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The Influence of *Syzygium aromaticum* Ethanolic Extract Against Streptozotocin-Induced Diabetic Rats

Amal Gouda Hussien[a]*, Amal Abd El-Azeem Fyiad[a], Mamdouh Moawad Ali[a], Abeer Essam El-Din Mahmoud[a]

[a]Biochemistry Department, Biotechnology Research Institute, National Research Centre, Dokki 12622, Giza, Egypt

Abstract

Diabetes Mellitus (DM) is characterized by hyperglycemia and increased level of reactive oxygen species (ROS). Our study verified *Syzygium aromaticum* (clove) ethanolic extract phytochemical composition by high-performance liquid chromatography (HPLC) analysis to assess clove extract possible impact on glucose status, liver and kidney functions, and oxidative stress in diabetic model. Dry *Syzygium aromaticum* buds obtained from the market, grinded, extracted by 70% ethanol. Thirty two adult male rats were classified into four groups (8per each). Controls (CG), *Syzygium aromaticum* group (SAG): rats received (500 mg/kg b.wt.) clove ethanolic extract, Diabetic rats (DG): injected with Streptozotocin [STZ, 75 mg/kg b.wt. (a single dose)], intraperitoneally (IP). Diabetic treated group (DSA): rats injected with STZ as in (DG) group and at the eighth day rats were treated with the ethanolic extract of clove as in (SAG) group. Animals were sacrificed after 14 days. Glucose level, liver enzymes, amylase activity, kidney function tests, total antioxidant capacity (TAC), malondialdehyde (MDA), albumin and total protein (TP) levels were measured. Also, the body weights of each rat were determined throughout the experimental period. Administration of STZ increased glucose level, hepatic markers, urea, creatinine, and MDA levels. Conversely, serum TAC, albumin, TP and α-amylase activity were significantly decreased. All the aforementioned parameters were improved due to the treatment of diabetic rats with clove ethanolic extract. Clove extract could be a promising therapeutic agent in the treatment of diabetic models.

Keywords: Syzygium aromaticum (Clove); Diabetes mellitus; Liver function; Oxidative stress; Antioxidant status; Kidney; rats.

1. Introduction

Diabetes mellitus (DM) is a chronic (long-lasting) health condition results from disturbed insulin emission or function or both [1, 2]. In DM hyperglycemia is accompanied by dysfunction, and failure of different organs and tissues, development of retinopathy, nephropathy, and neuropathy and cardiovascular disorders **[3, 4]**. Type 1 (DM1) resulting from destruction of pancreatic β-cells with absolute insulin insufficiency and type 2 (DM 2) mainly related to insulin resistance and relative insulin insufficiency **[5]**.

Numerous treatments for diabetics cause hypoglycemia. Therefore, propagation of studies on herbal plants used in traditional medicine are of great interest, as they may provide potential sources for novel therapeutic agents that could manage and prevent various diseases.**[6,7]**.

Herbs and spices rich in phytochemicals and polyphenolic fractions have antioxidant properties **[8]**, insulin-like effects in glucose utilization **[9]**, are good inhibitors of lipid peroxidation and diabetes linked enzymes in tissues **[10]**. *Syzygium aromaticum* [clove (species *aromaticum,* genus *Syzygium* and family *myrtacea*)] is an aromatic flower bud with intense brown color, aroma and hot taste used in different parts of the world in culinary and medicinal usages. Herbs own antioxidant, antimicrobial, antiviral, antifungal, antithrombotic, anti-inflammatory, antidiabetic, anesthetic and pain reliving properties. Also, clove used as a topical antiseptic and local anesthetic in

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dentistry **[11]**. **Atawodi *et al.* [12]** showed the ability of polyphenol constituents of clove bud to prevent oxidative stress diseases such as diabetes, cancer and cardiovascular disorders **[13, 14].** Interestingly, clove bud methanolic extract is capable of scavenging various free radicals and protect biomolecules from oxidative stress induced by copper ions **[15]**. Moreover, **Prasad *et al.* [16]** reported that in hepatocytes and hepatoma cells clove buds exert insulin-like actions via reducing glucose 6-phosphatase and phosphoenolpyruvate carboxykinase gene expression. Also, **Nassar *et al.* [17]** indicated the hepatoprotective activity of clove ethanolic extract against liver injury induced in female rats by paracetamol. Furthermore, **Tajuddin *et al.* [18]** illustrated the aphrodisiac effectivity of the clove extract on normal male rats, without gastric ulceration or any adverse effects. **Rabeh *et al.* [19]** demonstrated the pharmacologically potent effects of clove extract on diabetic rats with nephropathy. Hence this study investigates the potential anti-diabetic, antioxidant and hepatorenal protective properties of *Syzygium aromaticum* ethanol extract on Streptozotocin (STZ) induced diabetes.

