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Research article

Biochemical study on the antioxidant effect of some natural plants on the streptozotocin induced diabetic rats

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Abstract

Oxidative stress is a phenomenon associated with pathogenetic mechanisms of several diseases including diabetes mellitus. There are many medicinal herbs, which have been recommended for the treatment of diabetes. Cinnamon aqueous extract has many pharmacological properties, such as antioxidants activity; also, Coffee is rich in phenolic compounds with a strong antioxidant activity. The aim of the present study was to deduce of better methods of herbal treatment comparison between treatments by cinnamon, green and black coffee alone or by mixing herbs together on the Streptozotocin induced diabetic Rats. Sixty four female albino rats were weighed and divided into eight equal groups; 6 groups were treated orally one time daily for 90 days. Group 1: untreated controls; Group 2: diabetic control; Group 3: (0.6 ml black coffee extract /150g/day); Group 4: (0.6 ml green coffee extract /150g /day); Group 5: (0.6 ml cinnamon extract /150g /day); Group 6: (0.3ml of black coffee and 0.3 ml of cinnamon /150g/day); Group 7: (0.3 ml of green coffee and 0.3 ml of cinnamon/150g/day); Group 8: (0.3 ml of black coffee, 0.3 ml of green coffee and 0.3 ml of cinnamon/150g/day). The mean value level of nitric oxide and malonadialdehyde in the triple combination treated group was significantly decreased in compared with all treated groups while, the mean value level of serum reduced glutathione and paraoxinase in this group is significantly increased (p=0.001) compared with all treated groups. In conclusion: the triple combination treatment by cinnamon, green and black coffee is the best group that acts as antioxidant effect.

Key words: Streptozotocin, Cinnamon, green and black coffee, Malondialdehyde, Nitric oxide, reduced glutathione and paraoxinase.

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Introduction

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of

diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels[1].

DM results in additional vascular events that result in cardiac disease [2] atherosclerosis[3] and renal disorders [4] DM leads to immune system dysfunction[5], liver disorders [6], stroke [3], Alzheimer's disease [7], psychiatric disease [8], visual loss [3], and peripheral nerve impairment [9].

Symptoms of marked hyperglycemia include polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision. Impairment of growth and susceptibility to certain infections may also accompany chronic hyperglycemia. Acute, life-threatening consequences of uncontrolled diabetes are hyperglycemia with ketoacidosis or the non-ketotic hyperosmolar syndrome [1].

The vast majority of cases of diabetes fall into two broad etiopathogenetic categories .In one category, type 1 diabetes, the cause is an absolute deficiency of insulin secretion. Individuals at increased risk of developing this type of diabetes can often be identified by serological evidence of an autoimmune pathologic process occurring in the pancreatic islets and by genetic markers.

In the other, much more prevalent category, type 2 diabetes, the cause is a combination of resistance to insulin action and an inadequate compensatory insulin secretory response. In the latter category, a degree of hyperglycemia sufficient to cause pathologic and functional changes in various target tissues, but without clinical symptoms, may be present for a long period of time before diabetes is detected [1].

It is widely accepted that metabolism abnormality and hyperglycemia resulting in an increase in oxidative stress mark the progression of diabetes and its complications. Hyperglycemia is the cascade of reactions that cause an overproduction of free radicals. These abnormally high levels of free radicals result in reduced antioxidant defense mechanisms, which can lead to cellular organelle damage [10].

Oxidative stress is defined as an imbalance between production of free radicals (FR) and reactive metabolites (RM), so-called oxidants, and their elimination by protective mechanisms, referred to as antioxidative systems. This imbalance leads to damage of important bio-molecules and organs with potential impact on the whole organism.

Oxidative stress is a phenomenon associated with pathogenetic mechanisms of several diseases including atherosclerosis, neurodegenerative diseases, such as Alzheimer's and Parkinson's disease, cancer, diabetes mellitus, inflammatory diseases, as well as psychological diseases or aging processes [11].

Generation of free radicals and antioxidant capacity of the body have been observed to be modulated by environmental, physiological and nutritional factors. For example, physiological factors such as aging, alteration of body mass index and obesity and life style confounding factors such as smoking, drinking and high calorie diet have enhancing effect on oxidative stress and suppressive effect on antioxidants [12].

Nitric oxide (NO) is aparacrine factor that controls vascular tone, inhibits platelet function, prevents adhesion of leukocytes, and reduces proliferation of the intima. An enhanced inactivation and/or reduced synthesis of NO are seen in conjunction with risk factors for cardiovascular disease [13].

