats with 300 mg GC beans or 300 mg MO leaves extract in G3 and G4 Fig. (1c) and Fig. (1d) ,but in Fig. (1e) shows slight vacuolar degeneration of epithelial lining renal tubules and congestion of glomerular tuft by100 mg GC beans and 100 mg/kg MO extracts in G5 or 200 mg GC beans and 200 mg MO extracts in G6 Fig (1f) and Fig. (1g) nearly restored the renal tissues to their normal histology with no histopathological changes.

The sections of kidney tissue of control rats demonstrated normal architecture with normal glomeruli and tubules. On the other hand, section of kidney tissues of diabetic group of rats revealed visible distortion in the architecture of the kidney tissue showing features of glomerulopathy However, the treatment of diabetic rats with 300mg/kg b.wt. GC beans and

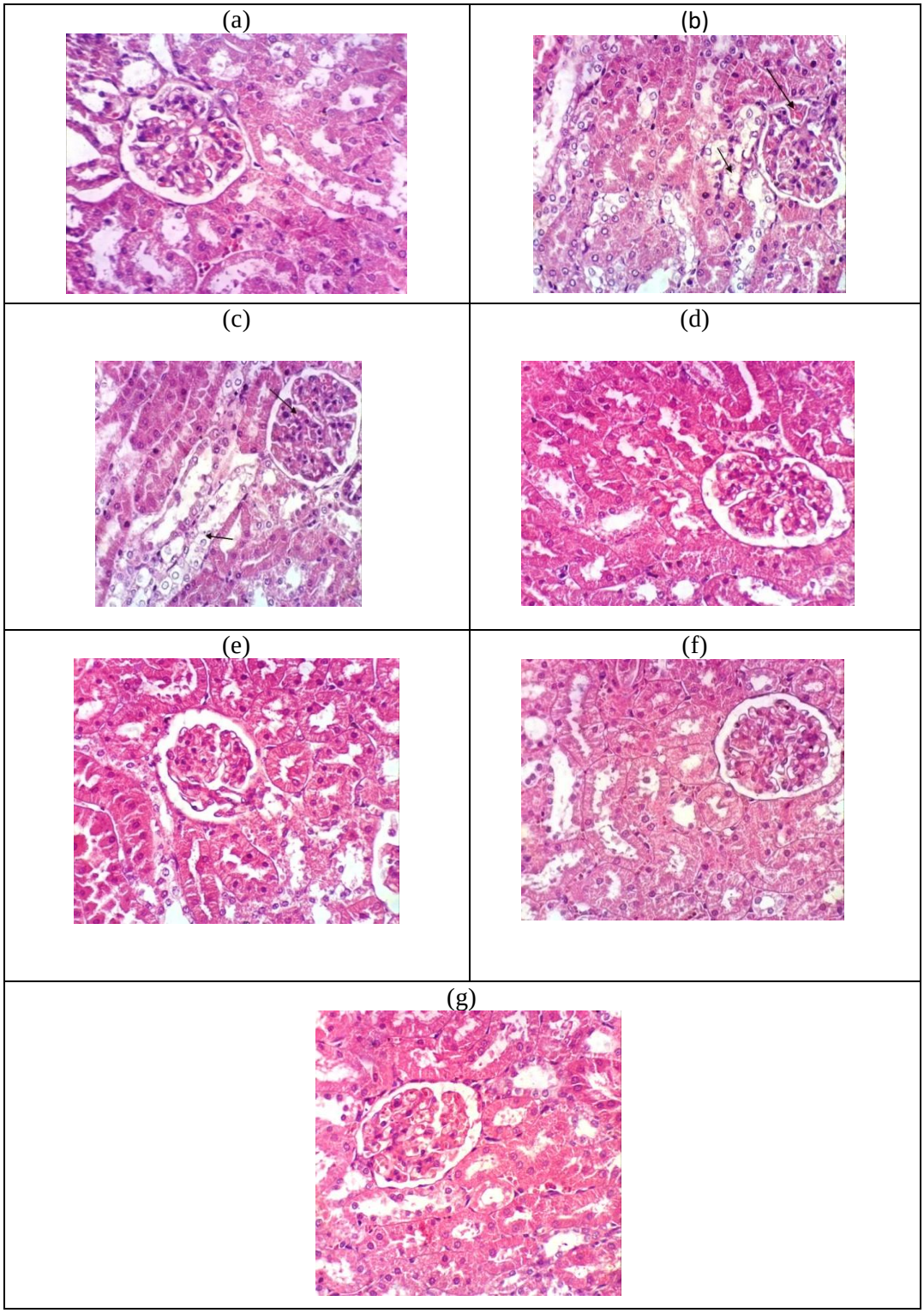

Fig. (1): Effects of *Moringa oleifera* **and/or green coffee extracts on kidney histopathological changes.**
a- kidney histopathological changes of negative control group. b- kidney histopathological changes of positive control group. c- kidney histopathological changes of diabetic group with 200 mg/kg b. wt. MO leaves extract. d- kidney histopathological changes of diabetic group with 300 mg/kg b.wt. MO extract. e- kidney histopathological changes of diabetic group with 300 mg/kg b.wt. GC beans extract. f- kidney histopathological changes of diabetic group with 400 mg/kg b.wt. GC beans extract. g- kidney histopathological changes of diabetic group with 300 mg/kg b.wt. MO leaves extract and 300 mg/kg b.wt GC beans extract.

Table (7): Effects of *Moringa oleifera* and/or green coffee extracts on antioxidant enzymes.

Antioxidant Parameters	Groups of rats						
	G1	G2	G3	G4	G5	G6	G7
MDA(nmol/dl)	2.56^{d} ± 1.02	6.34^{a} ± 0.97	4.36^{b} ±0.73	3.76^{c} ±0.61	3.91^{c} ± 1.04	4.83^{b} ± 1.12	2.94^{d} ± 0.26
SOD(u/ml)	168.34^{a} ±11.48	122.48^{d} ±8.41	150.94^{b} ±12.56	143.45^{cd} ±8.74	149.65^{b} ±13.38	142.83^{c} ±9.36	129.19^{d} ±7.28
GSH(mg/dl)	31.36^{a} ±3.76	17.92^{d} ±2.45	23.36^{b} ±4.34	19.53^{c} ±3.76	25.64^{b} ±6.43	22.24^{bc} ±3.54	18.94^{c} ±2.82

Each value is the mean of n=10 animals ± standard deviation.
Lowercase letters indicate significant differences between treatments when compared at $P \leq 0.05$.