2. Materials and Methods

2.1 Plant material and extraction

Syzygium aromaticum (clove) dry buds were purchased from herbal market (Giza, Egypt) and identified by a Plant Taxonomy Consultant at the Egyptian Ministry of Agriculture and Land Reclamation (Mrs. Trease Labib). In the Herbarium of the National Research Centre (CAIRC), Giza, Egypta voucher specimen coded (M174) was deposited by prof. Dr. Mona M. Marzouk. The extraction of clove was carried out by mixing its powder with 1:10 w/v in 70% ethanol (v/v) with continuous stirring for 24 hours at room temperature. Then, filtration of the extract was carried out and the filtrate concentrated by a rotary evaporator (BÜGHI Rotavapor R-205, Switzerland) at 30°C. The extract was dried in a hot water bath at 45°C. The yield of dried extract (21.05% w/w) packed in a container of wrapped glass and stored until used for the experiment at -20°C **[20]**.

2.2 HPLC analysis of phenolic compounds

The clove extract to hydrolyze glycosidic bonds was treated with 2N HCl. The dried extract was dissolved in ethanol, subjected to HPLC (for qualitative and quantitative analysis of phenolic contents) **[21]**. The HPLC system (Agilent Technologies Company), equipped with a dual lamp binary system, UV detector and C18 column (i.e. 4.6 mm×250mm, 5µm). The data was integrated using Agilent Chem Station software. Standards and samples were analyzed using the following gradient program. The mobile phase consisted of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate of 1 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A); 0–5 min (80% A); 5-8 min (60% A); 8-12 min (60% A); 12-15 min (82% A); 15-16 min (82% A) and 16-20 (82%A). The multi-wavelength detector was monitored at 280 nm. The injection volume for each of the sample solutions was 5 µl. The column temperature was maintained at 40 °C. The sample peak area (280 nm) is an index of the amount of component and the retention time of individual peaks is used to identify polyphenols by comparing with standard polyphenols – eugenol, caffeic acid, ellagic acid, ferulic acid, quercetin and kaempferol.

2.3. Experimental animals

From the Animal House of the National Research Centre (Giza, Egypt) 32adult Sprague-Dawley male rats (200-250g) were obtained. In compliance with The Principles of Laboratory Animal Care, all animals received humane care formulated by the National Society of Medical Research and Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (Publication No. 85-23, revised 1996) and by adopting ARRIVE guidelines **[22].** The study was taken approval by the Local Ethics Committee for Animal Care and Use at the National Research Centre, Egypt (Registration number 20/027). Rats were housed under standard laboratory conditions (24±3°C) with 12 hours of light-dark regimen, and monitored for the study duration. The animals were fed *ad libitum* with normal laboratory chow standard pellet diet.

2.4. Experimental design

After one week of acclimatization, rats were randomly assigned in four groups of eight rats each. Control group (CG): rats were received citrate buffer orally as a drug vehicle throughout the experimental period. *Syzygium aromaticum* group (SAG): rats were treated orally with the ethanolic extract of clove for then rats were followed up for one week. Diabetic treated group (DSA): rats were injected with STZ as in DG and at the eighth day rats were treated with the ethanolic extract of clove as in SAG. By using Touch Basic blood glucose monitoring system glycaemia was assessed everyday through the experimental period. Hyperglycemia (fasting blood glucose levels >250 mg/dL and statistically higher compared to control rats) was the confirmation of the effectiveness of inducing. Two minutes after IP administration of ketamine/xylazine mixture (10 mg ketamine/kg and 5 mg xylazine/kg) rats were sacrificed by decapitation. Blood samples were collected, clotted at room temperature, centrifuged at (6,000 × g, 15 minutes, 4°C). Biochemical analysis for measuring glucose level, liver marker enzymes; α-amylase activity; serum urea and creatinine levels; albumin; total protein level and markers of oxidant status (MDA& TAC) were determined. Also, body weights of each rat were evaluated through the experimental protocol.