Cells have different antioxidant systems such as glutathione and various antioxidant enzymes to protect various tissues from free radicals attacks. Apart from glutathione, the antioxidant enzymes including SOD, CAT and GSH dependent enzymes such as glutathione peroxidase (GPX), and glutathione transferase (GST) may minimize or remove the oxygen radical cascade and reduce cytotoxic oxidative damage in cells [14].

ROS interact with the lipid bilayer of the cell membrane resulting in lipid peroxidation. MDA is the end product of lipid peroxidation. An increased MDA level impairs the structural integrity of the cell membranes by decreasing membrane fluidity and changing the activity of membrane-bound receptors. The products of lipid peroxidation are associated with a variety of diseases, such as DM. Most published studies have found increased lipid peroxidation in DM patients [15].

Spices and some herbs are sources of many effective antioxidants. Herbal products can improve glucose metabolism in diabetic individuals not only by having hypoglycemic effect, but also by improving lipid metabolism, antioxidant status, and capillary function [16].

Cinnamon, a plant of the laurel family Lauraceae, has been used in China for thousands of years to treat many diseases, such as the "thirsty disease," which was an old term for diabetes in China before the term diabetes mellitus was coined in modern medicine. Recent studies demonstrated that cinnamon is effective in improving blood glucose control in patients with type 2 diabetes [17].

Cinnamon has many pharmacological properties, such as antioxidants activity, antibacterial effects, and natural insulin sensitizer [18].

It has been shown that the water soluble polyphenol polymers from cinnamon could markedly increase insulin-dependent glucose metabolism in vivo as well as lead to an elevated antioxidant activity [19].

Cinnamon increases body heat, and thereby speeds up metabolism in order to burn the extra calories or fats deposited in the body. Cinnamon has a marked antioxidant potential and may be beneficial in alleviating the complications of many illnesses related to oxidative stress in humans [20].

It is widely accepted that antioxidants may delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidizing chain reactions [21]. These involve absorption and neutralization of free radicals, quenching singlet and triplet oxygen, and decomposing peroxides [22].

Considerable attention has recently been focused on some nutritional factors such as polyphenols that could counteract oxidative damages and therefore be beneficial through their antioxidant properties [23]. Among plants that contain polyphenols is cinnamon that may be of special interest, since cinnamon and Cinnamon Aqueous Extract (CAE) has been documented to be *in vitro* and *in vivo* antioxidant [24].

Using of various aqueous cinnamon extracts and showing of insulin-enhancing properties in vitro in adipocytes, suggesting that isolated A-type doubly linked procyanidin oligomers of the catechins and/or epicatechins from cinnamon may be responsible for the observed effect [19].

Green coffee is a complex beverage with hundreds of components present in the grain or produced in the process and in the development of the beverage. Thus, there has been identified more than 700 volatile compounds from several categories in roasted coffee beans, as well as numerous nonvolatile components such as polysaccharides, melanoidins, chlorogenic acids, aldehydes, ketones, alkaloids such as caffeine and inorganic compounds such as nitrogen, potassium, calcium, magnesium, phosphorus and sulfur. To this is added the compounds formed during processing of the beverage [25].

Coffee is among the most widely consumed pharmacologically active beverages in the world. Caffeine is the most widely consumed psychoactive substance. Coffee is rich in phenolic compounds with a strong antioxidant activity [26].

Author found that regular drinking of coffee can reduce the oxidation of human low-density lipoprotein (LDL) and the oxidation of

LDL, decreasing the risk of atherosclerosis [27], coffee is rich in antioxidants [28].
Author reported that, phenolic compounds are widely distributed in fruits and vegetables [29]. Also, another author showed that, phenolic compounds have received considerable attention because of their potential antioxidant activities and free radical-scavenging abilities, which potentially have beneficial implications in human health [30].
At higher roasting degrees, damage to sensory characteristics and radical-scavenging activity of coffee beans is described as the main disadvantages of dark roasting. Therefore, degradation of polyphenol compounds by thermal process may result in releasing antioxidant compounds that have different chemical and biological properties [31].
Moreover, coffee contain compounds have several beneficial health properties largely explained by their potent antioxidant activity. In addition, they have exhibited hypoglycemic, antiviral, hepatoprotective and antispasmodic activities [32].
In the present study, we assess the antioxidant status in control and diabetic rats which received black and green coffee and cinnamon separately or together and deduce of better methods of herbal treatment comparison between treatments alone or by mixing herbs together.