G1- negative control group. G2- positive control group. G3- diabetic group with 200 mg/kg b. wt. MO leaves extract. G4- diabetic group with 300 mg/kg b.wt. MO extract. G5- diabetic group with 300 mg/kg b.wt. GC beans extract. G6- diabetic group with 400 mg/kg b.wt. GC beans extract. G7- diabetic group with 300 mg/kg b.wt. MO leaves extract and 300 mg/kg b.wt GC beans extract.

300 mg/kg MO extracts in G3 and G4 or 100 mg/kg GC beans and 100 mg/kg MO extracts, 200 mg/kg GC beans and 200 mg/kg MO extracts, 300 mg/kg GC beans and 300 mg/kg MO extracts in G5, G6 and G7, respectively the histological changes in the kidney of diabetic rats showing better patterned renal architecture with fairly normal glomeruli and tubules and mild inflammatory cells infiltration.

3.9. Effect of green coffee and/or *Moringa oleifera* extracts on hepatic Histopathological changes

The histological analysis of liver sample staining in liver tissue demonstrated the normal histological structure of hepatic lobule.

In the liver of control diabetic rats, treated with a physiological solution, the majority of hepatocytes contained empty vacuole-like spaces. Hepatocytes around Kiernan's spaces were more intensively vacuolized than the cells around central veins (Fig. 2b). In contrast, in normal liver showing the normal histological structure of hepatic lobule as shows in Fig. (2a).

Control rats showed liver parenchyma with general structures preserved, including hepatic lobules with normal hepatocytes surrounded by sinusoids and distributed radially toward the centrilobular veins with no inflammatory infiltration as shown in Fig. (2c) In contrast, untreated diabetic rats presented morphological changes in the liver that were characterized by hepatocytes that contained focal or generalized fatty vacuoles and micro- or macro vesicular features that were associated with the presence of dilated sinusoids and a progressive loss of general organ structure (disorganization of the lobular architecture) together with mild inflammatory cell infiltration Fig.(2b). Histological examination of the liver of diabetic rats treated with 300 mg GC beans or 300 mg MO extracts in G3 and G4 or by100 mg GC beans and 100 mg MO in G5 or 200 mg GC beans and 200 mg MO extracts in G6 or by 300 mg GC beans and 300 mg MO extracts in G7, show feathery degeneration, and only slight neutrophil infiltration almost comparable to the normal. Also, GC beans and / or MO treatment protected liver tissue from diabetes-induced damage, but to a lesser extent.

