International Journal of Theoretical and Applied Research (IJTAR)
ISSN: 2812-5878

Homepage: https://ijtar.journals.ekb.eg

Original article

Possible Effects of Dinitrophenol and Caffeine on Diabetic Adult Male Albino Rat

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ARTICLE INFO

Received 26/06/2023
Revised 11/09/2023
Accepted 26/09/2023

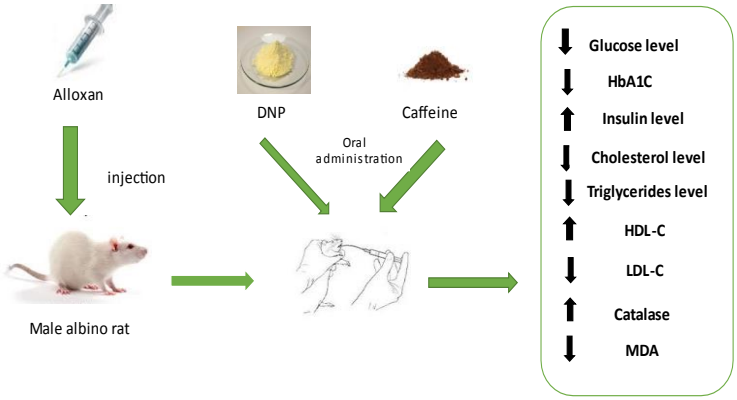
Keywords

Insulin
Hyperglycemia
Antioxidants
Oxidative stress
Lipid indicators

ABSTRACT

Diabetes is a major health problem characterized by inadequate hyperglycemia due to relative or absolute insulin deficiency at the cellular level. Dinitrophenol causes weight loss by uncoupling oxidative phosphorylation. While caffeine use on a regular basis is linked to a decreased risk of diabetes and Parkinson's disease. Dinitrophenol and caffeine decrease oxidative stress. Diabetes leads to increased oxidative stress so they can decrease blood glucose. In this research, 70 male albino rats were selected, with body weight ranging between 100-160 g, and divided into 7 groups, a control group, a dinitrophenol group, a caffeine group, and d diabetic group. Rats were given only one dosage of alloxan by intraperitoneal injection. Rats with diabetes were separated into the diabetic group, diabetic-dinitrophenol, diabetic-caffeine, and diabetic-dinitrophenol-caffeine treated groups. After 8 weeks, we measured glucose, insulin, lipid indicators, antioxidants, and oxidative stress markers. The diabetic group showed a very high elevation in glucose levels compared to the control group. The diabetic-dinitrophenol-caffeine group showed more reduction in blood glucose levels. All treated groups showed an increase in insulin levels. Lipid profiles showed variations in different groups. All treated groups had increased oxidative stress markers associated with increased antioxidant markers.

Graphical abstract



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DOI: 10.21608/IJTAR.2023.208830.1056

Special issue "selected papers from the 2nd International Conference on Basic and Applied Science (2nd ICBAS-2023)"

1. Introduction

Diabetes mellitus is a long-term condition that causes a boost in blood glucose levels. Diabetes affects about 463 million people worldwide in 2019 and causes great socioeconomic and medical burdens [1]. It has been considered one of the most pressing common health problems for humanity [2], [3].

One of the most essential elements of diabetes is that over half of all diabetics have hereditary factors. The inability of the pancreas to generate adequate insulin, as well as the body's inefficient use of insulin, are both pathologic causes of diabetes [4].

As diseases progress, various pathophysiological studies have advanced our understanding of insulin secretion and resistance. Insulin resistance develops early in individuals who are at risk of developing type II diabetes, which is compensated for by beta cell insulin hypersecretion. In contrast, the pancreatic functional reserve eventually loses its capacity to produce insulin, and by the time diabetes is recognized, beta cells can no longer secrete sufficient insulin [5].

Oxidative stress is caused by a relationship between the body's antioxidant capacity and oxygen-derived radicals. This disturbs the body's regular balance of radical production and defense. As a result, lipids, proteins, and nucleic acids are oxidatively damaged. Over time, oxidative stress causes a variety of disorders, including diabetes, atherosclerosis, inflammatory problems, hypertension, heart disease, neurological diseases, and cancer [6].