Materials and methods

Animals:

Sixty four normal female albino rats weighting 150-200 g were obtained from the animal house of Vacsera, Helwan, Egypt. The animals were housed in individual suspended stainless steel cages in a controlled environment (22-25 ^{o}C) and 12 hour light, 12 hour dark with food and water available in national research center and left for 1 week for acclimatization prior to the start of the experiment.
The experimental protocols were approved by the institutional animal ethics committee. All rats used in the following experiments were subject to the Guiding Principles for the care and use of laboratory animals and the recommendations of the declaration of Helsinki.
Diabetes was induced in the overnight fasted rats by a single subcutaneous injection of streptozotocin (50 mg/kg body weight) dissolved in citrate buffer (pH = 4.5). Normal control rats received 50 mg/kg body weight citrate buffer only as vehicle. Fasting blood sugar was estimated after 72 hours to confirm the development of diabetes [33].
The fasting blood sugar was estimated after 3 days from all rat tail veins by glucometer to confirm the development of diabetes mellitus and then rats were divided into eight groups control, diabetic control and 6 diabetic groups were treated with cinnamon, green and black coffee aqueous extracts single and mixed after diabetes was induced.

Chemicals:

Streptozotocin (STZ) was purchased from Sigma Chemicals Company, St, Louis, USA, malondialdehyde (MDA), reduced glutathione (GSH), nitric oxide (NO) and paraoxinase were purchased from Bio diagnostics company, GIZA, EGYPT and cinnamon, Green coffee and black coffee were purchased from local market (Cairo, Egypt).

Preparation of natural compounds extracts:

10grams of each ground natural compound (black and green coffee, cinnamon) are placed in Whatman filter paper no.3 and then 100 ml of distilled water are poured at 90^{o}C into the powder contained in the filter and each rat is administered by gavage (4 ml /kg/day) for 90 days but we suggest treatment by common way to be easily used by public [34].
For rats administrated single herbs, 10grams of each ground natural compound (black and green coffee, cinnamon) were added to 100 ml of boiling distilled water then lift to cool and each rat is administered by gavage (4 ml supernatant /kg/day for 90 days .

For rats administrated combined herbs, 10grams of each ground natural compound (black and green coffee, cinnamon) were added to 50 ml of boiling distilled water then lift to cool and each rat is administered by gavage (4 ml supernatant /kg/day for 90 days.

Experimental design and animal grouping:
Sixty four female albino rats were weighed and divided into eight equal groups; all were treated orally one time daily for 90 days.
Group 1: untreated controls; Group 2: diabetic control; Group 3: group will be rendered STZ induced diabetic rats; Group 4: group will be rendered STZ induced diabetic rats; Group 5: group will be rendered STZ induced diabetic rats; Group 6: group will be rendered STZ induced diabetic; Group 7: group will be rendered STZ induced diabetic, Group 8: group will be rendered STZ induced diabetic rats

Blood Collection:
After 3 months of the experiment, from the treatments, 6 rats from each group were fasted overnight before blood sampling and sacrificed under diethyl ether anesthesia. Blood samples were collected and allowed to coagulate at room temperature then centrifuged at 3000 rpm for 30 minutes. The clear, non haemolysed supernatant sera were quickly removed and stored at -20C° for subsequent biochemical analysis. The following parameters were estimated:-

Determination of lipid peroxidation product (MDA):
This was performed according to the method of [35]**.**The kit was supplied from Bio-diagnostic Company.

Estimation of serum antioxidant parameters:
GSH concentration, Nitric oxide and paraoxinase activities were determined according to [36**,** 37] and [38] respectively.

Statistical analysis:
The collected data were coded, tabulated, and statistically analyzed using IBM SPSS statistics (Statistical Package for Social Sciences) software version 22.0, IBM Corp., Chicago, USA, 2013.

Result and Discussion

Results
Estimation of serum Malondialdehyde (µM/L) after treatment:
The mean value level of serum malondialdehyde (MDA) in diabetic group was significantly increased (p=0.001) compared to control group (table 1, figure 1).
In treated groups, the mean value levels of serum MDA in the single treated groups (black coffee or green coffee or cinnamon) were significantly increased (p=0.001) compared with control group (table 1, figure 1).
Alternatively, the mean value levels of serum MDA in the single treated groups (black coffee or green coffee or cinnamon) were significantly decreased (p=0.001) compared with diabetic group (table 1, figure 1).
Also, no significant difference were observed in the mean value levels of serum MDA in the single treated groups with each other black coffee or green coffee or cinnamon (table 1, figure 1).
On the other hand, no significant difference were observed in the mean value levels of serum MDA in the double combination treated groups (black coffee and cinnamon or green coffee and cinnamon) when compared with control group (table 1, figure 1).
In contrast, the mean value levels of serum MDA in the double combination treated groups (black coffee and cinnamon or green coffee and cinnamon) were significantly decreased (p=0.001) compared to diabetic group (table 1, figure 1).
However, the mean value levels of serum MDA in the double combination treated groups (black coffee and cinnamon or green coffee and cinnamon) were significantly decreased

(p=0.001) compared with single treated groups (black coffee, green coffee and cinnamon) (table 1, figure 1).