2.1. Biochemical analyses

Hepatic marker levels [Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP)] and α-amylase activity were analyzed by Reactivos GPL, Spain kits. Also, serum urea and creatinine levels were analyzed by Reactivos GPL kits (Spain). Malondialdehyde and TAC serum levels were

seven days (500 mg/kg b.wt.) **[17]**. Diabetic group (DG): rats were starved overnight, injected intraperitoneally with a single dose of STZ [Sigma chemical Co., USA, (75mg/kg b.wt)] dissolved in citrate buffer (0.1M, pH 4.5) according to **[23].** After 72 hours of STZ injection, serum glucose level >250 mg/dL was considered as a confirmation of DM, and

determined using Elabscience kits (USA). Serum albumin and total protein levels were determined by chemadiagnostica kits (Monsano, Italy). Blood glucose level was determined by Glucose Oxidase Method (mg/dL) **[24]**.

2.2. Statistical analysis

One-way analysis of variance (ANOVA) and independent t-test were applied for determining between-group differences based on a two-tailed analysis, and P<0.05 was set for the level of significanc.

3. Results

The clove ethanolic extract was analyzed for the presence of phytochemicals with antioxidant activity. The clove extract as shown in Table 1 had considerable levels of gallic acid, catechin and syringic acid. The levels of gallic acid, catechin, and syringic acid were 74902.33, 15531.67, and was14441.40 µg/g dry weight, respectively. Whereas, Coffeic acid was only 37.19 µg/g dry weight. Quantitative analysis of the phenolic compounds by using HPLC showed the presence of ellagic acid (13385.82µg/g dry weight), chlorogenic acid (10758.79 µg/g dry weight), kaempferol (966.98 µg/g dry weight) and quercetin (508.45 µg/g dry weight) in ethanol clove extract (Figure 1).

Table (1): Concentration of various compounds in clove ethanolic extract by using HPLC analysis

Compounds	Conc. (µg/g dry wt.)
Gallic acid	74902.33
Catechin	15531.67
Syringic acid	14441.40
Chlorogenic acid	10758.79
Ellagic acid	13385.82

Pyrocatechol	2719.63
Naringenin	1626.14
Kaempferol	966.98
Coumaric acid	881.72
Methyl gallate	738.75
Rutin	679.19
Querectin	508.45
Hesperetin	97.06
Cinnamic acid	88.25
Coffeic acid	37.19

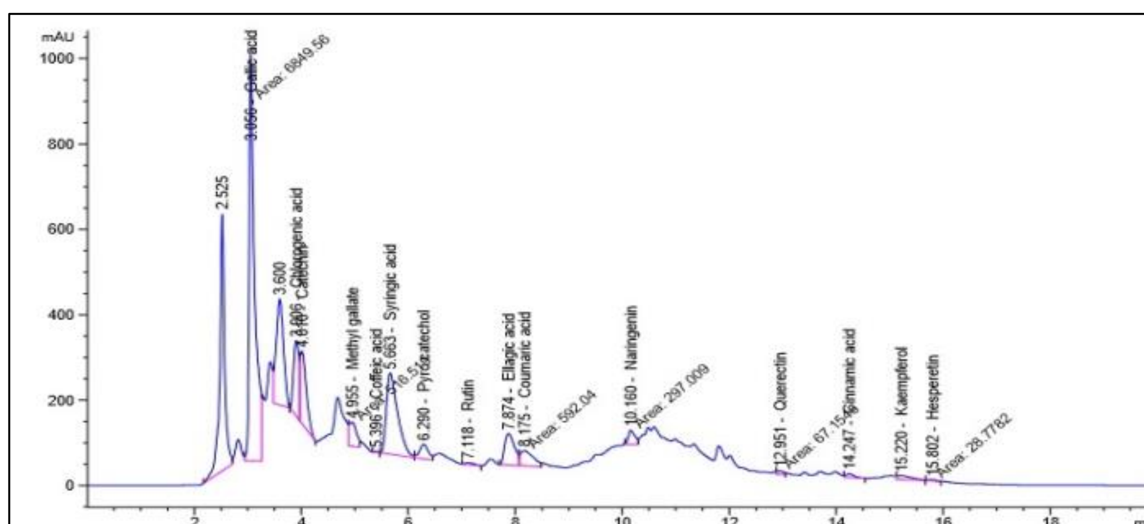

Figure (1): Clove extract HPLC chromatogram; peak at retention time 14.247, 15.220 and 15.802 min are identified as cinnamic acid, kaempferol and hesperetin, respectively.