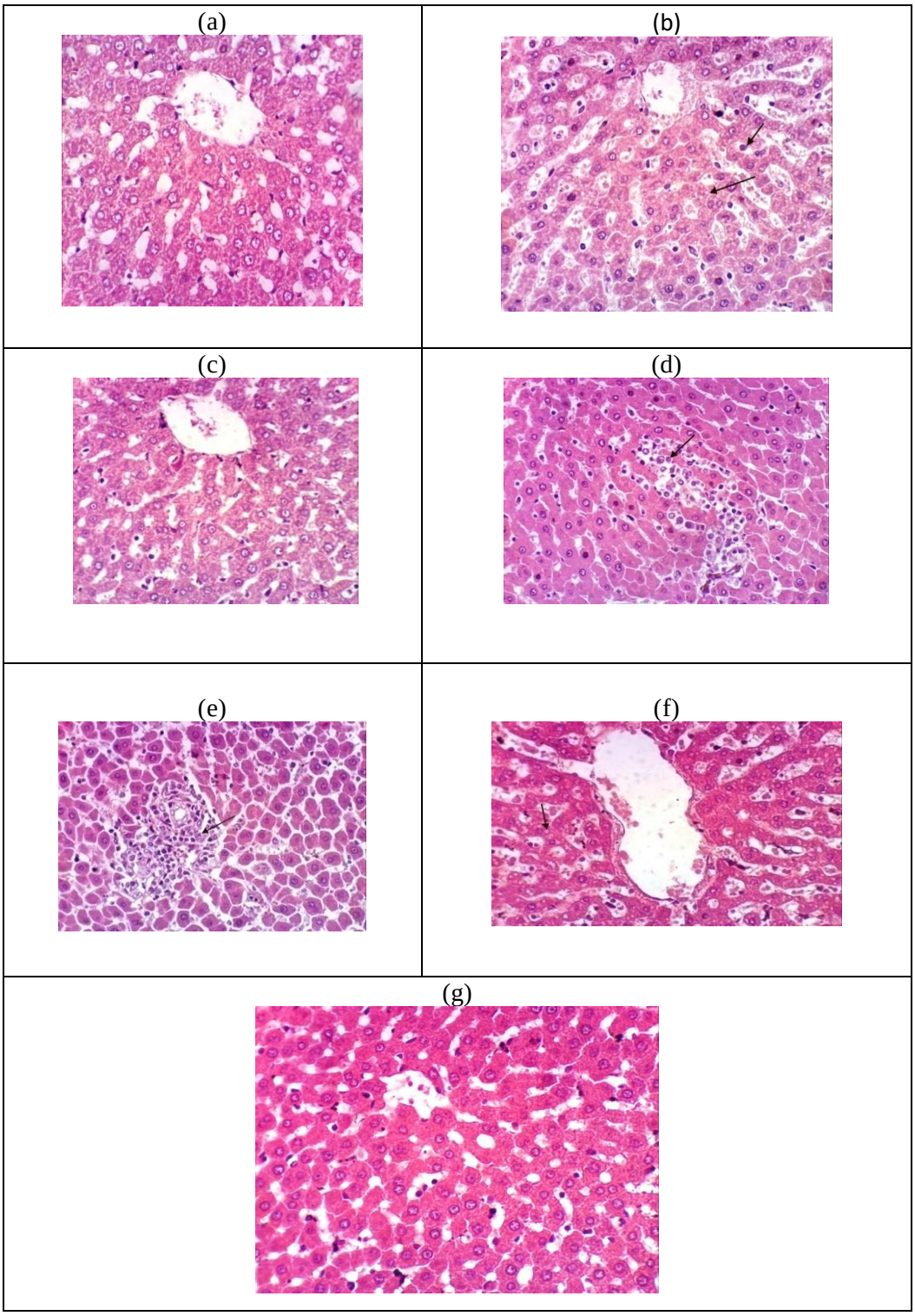

Fig. (2): Effects of green coffee and/or *Moringa oleifera* extracts on hepatic histopathological changes.
a- liver histopathological changes of negative control group. b- liver histopathological changes of positive control group. c- liver histopathological changes of diabetic group with 200 mg/kg b. wt. MO leaves extract. d- liver histopathological changes of diabetic group with 300 mg/kg b.wt. MO extract. e- liver histopathological changes of diabetic group with 300 mg/kg b.wt. GC beans extract. f- liver histopathological changes of diabetic group with 400 mg/kg b.wt. GC beans extract. g- liver histopathological changes of diabetic group with 300 mg/kg b.wt. MO leaves extract and 300 mg/kg b.wt GC beans extract.