No significant difference were observed in the mean value levels of serum MDA in the double combination treated groups (black coffee and cinnamon or green coffee and cinnamon) when compared with each other (table 1, figure 1).

Additionally, no significant difference were observed in the mean value level of serum MDA in the triple combination treated group (black coffee, green coffee and cinnamon) when compared with control group (table 1, figure 1).

In contrast, the mean value level of serum MDA in the triple combination treated group (black coffee, green coffee and cinnamon) is significantly decreased (p=0.001) compared with diabetic group (table 1, figure 1).

However, the mean value level of serum MDA in the triple combination treated group (black coffee, green coffee and cinnamon) is significantly decreased (p=0.001) compared with single treated groups (table 1, figure 1).

On the other hand, no significant difference were observed in the mean value level of serum MDA in the triple combination treated group (black coffee, green coffee and cinnamon) when compared with double combination treated groups(table 1, figure 1).

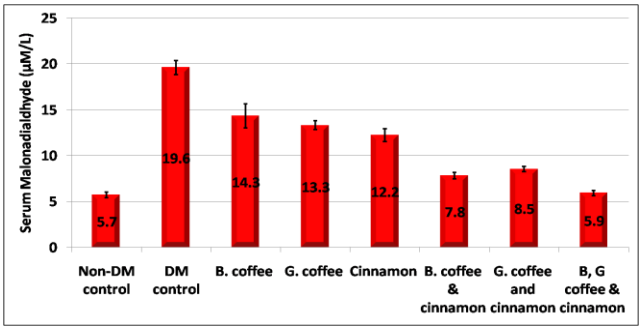

Figure 1. Mean value levels of serum malonadialdehyde µM/L in different studied groups.

Table 1. Serum malonadialdehyde (µM/L) after treatment in different studied groups.

Group	Mean±SD	Range	HG
Non-DM control	5.7±0.7	4.7–6.9	a
DM control	19.6±1.9	17.1–21.8	b
B. coffee	14.3±3.2	10.9–20.4	c
G. coffee	13.3±1.2	11.8–14.7	c
Cinnamon	12.2±1.7	10.1–14.1	c
B. coffee & cinnamon	7.8±0.9	6.4–8.7	a
G. coffee and cinnamon	8.5±0.7	7.5–9.4	a
B, G coffee & cinnamon	5.9±0.7	4.9–6.9	a

HG: Homogenous groups have the same letter(Tukey HSD post ANOVA test) that between groups which non-significant difference between them.

Number of cases= 8

Significant P value = 0.001.

P^a significant difference in diabetic, single treated group.

P^b significant difference in control group, single treated groups, double combination treated groups and triple combination treated group.

P^c significant difference in control group, diabetic, double combination treated groups and triple combination treated group.

Estimation of serum nitric oxide (µM/L) after treatment:

The mean value level of Nitric oxide (NO) after treatment in diabetic group was significantly increased (p=0.001) compared to control group (table 2, figure 2).

In treated groups, the single treated groups (black coffee or green coffee or cinnamon), the mean value level of NO are significantly decreased (p=0.001) compared to diabetic group (table 2, figure 2).

Additionally, there was still a significant increased (p=0.001) in the mean value levels of NO in single treated groups (black coffee, green coffee or cinnamon) compared to control group (table 2, figure 2).

In contrast, no significant difference was observed in the mean value levels of NO in the single treated groups (black coffee, green coffee or cinnamon) compared with each other (table 2, figure 2).

The mean value level of NO of double combination treated groups (Black coffee & cinnamon and green coffee & cinnamon) were significantly decreased (p=0.001) compared to diabetic group (table 2, figure 2).

However, the mean value level of NO of Black coffee & cinnamon double combination treated group was significantly increased (p=0.001) compared to control group (table 2, figure 2).

While, no significant difference was observed in the mean value level of NO in the double combination treated group green coffee and cinnamon in compared with control group(table 2, figure 2).

The mean value level of NO of double combination treated groups (Black coffee & cinnamon and green coffee & cinnamon) were significantly decreased (p=0.001) compared to black coffee, green coffee and cinnamon single treated groups(table 2, figure 2).

Table 2. Nitric oxide (µM/L) after treatment in different studied groups.

Group	Mean±SD	Range	HG
Non-DM control	3.6±0.5	2.9–4.2	a, b
DM control	8.3±0.7	7.3–9.1	c
B. coffee	6.9±0.8	6.1–7.9	d
G. coffee	6.9±0.7	6.0–7.8	d
Cinnamon	7.1±1.0	5.9–8.8	d
B. coffee & cinnamon	5.3±0.4	4.8–5.9	e
G. coffee and cinnamon	4.8±0.4	4.4–5.5	b, e
B, G coffee & cinnamon	3.6±0.6	2.8–4.4	a

HG: Homogenous groups have the same letter(Tukey HSD post ANOVA test) that between groups which non-significant difference between them.