2-4-Dinitrophenol causes weight loss via uncoupling oxidative phosphorylation, which increases metabolic rate and fat metabolism. The fast calorie consumption was assumed to be caused by a change in the proton electrochemical gradient, which results in energy [7].

Caffeine (1,3,7-trimethylxanthine) is a plant alkaloid found in a variety of plants, including coffee beans (*Coffea arabica and Coffea robusta*). It has been found to be a protective chemical against cellular damage with antioxidant properties. It also functions as an antioxidant in several organs by raising the concentration and activity of antioxidant enzymes [8]. Caffeine is ingested on a regular basis by humans, including those with type I or type II diabetes [9].

The aim of this study: This study aimed to investigate the effect of dinitrophenol and caffeine on diabetic adult male albino rats.

2. Materials and methods

The present research was conducted at the Medical Physiology Department, Faculty of Medicine (Boys), Al-Azahar University, Cairo.

1- **Animals:** In this study, 70 adult male albino rats of a common strain weighing 100–160 g (by sensitive scale) were used. Animals were kept under standard conditions in the lab, exposed to (12/12h) light/dark cycle, humidity of 60 ± 5 %, and a temperature of 25 ± 2 °C. Animals were housed in cages (5 rats/cage) with free access to standard laboratory chow diets from (El-Nasr Company, Cairo, Egypt) and water *ad libitum*. The study was conducted according to international guidelines for animal experiments and consent by the Ethical Committee of the Medical Physiology Lab, Faculty of Medicine, Al-Azhar University in Cairo, the Egyptian capital.

2- Drugs and chemicals:

a- Dinitrophenol: DNP **(Nile Pharmaceutical-Egypt)** was dispersed into the water and given orally for 8 weeks through gastric intubation at an amount of 16 mg per kg b.w.10].

b- Caffeine: Caffeine (1,3,7 trimethylxanthine) **(Nile Pharmaceutical-Egypt)** was supplied continuously for a period of 8 weeks in water at a dosage of 20 mg per kg b.w.[11].

c- After an overnight fast, Alloxan was mixed in 0.9% sodium chloride solution and given only one intraperitoneal injection of 140 mg per kg b.w. [12]. Alloxan was administered intraperitoneally in only one dose of 140 mg per kg b.w. For 18 hours, rats were fed a 5% glucose solution to prevent hypoglycemic shock [12]. Fasting blood glucose estimations after two days of alloxan administration with an Accu-Chek glucometer (Roche, Germany) confirmed the development of hyperglycemia in rats. In the study, animals that had blood glucose values of 200 mg per dL or higher were designated as hyperglycemic [13].

3. Experimental design:

3.1- Animal groups:

In this study animals were divided into 7 groups equally:

Control group: rats were supplied normal animal pellets and drinking water (no. =10 rats/100-160g).

Control DNP group: rats were given DNP (16 mg per kg b.w) daily for 8 weeks orally by gastric intubation (no. =10 rats/100-160g).

Control caffeine group: rats were given caffeine (20 mg per kg b.w) orally for 8 weeks by gastric intubation (no.10= rats/ 100-160g).

Control diabetic group: With just one intraperitoneal administration of alloxan (140 mg per kg b.w) in sterile saline and a typical rat chow diet, rats have been given diabetes (no. =10 rats/ 100- 160g).

Diabetic-DNP-treated group: After inducing diabetes, diabetic rats were given DNP (16 mg per kg b.w) orally to for 8 weeks (no. =10 rats/100-160g).

Diabetic-caffeine-treated group: rats were given caffeine (20 mg per kg b.w) for 8 weeks orally after diabetes was induced (no. =10 rats/100-160g).

Diabetic-DNP-caffeine-treated group: After diabetes induction, rats were given DNP & caffeine at the same dosage as before for 8 weeks orally (no. =10 rats/100-160g).

3.2- **Blood Sampling**: Using a heparinized capillary tube added into the medial canthus, blood was taken out of the retro-orbital plexus. Serum was obtained by placing blood in a clean, dry, graduated glass centrifuge tube. It was quickly turned on for centrifugation at 3000 r.p.m. for fifteen minutes. A little less than fifty percent of the serum was moved to Eppendorf tubes for storage at -20ºC, for later use. EDTA was used to collect a second blood sample in order to measure glycosylated hemoglobin (HbA1C).