3.1. Body weight and fasting blood glucose

Data presented in (Table 2; Figure 2A&2B) summarizes the mean data of body weights and the glucose level in all groups at the start and at the end of the experiment. At the beginning of the experiment, our results revealed that there was no significant change (P>0.05) in the body weight. While, there was a significant decrease (P<0.05) in the body weight of the diabetic group at the end of the experiment compared to normal controls. On the other hand, DSA group showed a significant increase in body weight compared to diabetic group. Meanwhile, at the start of the experiment, results in (table 2) showed that there was no significant change in the glucose level (P>0.05) between all groups. While, there was a significant elevation in the glucose level in the DG group and a slight significant increase in glucose level in DSA group compared to the healthy control group (P<0.05)at the end of the experiment. In contrast, a significant decrease in glucose level was noticed in DSA group compared to DG group.

Table (2): The change of body weight (g) as well as the levels of glucose (mg/dl) in different groups of rats.

Groups	Mean body weight (g)		Glucose level (mg/dl)	
	Beginning	Ending	Beginning	Ending
CG gp	163.67^{a}±3.40	208^{a}±6.85	123.44^{a}±1.70	123.59^{a}±1.71
SAG gp	168.83^{a}±3.38	214.17^{a}±4.28	124.17^{a}±5.02	122.17^{a}±2.12
DG gp	163.17^{a}± 4.11	142.5^{b}±3.89	120.93^{a}±3.23	469.17^{b}±13.32
DSA gp	159.5^{a}±2.59	211.67^{a}±5.70	119.5^{a}±5.06	187.34^{c}±5.17

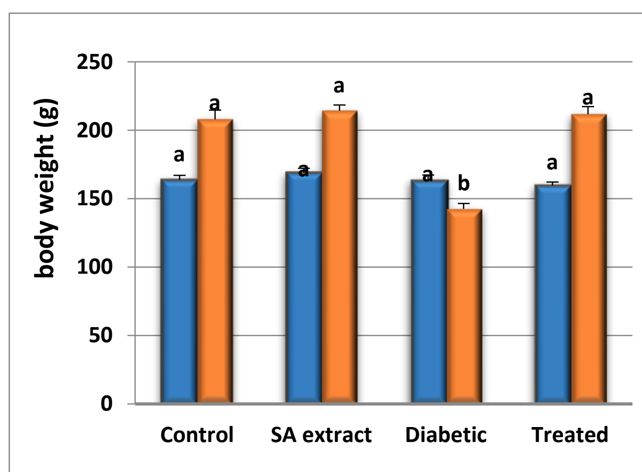

Fig (2A): Statistical comparison of body weight at the start and at the end of the experiment in different groups. Values are expressed as mean ± SE. Groups sharing the same letters are not significantly different.

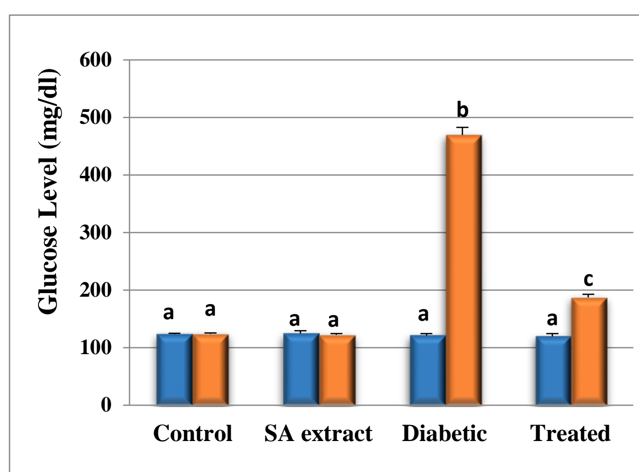

Fig (2B): Statistical comparison of the glucose level at the beginning and at the end of the experiment in different groups. Values are expressed as mean ± SE. Groups sharing the same letters are not significantly different.

3.2. Liver function tests

Data presented in (Table 3 and Figure 3A, 3B & 3C) revealed that treatment of rats with SA alone showed non-significant changes in the activities of serum liver marker enzymes, compared to controls. In diabetic rats (DG) a significant elevation in liver markers (AST, ALT, and ALP) was recorded when compared to control rats. While, a significant decrease was observed in all of the aforementioned parameters in the DSA group compared to the diabetic rats ($P<0.05$).

Table (3): Activities of AST, ALT, ALP and α-amylase in all groups.