Number of cases=8

Significant P value = 0.001.

P^a No significant difference in control group and triple combination treated group

P^b No significant difference in control group and green coffee and cinnamon double combination treated group.

P^dNo significant difference in single treated groups.

P^e No significant difference in double combination treated groups.

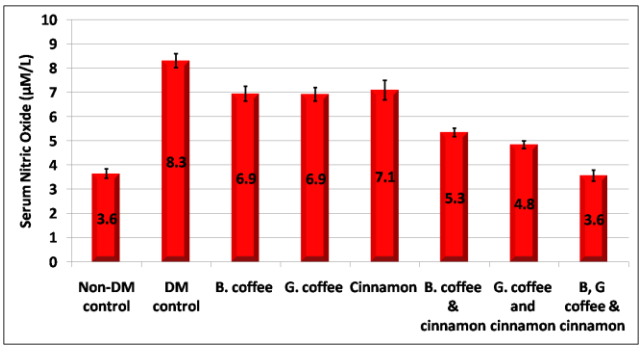

Figure 2. Mean value levels of Nitric oxide (µM/L) in different studied groups.

Also, the triple combination treated group (black, green and cinnamon), the mean value level of NO was significantly decreased in compared to diabetic control(table 2, figure 2).
In contrast, the triple combination treated group (black, green and cinnamon), the mean value level of NO was significantly decreased in compared with black coffee, green coffee and cinnamon single treated groups (table 2, figure 2).

On the other hand, the triple combination treated group (black, green and cinnamon), the mean value level of NO was significantly decreased in compared with black coffee & cinnamon and green coffee & cinnamon double combination treated groups (table 2, figure 2).

Estimation of serum reduced glutathione (µM/L) after treatment:

The mean value level of serum reduced glutathione (GSH) in diabetic group was significantly decreased (p=0.001) compared with control group (table 3, figure 3).
In treated groups, the mean value levels of serum GSH in the single treated groups (black coffee or green coffee or cinnamon) were significantly decreased (p=0.001) compared with control group (table 3, figure 3).
Alternatively, the mean value levels of serum GSH in the single treated groups (black coffee or green coffee or cinnamon) were significantly decreased (p=0.001) compared with diabetic group (table 3, figure 3).

Table 3. Serum Reduced glutathione (µM/L) after treatment in different studied groups.

Group	Mean±SD	Range	HG
Non-DM control	3.0±0.2	2.7–3.2	a
DM control	1.2±0.1	0.9–1.0	b
B. coffee	2.0±0.5	1.6–2.8	c
G. coffee	2.1±0.3	1.8–2.6	c
Cinnamon	2.0±0.2	1.7–2.3	c
B. coffee & cinnamon	2.6±0.5	1.9–3.3	a, c
G. coffee and cinnamon	2.5±0.3	2.1–2.9	a, c
B, G coffee & cinnamon	2.8±0.3	2.3–3.2	a

HG: Homogenous groups have the same letter(Tukey HSD post ANOVA test) that between groups which non-significant difference between them.
Number of cases= 8
Significant P value = 0.001.
P^a significant difference in diabetic, single group and double combination treated group
P^b significant difference in control group, single, double and triple combination treated group.
P^c significant difference in control group, diabetic, single treated groups, double combination treated group and triple combination treated group.

Also, no significant difference were observed in the mean value levels of serum GSH in the single treated groups with each other black coffee or green coffee or cinnamon(table 3, figure 3).
On the other hand, no significant difference were observed in the mean value levels of serum GSH in the double combination treated groups (black coffee and cinnamon or green coffee and cinnamon) when compared with control group (table 3, figure 3).
In contrast, the mean value levels of serum GSH in the double combination treated groups (black coffee and cinnamon or green coffee and cinnamon) were significantly increased (p=0.001) compared to diabetic group (table 3, figure 3).
However, no significant difference were observed in the mean value levels of serum GSH in the double combination treated groups (black coffee and cinnamon or green coffee and cinnamon) when compared with single treated groups (black coffee, green coffee and cinnamon) (table 3, figure 3).
No significant difference were observed in the mean value level of serum GSH in the triple combination treated group (black coffee, green coffee and cinnamon) when compared with control group (table 3, figure 3).
Additionally, the mean value level of serum GSH in the triple combination treated group (black coffee, green coffee and cinnamon) is significantly increased (p=0.001) compared with diabetic group (table 3, figure 3).
However, the mean value level of serum GSH in the triple combination treated group (black coffee, green coffee and cinnamon) is significantly increased (p=0.001) compared with single treated groups (table 3, figure 3).
Also, the mean value level of serum GSH in the triple combination treated group (black coffee, green coffee and cinnamon) is significantly increased (p=0.001) compared with double combination treated groups (table 3, figure 3).