3.3- Biochemical parameters: The measured biochemical parameters are:

I. Fasting glucose level (The method of Burtis by using Spinreact kits) [14].

II. Glycosylated hemoglobin (HbA_1c) (The method of Nathan) [15].

III. Insulin (using Insulin ELISA Assay Kit to the method of Rudovich) [16].

IV. Lipid profile:

a- Total cholesterol (The colorimetric method according to Naito by using spinreact kits) [17].

b- Triglycerides (The method of Burtis by using Spinreact kits) [14].

c- HDL-C (The colorimetric method according to Williams by using beacon kits) [18].

d- LDL-C levels (The equation used by Tietz by using the Spinreact kit) [19].

V. Serum malondialdehyde (MDA) levels as a result of lipid peroxidation (determined by Ohkawa) [20] and catalase (CAT) levels as an antioxidant (determined by Aebi) [21].

Statistical Analysis: The Independent Samples T-Test was used to assess statistical differences between control and test groups using the statistical software SPSS (Statistical Package for Social Science) version 22.0.

Ethical Approval: This study was conducted in accordance with ethical procedures and policies approved by the Animal Care and Use Committee of the Faculty of Science, Al-Azhar University.

4. Result

In this study, we found that fasting blood glucose levels and HbA1c raised significantly ($P<0.01$) in the diabetic, diabetic- DNP- treated group, diabetic -caffeine- treated group, and diabetic- DNP -caffeine -treated group in comparison with the control group. DNP and caffeine groups were not substantially different in blood glucose and HbA1c from the control group table (1). Meanwhile, there was a substantial reduction ($P<0.05$) in insulin levels in the caffeine group as compared to the control group as described in table (1). Meanwhile, there was a substantial reduction ($P<0.05$) in insulin levels in the caffeine group as compared to the control group as described in table (1).

When compared to the control group, the diabetic-DNP-caffeine-treated group had a substantial ($P<0.05$) decrease in insulin levels, while a high substantial reduction ($P<0.01$) appeared in insulin levels in the diabetic group, diabetic- DNP- treated group and diabetic -caffeine- treated group as compared to the control. No significant change was found in insulin level in the DNP group as described in table (1).

Fasting blood glucose levels were significantly reduced ($P<0.01$) in the diabetic- DNP-treated group and diabetic-DNP-caffeine-treated group when compared to the diabetic group. When compared to the diabetic group, there was a significant reduction in HbA1C ($P<0.01$). While a significant decline ($P<0.05$) in HbA1C in the diabetic-DNP-treated group when compared to the diabetic group. While fasting blood glucose and HbA1C levels were significantly lower ($P<0.05$) in the diabetic-caffeine-treated group than the diabetic group, as illustrated in table (2).

A highly significant rise appeared ($P<0.01$) in insulin levels in the diabetic-DNP-caffeine-treated group in comparison with the diabetic group. While a significant rise ($P<0.05$) in insulin levels in the diabetic- DNP -treated group and diabetic-caffeine-treated group when compared to the diabetic group as illustrated in table (2).

A significant rise appeared ($P<0.05$) in cholesterol and LDL-C levels, while a highly significant rise ($P<0.01$) in triglyceride levels in the diabetic group in comparison with the control group. No significant change was found in cholesterol level in the DNP group, caffeine group, diabetic-DNP-treated group, diabetic-caffeine-treated group, and diabetic-DNP-caffeine-treated group as compared to the control group as illustrated in table (3). In comparison with the control group, Triglyceride levels increased significantly ($P< 0.01$) in the diabetic-DNP-treated, diabetic-caffeine-treated, and diabetic-DNP-caffeine-treated groups. As observed in table (3), there was no significant difference in triglyceride levels in DNP and caffeine groups as opposed to the control group. A significant reduction appeared ($P<0.05$) in HDL-C in the diabetic-DNP-treated group and diabetic-caffeine-treated group while a highly significant reduction ($P<0.01$) in the diabetic as compared to the control group was observed in table (3). As demonstrated in table 3, there was no significant change in LDL-C levels in the DNP and caffeine groups as compared to the control group.