4. DISSCUSION

Diabetes mellitus is a metabolic disorder that usually affects carbohydrate, fat, and protein metabolism, followed by multiorgan dysfunction in the later period, and hyperlipidemia associated with hyperglycemia (Chawla *et al.*, 2016).

Data in Table (1) showed that the total phenolic, polyphenols, total flavonoids and chlorogenic acid of GC beans were 15, 35, 29 and 110, respectively and these results are in harmony with that reported by Priftis *et al.* (2015).

Also, results in Table (1) showed that the total phenoilic, polyphenols and total flavonoids of *Moringa oleifera* leaves were 32, 28, 9.30, and 0.36, respectively. The concentrations of the total phenoilic, polyphenols and flavonoids are in agreement with those reported by Yassa and Tohamy (2014).

Cholorogenic acid of MO leaf extract have been shown to have antioxidant, anti-inflammatory and anti-hyperglycemic properties (Stohs and Hartman, 2015).

One of the most sensitive indicators of hepatocyte injury is the release of intracellular enzymes, such as transaminases (ALT and AST and ALP). The enhanced activities of these enzymes are indicative of cellular leakage and loss of the functional integrity of the cell membranes in the liver (Shanmugasundaram *et al.*, 1983; El Arem *et al.*, 2014).

Enzymes indicating liver damage, such as AST, ALT and ALP levels increased in diabetic rats. The elevated serum level of these enzymes were significantly reduced by GC beans *and / or* MO extracts.There is an evidence that the diabetic complications such as increased gluconeogenesis and ketogenesis may be due to elevated enzymes (Shanmugasundaram *et al.*, 1983). The restoration of transaminases to their normal levels after treatment indicates revival of insulin secretion and regenerative activities of islets of Langerhans cells of pancreas after administration of the plant material. MO leaves and/ or GC beans extracts also improved renal functions in diabetic rats by reducing serum urea and creatinine levels. Therefore, our results demonstrate that MO is able to normalize vital organs function in rats. The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia (Jacobson *et al.*, 2007) and this is the primary cause of cardiovascular disease (Bertoni *et al.*, 2004). By administration with MO, the level of triglycerides and LDL-cholesterol were significantly decreased whereas HDL-cholesterol level significantly increased compared to that of diabetic rats (G2). The decrease in triglycerides is most likely due to the insulin stimulatory effect of MO, as insulin plays major role in the inhibition of lipolysis. The HDL-cholesterol level was increased due to improvement in insulin secretion by MO. The reduction in LDL-cholesterol is possibly because of the inhibition of glycosylation of LDL-cholesterol, as MO reduced the elevated blood glucose in the diabetic rats. Increased HDL and reduction in LDL suggested possible conversion of LDL to HDL and clearance of circulating lipids. Also, the observed antihypelipidemic effect may be due to the decrement in cholesterogenesis and fatty acid synthesis through inhibition of pancreatic cholesterol esterase and pancreatic lipase inhibition effect (Heidrich *et al.*, 2004 ; Helmy *et al* ., 2016 and 2017).

In the present study, the alloxane-induced diabetic rats exhibited impairment in kidney function that was manifested by a significant elevation of serum urea, uric acid and creatinine levels as well as derangement in kidneys histological architecture and integrity which were marked by severe glomerular congestion, tubular necrosis and intertubular hemorrhage. These results are in accordance with Ahmed (2001).

The treatment of diabetic rats with GC beans and / or MO, in the present study, resulted in a marked improvement of kidney function represented by a significant decrease in the elevated serum urea, uric acid and creatinine levels along with a remarkable amelioration of the deteriorated kidney histological changes. These ameliorations in kidney function and histological architecture and integrity are associated with the improvements in the glycemic state, serum insulin and C-peptide levels, islets histological changes, kidney oxidative stress and antioxidant defense system as reported by Hu *et al.* (2018).

Body weight was rapidly reduced in animals treated with alloxan alone; the fall was the largest between 3 and 10 days, and then body weight started to recover easily. In diabetic animals treated with MO and /or GC extracts body weight was slightly reduced. It is likely that decreased body weight in diabetic animals is due to dehydration and catabolism of fats and proteins (Hakim *et al.*, 1997).