Estimation of serum paraoxinase (µM/L) after treatment:

The mean value level of serum paraoxinase (PON) in diabetic group was significantly decreased (p=0.001) compared to control group (table 4, figure 4).

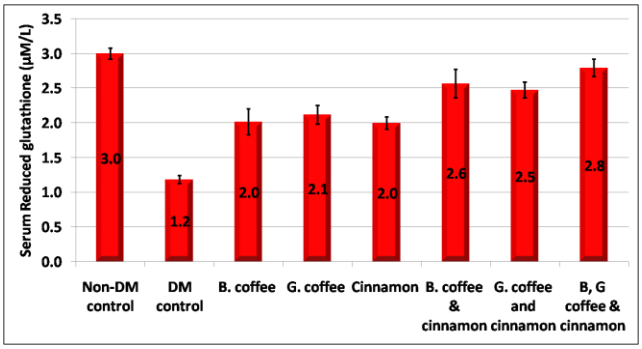

Figure 3. Mean value levels of serum reduced glutathione µM/L in different studied groups.

In treated groups, the mean value levels of serum PON in the single treated groups (black coffee or green coffee or cinnamon) were significantly decreased (p=0.001) compared with control group (table 4, figure 4).
Alternatively, the mean value levels of serum PON in the single treated groups (black coffee or green coffee or cinnamon) were significantly increased (p=0.001) compared with diabetic group(table 4, figure 4).
Also, no significant difference were observed in the mean value levels of serum PON in the single treated groups with each other black coffee or green coffee or cinnamon (table 4, figure 4).
On the other hand, no significant difference were observed in the mean value levels of serum PON in the double combination treated groups (black coffee and cinnamon or green coffee and cinnamon) when compared with control group (table 4, figure 4).
In contrast, the mean value levels of serum PON in the double combination treated groups (black coffee and cinnamon or green coffee and cinnamon) were significantly increased (p=0.001) compared to diabetic group (table 4, figure 4).
However, the mean value levels of serum PON in the double combination treated groups (black coffee and cinnamon or green coffee and

cinnamon) were significantly increased (p=0.001) compared with single treated groups (black coffee, green coffee and cinnamon) (table 4, figure 4).
No significant difference were observed in the mean value levels of serum PON in the double combination treated groups (black coffee and cinnamon or green coffee and cinnamon) when compared with each other (table 4, figure 4).
Additionally, no significant difference were observed in the mean value level of serum PON in the triple combination treated group (black coffee, green coffee and cinnamon) when compared with control group(table 4, figure 4).
In contrast, the mean value level of serum PON in the triple combination treated group (black coffee, green coffee and cinnamon) is significantly increased (p=0.001) compared with diabetic group(table 4, figure 4).
However, the mean value level of serum PON in the triple combination treated group (black coffee, green coffee and cinnamon) is significantly increased (p=0.001) compared with single treated groups(table 4, figure 4).
On the other hand, no significant difference were observed in the mean value level of serum PON in the triple combination treated group (black coffee, green coffee and cinnamon) when compared with double combination treated groups, (table 4, figure 4).

Discussion:

Type 2 diabetes mellitus is a major endocrine disorder and a deadly disease in human beings [39].According to the recent estimations, the prevalence of diabetes in the world, would reach to 552 million people in 2030 [40].

Table 4. Serum paraoxinase (µM/L) after treatment in different studied groups.

Group	Mean±SD	Range	HG
Non-DM control	129.5±20.0	103.1–151.2	a
DM control	52.3±12.6	30.8–68.5	b
B. coffee	93.0±15.8	75.5–113.7	c
G. coffee	87.1±14.6	69.5–103.0	c
Cinnamon	89.5±18.7	69.3–114.2	c
B. coffee & cinnamon	131.6±19.7	107.6–155.7	a
G. coffee and cinnamon	131.5±12.4	107.1–141.5	a
B, G coffee & cinnamon	124.0±15.7	98.5–140.1	a

HG: Homogenous groups have the same letter (Tukey HSD post ANOVA test) that between groups which non-significant difference between them.
Number of cases= 8
Significant P value = 0.001.
P^a significant difference in diabetic, single treated group.
P^b significant difference in control group, double combination treated groups and triple combination treated group.
P^c significant difference in control group, diabetic, double combination treated group and triple combination treated group

Dyslipidemia, a main risk factor for cardiovascular diseases as well as increase in generation of reactive oxygen species (ROS) and occurrence of oxidative stress which results in destruction of insulin producing β-cells in pancreatic langerhans islets, all have critical role

in pathogenesis and progression of diabetes mellitus [41].