A significant reduction clearly appeared ($P<0.01$) in cholesterol and triglyceride levels in diabetic-DNP-treated and diabetic-DNP-caffeine-treated groups compared to the diabetic group. LDL-C levels were significantly decreased ($P<0.05$) in the diabetic-DNP-treated group and diabetic-DNP-caffeine-treated group compared to the diabetic group. A high significant rise clearly appeared ($P<0.01$) in HDL-C in the diabetic-DNP-caffeine-treated group while a substantial rise in the diabetic-DNP-treated group as contrasted to the diabetic group. In the diabetic-caffeine-treated group, cholesterol, triglycerides, and LDL-C levels were significantly lower ($P<0.05$) than in the diabetic group, while a significant rise ($P<0.05$) in HDL-C in the

diabetic-caffeine-treated group when compared to diabetic group as explained in table 4.

Table (1): The levels of fasting blood glucose, HbA1c, and insulin in diabetic and different treated groups in comparison with control.

Groups / Parameters	Control group	DNP group	Caffeine group	Diabetic Group	Diabetic-DNP-treated group	Diabetic-caffeine-treated group	Diabetic-DNP-caffeine-treated group
Fasting blood glucose (mg/dl)	84.90±1.37	82.70±1.01	81.60±1.21	374.20±7.11**	226.50±9.15**	279.30±5.03**	174.70±4.79**
% change from control		-2.59	-3.89	340.75	166.78	228.98	105.77
HbA1C (%)	3.75±0.11	3.86±0.08	3.79±0.09	8.00± 0.18**	6.65± 0.22**	6.55±0.19**	5.38± 0.16**
% change from control		2.93	1.07	133.33	77.33	74.67	43.47
Insulin (U/L)	5.48±0.24	5.69±0.14	4.58±0.16*	2.62±0. 13**	3.50± 0. 13**	3.35±0.11**	4.36±0.12*
% change from control		3.83	-16.42	-52.19	-36.13	-38.87	-20.44

- Results were provided as M ± SEM (standard error of the mean) of ten in each group of rats with *P< 0.05 & **P<0.01 comparison with the control group.

Table (2): The levels of fasting blood glucose, HbA1c, and insulin in diabetic rats treated with DNP and/or caffeine were compared to the diabetic group.

Groups / Parameters	Diabetic Group	Diabetic-DNP-treated group	Diabetic-caffeine-treated group	Diabetic-DNP-caffeine-treated group
Fasting blood glucose (mg/dl)	374.20±7.11	226.50±9.15**	279.30±5.03*	174.70±4.79**
% change from diabetic group		-39.48	-25.36	-53.27
HbA1C (%)	8.00±0.18	6.65±0.22*	6.55±0.19*	5.38±0.16**
% change from diabetic group		-16.88	-18.13	-32.75
Insulin (U/L)	2.620±0. 13	3.500± 0. 13*	3.350±0.11*	4.360±0.12**
% change from diabetic group		33.59	27.86	66.41

-Results were provided as M ± SEM of ten in each group of rats with *P< 0.05 & **P<0.01 in comparison to the diabetic group

Table 3: Changes in the lipid profile in the DNP, caffeine on diabetic induced rats groups.

Groups / Parameters	Control group	DNP group	Caffeine group	Diabetic Group	Diabetic-DNP-treated group	Diabetic-caffeine-treated group	Diabetic-DNP-caffeine-treated group
Cholesterol (mg/dl)	96.90±2.61	104.30±2.83	98.10±2.83	141.50±13.02*	98.00±3.07	102.60±3.27	95.60±1.82
% change from control		7.64	1.24	46.03	1.14	5.88	-1.34
Triglycerides (mg/dl)	77.50±1.53	77.80±1.68	77.00±1.37	218.10±7.04**	163.20±4.36**	192.00±6.09**	135.90±1.97**
% change from control		0.39	-0.65	181.42	110.58	147.74	75.35
HDL-C (mg/dl)	24.90±0.59	25.10±0.48	23.40±0.60	19.00±0.75**	22.50±0.60*	21.50±0.79*	23.50±0.50
% change from control		0.80	-6.02	-23.69	-9.64	-13.65	-5.62
LDL -C (mg /dl)	56.50±2.45	63.64±2.69	59.30±2.97	72.15±3.87*	42.86±3.67*	42.70±2.79*	44.92±2.19*
% change from control		12.64	4.96	27.70	-24.14	-24.42	-20.50