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	α-amylase(U/L)
CG gp	74.06^{a}±4.64	27.50^{a}±1.43	343.73^{a}±37.28	79.09^{a}±1.75
SAG gp	78.12^{a}±3.51	27.46^{a}±1.50	359.38^{a}±36.49	78.71^{a}±1.60
DG gp	179.59^{b}±17.23	60.26^{b}±3.80	700.81^{b}±48.02	52.97^{b}±3.75
DSA gp	107.63^{a}±2.88	35.02^{a}±1.08	370.04^{a}±27.27	74.71^{a}±1.56

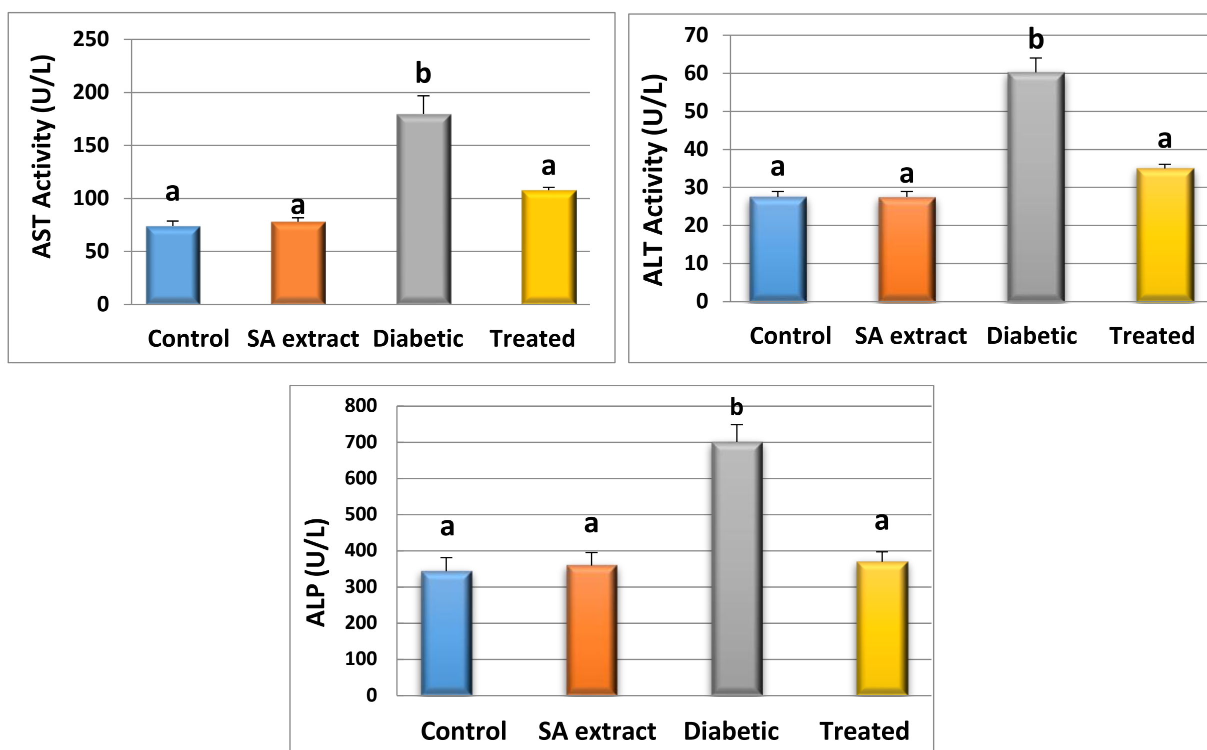

Fig (3A, 3B & 3C): Statistical comparison of serum AST, ALT and ALP activities in different groups. Values are expressed as mean ± SE. Groups sharing the same letters are not significantly different.

3.3. Amylase activity

Data presented in Table (3) and Figure (4) showed no significant change in amylase activity in SAG group compared to controls. In diabetic group, the amylase activity was significantly decreased when compared with control group. By contrast, a significant elevation in the amylase activity in DSA group was observed compared to diabetic group (DG) ($P<0.05$).