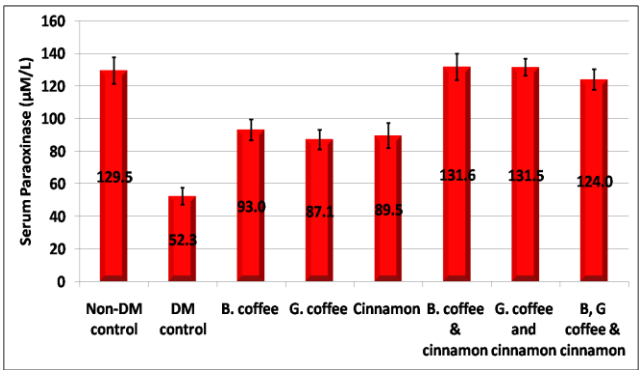

Figure 4. Mean value levels of serum paraoxinase µM/L in different studied groups.

Since using of medicinal plants is more financial, and they have different varieties of effective compounds as well as lower side effects in comparison to synthetic drugs and also because of recommendations of World Health Organization (WHO), in recent years, there has been renewed interest in using hypoglycemic traditional plants [42]. There are many medicinal herbs, which have been recommended for the treatment of diabetes [43].

Markers of oxidative stress in treated groups were assessed to elucidate and compare the effects of these natural products used alone or together against diabetic and non-diabetic control.

Streptozotocin induced diabetes is well documented model of experimental diabetes [44]**.** It can begin an autoimmune process that results in the destruction of the Langerhans islets beta cells and results in the toxicity of beta cells [45].Increased oxidative stress could be one of the common pathogenic factors of diabetic complications [46].

The mean value level of Nitric oxide of double combination treated groups (Black coffee & cinnamon and green coffee & cinnamon) were significantly decreased compared to diabetic group. However, the mean value level of Nitric oxide of Black coffee & cinnamon double combination treated group was significantly increased compared to control group.

While, no significant change was observed in the mean value level of Nitric oxide in the double combination treated group green coffee and cinnamon in compared with control group.

The mean value level of Nitric oxide of double combination treated groups (Black coffee & cinnamon and green coffee & cinnamon) were significantly decreased compared to black coffee, green coffee and cinnamon single treated groups.

Also, the triple combination treated group (black, green and cinnamon), the mean value level of Nitric oxide was significantly decreased in compared to diabetic control.

In contrast, the triple combination treated group (black, green and cinnamon), the mean value level of Nitric oxide was significantly decreased in compared with black coffee, green coffee and cinnamon single treated groups.

On the other hand, the triple combination treated group (black, green and cinnamon), the mean value level of Nitric oxide was significantly decreased in compared with black coffee & cinnamon and green coffee & cinnamon double combination treated groups.

In our study, the mean value levels of serum malonadialdehyde (MDA) in the cinnamon single treated groups was significantly decreased ($p=0.001$) compared with diabetic group, this results in agreement with [47], confirmed the previously recorded antioxidant effect of cinnamon *in vitro* [48] and *in vivo* [24] that was indicated by decreased MDA levels and increased antioxidant enzymes activities.

Additionally, cinnamon extract exhibited protective capacity against irradiation-induced LPO in liposomes, and quenched hydroxyl radicals (OH•) and H_2O_2 [49].

The protective action of cinnamon extract against radiation-induced oxidative and inflammatory damages was attributed to its suppressive effect on ROS generation. This is due to its phenolic and flavonoids contents, in addition to modification of gene expression by

inhibiting nuclear factor-kappa B (NF-Kb) activation [50].

Polyphenols act as reactive oxygen and nitrogen species scavengers, redox-active transition metal chelators and enzyme modulators [51]. The high reactivity of the hydroxyl substituent of flavonoids with the number of hydroxyl groups on the B-ring being correlates with ROS scavenging capability [52].

The mean value levels of serum MDA in the black coffee or green coffee single treated groups was significantly decreased compared with diabetic group, this result was in agreement with author who found that, diet supplemented with Arabic coffee beans induced a significant decrease of MDA levels in plasma compared to the diabetic control group. In diabetic group, a significant decrease of GSH levels was observed in plasma as compared to the control group [53].

On the other hand, no significant change were observed in the mean value levels of serum MDA in the double combination treated groups (black coffee and cinnamon or green coffee and cinnamon) when compared with control group.

In contrast, the mean value levels of serum MDA in the double combination treated groups (black coffee and cinnamon or green coffee and cinnamon) were significantly decreased compared to diabetic group.