-Results were provided as M ± SEM of ten in each group of rats with *P< 0.05 & **P<0.01 in comparison with the control group

Table 4: Change in the lipid profile in the DNP, caffeine on diabetic induced rats groups were compared to the diabetic group.

Groups / Parameters	Diabetic Group	Diabetic-DNP-treated group	Diabetic-caffeine-treated group	Diabetic-DNP-caffeine-treated group
Cholesterol (mg/dl)	141.50±13.02	98.00±3.07**	102.60±3.27*	95.60±1.82**
% change from diabetic group		-30.74	-27.49	-32.44
Triglycerides (mg/dl)	218.10±7.04	163.20±4.36**	192.00±6.09*	135.90±1.97**
% change from diabetic group		-25.17	-11.97	-37.69
HDL (mg/dl)	19.00±0.75	22.50±0.60*	21.50±0.79*	23.50±0.50**
% change from diabetic group		18.42	13.16	23.68
LDL (mg /dl)	72.15±3.87	42.86±3.67*	42.70±2.79*	44.92±2.19*
% change from diabetic group		-40.60	-40.82	-37.74

-Results were shown as M ± SEM of ten in each group of rats with *P< 0.05 & **P<0.01 in comparison to the diabetic group.

As illustrated in table (5), there was a substantial increase (P<0.05) in MDA levels in the diabetic-DNP-treated group and a very significant increase (P<0.01) in the diabetic as compared to the control group. A significant reduction (P<0.05) appeared in the MDA level in the DNP group in comparison with the control group. No significant change was noticed in MDA level in the caffeine group, diabetic-caffeine-treated group, and diabetic-DNP-caffeine-treated group when compared to the control group. A high significant decline (P<0.01) in catalase levels in the diabetic group, diabetic-DNP-treated group, diabetic-caffeine-treated group, and diabetic- DNP-caffeine-treated group in comparison with the control group. Catalase levels in the DNP group increased significantly (P<0.05) as compared to the control group. There was no significant difference in catalase levels between the caffeine and control groups.

A high significant rise (P<0.01) in catalase level and a high significant reduction (P<0.01) in MDA in the diabetic-DNP-treated group and the diabetic-DNP-caffeine-treated group in comparison with the diabetic group. There was a highly significant decline (P<0.01) in MDA levels and high significant rise in the diabetic-caffeine-treated group when compared to the diabetic group as seen in table 6.

5. Discussion

Diabetes is a peptide hormone synthesized by the β-cells of the pancreas that regulates the storage and release of energy and controls blood glucose levels [22]. In this study, we showed the effect of DNP and caffeine on diabetic male albino rats and measured blood glucose levels and some biochemical parameters.

Table 5: Levels of catalase and MDA in the DNP, caffeine on diabetic induced rats groups.

Groups / Parameters	Control group	DNP group	Caffeine group	Diabetic Group	Diabetic-DNP-treated group	Diabetic-caffeine-treated group	Diabetic-DNP-caffeine-treated group
Catalase (U/L)	25.60±0.70	28.40±0.78*	26.20±0.59	7.08±0.34**	12.40±0.50**	11.20±0.71**	15.61±0.53**
% change from control		10.94	2.34	-72.34	-51.56	-56.25	-39.02
MDA (µg/Ml)	21.81±0.62	18.61±0.55*	20.35±0.53	31.43±0.78**	25.08±0.80*	22.93±0.59	20.66±0.40
% change from control		-14.67	-6.69	44.11	14.99	5.14	-5.27

Results were shown as M± SEM of ten in each group of rats *P< 0.05 & **P<0.01 in comparison to the control.

Table 6: Levels of catalase and MDA in the DNP, and caffeine in diabetic-induced rats groups were compared to the diabetic group.