It is noteworthy that the total polyphenolic compounds and total flavonoids, may contribute to the pleiotropic effects of *Moringa olefiera* leaves that support the use of the plant for different metabolic disorders and this is agreement with (Harnly *et al.*, 2006). On the other hand, increased generation of reactive oxygen species (ROS) is an important aspect in the pathophysiology of diabetes. ROS can damage cellular components, such as proteins, DNA and lipids, resulting in the development of diabetic complications and worsening glycemic control (Nishikawa *et al.*, 2000). Products of lipid peroxidation, such as malondialdehyde (MDA), are elevated with reactive oxygen species (ROS) increase being frequently used as markers of oxidative stress. Superoxide dismutase (SOD) and catalase (CAT) are important antioxidant enzymes that prevent this process by decreasing the level of reactive oxygen species (Del Rio *et al.*, 2005).

The effects of MO leaves and GC beans extracts on antioxidant enzymes in the diabetic rats showed significantly ($p < 0.05$) increased in malondialdehyde (MDA) levels when compared with normal control group. Subsequent treatment of diabetic rats with MO extracts led to a significant ($p < 0.05$) decrease in MDA when compared with non-treated diabetic control. MDA levels decreased in MO-treated control when compared to normal control rats. Activities of catalase (CAT), superoxide dismutase (SOD) decreased in diabetic rats when compared to normal control and a significant ($p < 0.05$) decrease was observed only in CAT as compared to normal control. MO extracts administration to diabetic rats led to significantly ($p < 0.05$) increased in the activities of CAT, while SOD increased but not significantly when compared with diabetic control group. (Verma *et al.*, 2009). SOD represents the first line of defense against oxygen derived radicals (ROS), as it is responsible for the dismutation of superoxide radicals to H_2O, whereas catalase metabolically removes hydrogen peroxide (H_2O_2) and hydroxyl radical generation (McCune and Johns, 2002).

Histopathological examination of the kidney sections of non-diabetic and diabetic rats revealed the protective effect of MO leaves and GC beans extracts on the kidneys. However, histopathological sections of the kidney of non-diabetic rats indicated normal cell structure. Kidney sections of diabetic rats demonstrated severe renal damage showing interstitial nephritis at the cortical area of the kidney (Donath and Shoelson, 2011).

Conclusion

The present study has demonstrated the promising activity of ethanolic extracts of *M. oleifera* leaves and green coffee beans in an animal model of hyperglycemia through improved blood glucose level.

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دراسة النشاط البيولوجى للمورينجا و حبوب القهوة على وظائف وانسجة الكبد والكلى فى الفئران المصابة بالسكرى

عزيزة على صلاح الدين – داليا محمد عبد الله حسن

قسم الأغذية الخاصة – معهد بحوث تكنولوجيا الأغذية- مركز البحوث الزراعية – الجيزة - مصر

ملخص

يرتبط مرض السكري (DM) بإرتفاع سكر الدم و الدهون غيرالطبيعية و إضطرابات إلتهابية. لذلك تم إستخدام مستخلصات أوراق المورينجا أوليفيرا و حبوب البن الأخضر لعلاج اضطرابات التمثيل الغذائي بسبب كونها مصدرا جيدا لمركبات البوليفينول والفلافونويد. كما أن البن يعتبر مصدرا غنيا بمضادات الأكسدة وحمض الكلوروجينيك. هدفت الدراسة الحالية إلى تقييم التأثير الوقائي للمستخلص الايثانولي لأوراق المورينجا أوليفيرا و حبوب البن الأخضر بعد حقن مادة الألوكسان للفئران التي تسبب السكرى و الإجهاد التأكسدي والخلل في وظائف الكبد والكلى والتغيرات النسيجية في كلا من الكبد والكلى لمجموعات تجريبية مختلفة من الفئران. أدى تناول المستخلص الإيثانولي لحبوب القهوة الخضراء وأوراق المورينجا إلى خفض مستوى الجلوكوز في الدم بشكل ملحوظ ($P < 0.05$) ، وتحسين مستوى الدهون في الدم ، و تحسن ملحوظ فى إنزيمات الكبد ووظائف الكلى في الفئران المصابة بداء السكري بعد 28 يومًا. كذلك أظهرت الدراسات النسيجية لأنسجة الكبد والكلى تأثيرات وقائية لكل من اوراق المورينجا اوليفيرا و حبوب البن الاخضر في الفئران المعالجة.