However, the mean value levels of serum MDA in the double combination treated groups (black coffee and cinnamon or green coffee and cinnamon) were significantly decreased compared with single treated groups (black coffee, green coffee and cinnamon.

No significant different were observed in the mean value levels of serum MDA in the double combination treated groups (black coffee and cinnamon or green coffee and cinnamon) when compared with each other.

Additionally, no significant different were observed in the mean value level of serum MDA in the triple combination treated group (black coffee, green coffee and cinnamon) when compared with control group.

In contrast, the mean value level of serum MDA in the triple combination treated group (black coffee, green coffee and cinnamon) is significantly decreased compared with diabetic group.

However, the mean value level of serum MDA in the triple combination treated group (black coffee, green coffee and cinnamon) is significantly decreased compared with single treated groups.

On the other hand, no significant different were observed in the mean value level of serum MDA in the triple combination treated group (black coffee, green coffee and cinnamon) when compared with double combination treated groups.

The mean value levels of serum reduced glutathione in the black coffee or green coffee single treated groups were significantly increased when compared with diabetic group, this results was in agreement with author who demonstrated that, Diet supplemented with Arabic coffee beans improved, GSH levels in plasma as compared to those of the diabetic group [53].

The mean value levels of serum reduced glutathione in the cinnamon single treated groups was significantly decreased compared with diabetic group this results was in agreement with [47]**,** confirmed the previously recorded antioxidant effect of cinnamon *in vitro*.

On the other hand, no significant change were observed in the mean value levels of serum reduced glutathione in the double combination treated groups (black coffee and cinnamon or green coffee and cinnamon) when compared with control group.

In contrast, the mean value levels of serum reduced glutathione in the double combination treated groups (black coffee and cinnamon or green coffee and cinnamon) were significantly increased compared to diabetic group.

However, no significant change were observed in the mean value levels of serum reduced glutathione in the double combination treated groups (black coffee and cinnamon or green

coffee and cinnamon) when compared with single treated groups (black coffee, green coffee and cinnamon).

No significant change were observed in the mean value level of serum reduced glutathione in the triple combination treated group (black coffee, green coffee and cinnamon) when compared with control group.

Additionally, the mean value level of serum reduced glutathione in the triple combination treated group (black coffee, green coffee and cinnamon) is significantly increased compared with diabetic group.

However, the mean value level of serum reduced glutathione in the triple combination treated group (black coffee, green coffee and cinnamon) is significantly increased compared with single treated groups.

Also, the mean value level of serum reduced glutathione in the triple combination treated group (black coffee, green coffee and cinnamon) is significantly increased compared with double combination treated groups.

the mean value levels of serum paraoxinase (PON) in the single treated groups (black coffee or green coffee or cinnamon) were significantly decreased compared with diabetic group, this results were in agreement with author**,** suggested that cinnamon, green and black aqueous extract may provide beneficial effects on antioxidant enzyme activities [54].

On the other hand, no significant change were observed in the mean value levels of serum PON in the double combination treated groups (black coffee and cinnamon or green coffee and cinnamon) when compared with control group.

In contrast, the mean value levels of serum PON in the double combination treated groups (black coffee and cinnamon or green coffee and cinnamon) were significantly increased compared to diabetic group.

However, the mean value levels of serum PON in the double combination treated groups (black coffee and cinnamon or green coffee and cinnamon) were significantly increased compared with single treated groups (black coffee, green coffee and cinnamon).

No significant different were observed in the mean value levels of serum PON in the double combination treated groups (black coffee and cinnamon or green coffee and cinnamon) when compared with each other.

Additionally, no significant different were observed in the mean value level of serum PON in the triple combination treated group (black coffee, green coffee and cinnamon) when compared with control group.

In contrast, the mean value level of serum PON in the triple combination treated group (black coffee, green coffee and cinnamon) is significantly increased compared with diabetic group.

However, the mean value level of serum PON in the triple combination treated group (black coffee, green coffee and cinnamon) is significantly increased compared with single treated groups.

On the other hand, no significant different were observed in the mean value level of serum PON in the triple combination treated group (black coffee, green coffee and cinnamon) when compared with double combination treated groups.

There are no references about effect of the double combination treated groups and triple combination treated groups on hyperglycemia, hyperlipidemia and oxidative stress in the recent studies.

Our study was unique in that it was also designed to examine deduction of better methods of herbal treatment comparison between treatments alone or by mixing herbs together.

In this study, we found that the mix of herbs is better than using herbs alone where the triple combination treated group is more effective than double combination treated groups (cinnamon& green coffee and cinnamon & black coffee) than single treated groups in the same time period on Streptozotocin induced diabetic rats.

Conclusion

The recommendation of the present study to use the triple combination treatment by cinnamon, green and black coffee is the best group that acts as antioxidant effect.

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