Groups / Parameters	Diabetic Group	Diabetic-DNP-treated group	Diabetic-caffeine-treated group	Diabetic-DNP-caffeine-treated group
Catalase (U/L)	7.08±0.34	12.40±0.50**	11.20±0.71**	15.61±0.53**
% change from diabetic group		75.14	58.19	120.48
MDA (µg/mL)	31.43±0.78	25.08±0.80**	22.93±0.59**	20.66±0.40**
% change from diabetic group		-20.20	-27.04	-34.27

-Results were shown as M ± SEM of ten in each group of rats with **P<0.01 in comparison to the diabetic.

The current research showed an increase in blood glucose levels and HbA1C in diabetic groups as compared to the control group. High blood glucose levels are induced by the activity of alloxan, which reacts by destroying the important component in pancreatic β-cells resulting in reduced insulin-carrying granules in pancreatic β-cells [23]. The toxic action of alloxan in β-cells; is beginning by free radicals produced by redox processes. High-stimulated free radicals destroy β- cells so blood glucose level is raised [24].

Alloxan induces oxidative stress, which increases the formation of reactive oxygen species (ROS) [25]. These findings were compatible with Yogal et al. [26] who reported a substantial rise in HbA1c in diabetic animals.

Insulin levels in the caffeine group were reduced as compared to the control group. Caffeine intake in normal rats may reduce insulin levels due to increased levels of epinephrine and free fatty acids that increase insulin resistance [27].

Diabetic groups showed a decrease in insulin levels as compared to the control group. Diabetes caused by alloxan is related to selective reduction of insulin production via glucokinase inhibition and promotion of ROS generation. As a result, such a poison will cause necrosis of the pancreatic β-cells [28], [29].

Current research showed all treated groups decreased glucose levels, Hba1c, and elevated insulin levels as compared to the diabetic group.

DNP acts as a mitochondrial uncoupler, disrupting the gradient of the proton, which might be implemented to create ATP through single protons passing by the ATP synthase process, instead, it spills and is wasted as energy. It reduces the membrane hypothetical, distributes heat as well as raises ADP. The corresponding rise in ADP induces mitochondria to accelerate energy consumption by increasing the absorption of glucose through the process of glycolysis [30]. These findings were compatible with Geisler [30] who mentioned that DNP significantly reduces glucose levels and HbA1c. Abunasef *et al*., [31] observed that caffeine significantly reduced glucose levels and increased insulin levels in diabetic rats. Caffeine decreases blood glucose levels due to decreased insulin resistance coupled with a decrease in hyperinsulinemia [32]. Goedeke *et al.* [33] showed that DNP significantly increases insulin levels in mice. DNP improves energy distribution by decoupling the mitochondrial proton gradient from ATP synthesis.

Mitochondrial uncoupling reduces ROS generation by reducing the proton gradient [34]. Abunasef *et al.* [31] reported that caffeine significantly increases insulin levels in diabetic rats. Caffeine may improve insulin secretion. Caffeine changes the expression of glucose transporter 2 (GLUT2) and glucokinase in cells, which are involved in the phosphorylation of the glucose mechanism and, as a result, insulin production [35].

In current study showed an elevation in cholesterol, triglycerides, and LDL-C levels in the diabetic group. Lipolysis and hypertriglyceridemia were caused by alloxan administration. Alloxan induction caused an elevation in ROS that resulted in damaged β-cells, resulting in reduced insulin production and hyperglycemia; this state induces lipolysis, resulting in an increase in fatty acids in the blood [36]. Lipolysis promotes the production of free fatty acids. These fatty acids will reach adipose tissue or muscle cells by damaging the endothelium and subsequently re-oxidize or change back to triglycerides [37].

Lipoprotein lipase is activated by insulin, which hydrolyzes triglycerides and prevents lipolysis. Diabetes, on the other hand, causes an increase in lipolysis, which eventually leads to hyperlipidemia [38]. The elevated triglycerides in the diabetic group resulted from inhibiting lipoprotein lipase in fatty tissues; because of insulin declination [39]. Insulin deficiency causes an increase in free fatty acid mobilization from adipose tissue which results in increased production of LDL-C and dyslipidemia. [36]. These findings were compatible with Airaodion *et al.* [40] who mentioned the most significant elevation in total cholesterol and serum triglycerides in diabetic animals.

This study showed a reduction in HDL-C in diabetic groups. This reduction is superior; because suppression of lipoprotein lipase caused by alloxan and lipid peroxidation is due to stress oxidation. Oxidative stress causes lipid degradation and a decrease in HDL-C which is important for cholesterol transport from tissues into the liver [41]. These findings were compatible with Daniel *et al*. [42] who mentioned that HDL-cholesterol levels declined in diabetic animals.

All treated groups showed an increase in HDL-C and a decrease in cholesterol, triglycerides, and LDL-C (especially the combined group) as compared to the diabetic group. DNP serves as a mitochondrial uncoupler, interrupting the proton that might be utilized to create ATP through the unique protons getting via the ATP synthase pathway, but ultimately leaked and squandered as energy. A corresponding rising rate of ADP causes the mitochondria to accelerate the consumption of energy by boosting the degradation of lipids during β-oxidation [30]. Additionally, Belosludtseva *et al.* [41] showed that DNP decreases serum triglyceride levels in /mice. These findings were compatible with those of Goedeke *et al*. [33] who showed that DNP decreases triglycerides and LDL-cholesterol by increasing the rate of hepatic mitochondrial oxidation.

These findings were compatible with Mansour *et al*. [43] who recorded that caffeine reduces total cholesterol levels in the patient. These findings were compatible with Xu *et al*. [44] who showed that improving lipid profile decreases triglyceride, cholesterol, and LDL-C and increases HDL-C. Caffeine increases fat oxidation, and lipolysis and decreases body fat [45]. The triglyceride reduction is almost certainly caused by insulin stimulation, as insulin plays an important role in lipolysis inhibition. The HDL-cholesterol level was boosted due to an improvement in insulin secretion. LDL-cholesterol can be reduced by inhibiting LDL-cholesterol glycosylation [46].

In current study showed an increase in MDA and reduced catalase in the diabetic group. This result is compatible with Ogochukwu *et al.* [47] who reported that increased ROS formation is responsible for the raised levels of MDA, as an end product of lipid peroxidation in diabetic rats.

These results are compatible with those of Awan *et al*. [48] who stated that ROS formation is a direct result of hyperglycemia and has been linked to vascular complications in diabetic patients. These results are also compatible with Daniel *et al.* [42], who demonstrated that elevated levels of ROS in diabetes result in decreased levels of catalase making a number of cells susceptible to free radicals. All treated groups showed reduced MDA and increased catalase (especially the combined group). This result is compatible with *Samaiya et al.* [49] who showed that DNP significantly raised serum catalase levels. DNP prevents the development of oxidative stress by decreasing ROS levels in mice which helps to increase catalase [50]. This result is compatible with Abdel-Salam *et al*. [51] who showed that DNP significantly decreased MDA levels in rats. DNP helps to decrease ROS which decreases oxidative stress that leads to reduced MDA levels in rats. [52].

These findings were compatible with Kaczmarczyk-Sedlak *et al.* [8] who recorded that; caffeine decreases MDA in rats due to reduced oxidative stress by caffeine [53]. These findings were compatible with Reddy [53] who showed that caffeine increases catalase levels and decreases MDA. The enhanced activity of catalase in pancreatic tissue after caffeine treatment demonstrates its antioxidative efficiency. The inclusion of polyphenols and melanoidins in pure caffeine may contribute to improved endogenous antioxidant status [54].

In our study, we studied the efficacy of DNP with a low dose and caffeine on diabetic rats separately and combined while previous studies investigated separately only. Many parameters were measured as glucose, HbA1C, insulin, catalase, and MDA while previous studies measured only glucose levels and this gives a wide picture to understanding diabetes mellitus disease and improving the choice of treatment in animals.

6. Conclusion

According to the findings of this study, caffeine and a very low dose of dinitrophenol reduced glucose levels in diabetic rats. They also raised insulin and catalase as antioxidants and reduced MDA. They improved their lipid profile. Therefore, caffeine can be considered a safe and effective natural medicine that may help reduce blood glucose.

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