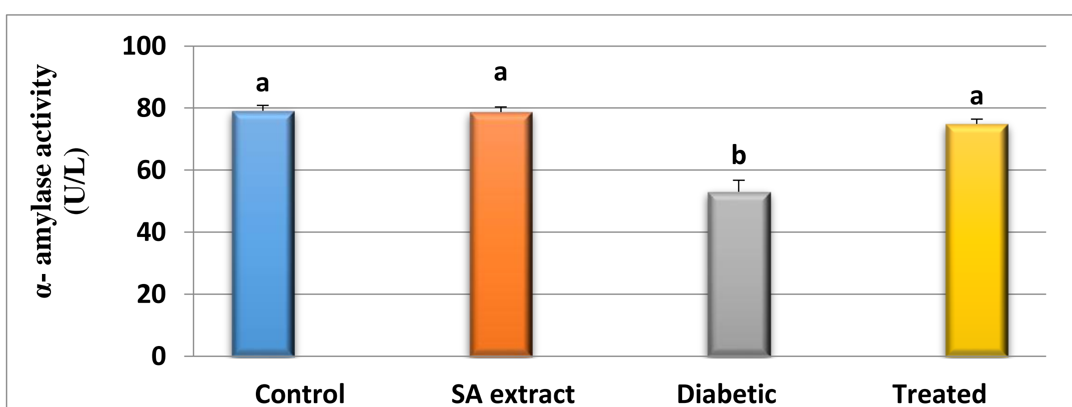

Fig (4): Statistical comparison of serum amylase activity in different groups. Values are expressed as mean ± SE. Groups sharing the same letters are not significantly different.

3.4. Renal function tests

The results of the current study demonstrated that rats treated with SA alone had no significant influence on urea and creatinine levels in serum compared to the control group (Table 4 and Figures 5A&5B). On the other hand, their levels were significantly increased in the diabetic rats (DG) compared to controls. While, levels of urea and creatinine were reduced in diabetic treaded group (DSA), compared to the untreated diabetic (DG) rats (*P*<0.05).

Table (4): Kidney function tests in different groups.

Groups	Urea (mg/dl)	Creatinine (mg/dl)
CG gp	63.12[a]±2.36	0.28[a]±0.028
SAG gp	64.89[a]±1.68	0.28[a]±0.025
DG gp	85.54[b]±3.66	0.54[b]±0.011
DSA gp	68.38[a]±1.48	0.35[a]±0.019

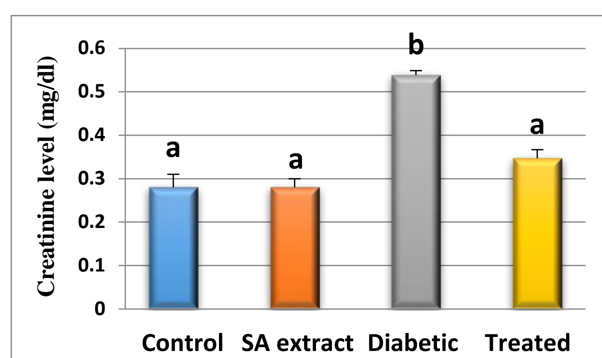

Fig (5A): Statistical comparison of serum Creatinine level in different groups. Values are expressed as mean ± SE. Groups sharing the same letters are not significantly different.

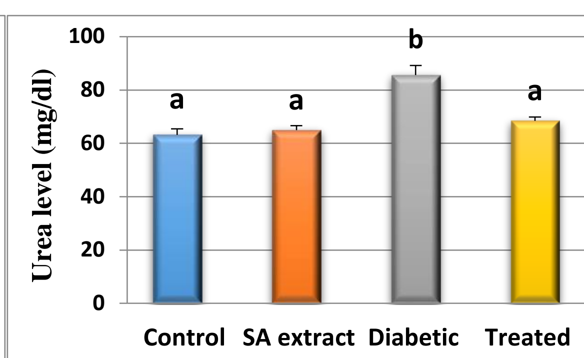

Fig (5B): Statistical comparison of serum Urea level in different groups. Values are expressed as mean ± SE. Groups sharing the same letters are not significantly different.

3.1. Serum oxidative stress markers

The results of our study showed that the administration of SA alone had no effect on TAC and MDA levels compared to the control group (Table 5 and Figure 6A&6B). Whereas, TAC level was significantly depleted and serum MDA level was significantly elevated in diabetic group (DG), compared to normal controls (*P*<0.05). While, oral administration of SA to diabetic rats (DSA group) normalized the levels of TAC and MDA.

Table (5): Oxidative stress markers, total antioxidant capacity (TAC) and malondialdehyde (MDA) in different groups.

Groups	TAC (U/ml)	MDA (nmol/ml)
CG gp	7.40[a]±0.18	30.91[a]±1.24
SAG gp	7.44[a]±0.28	31.14[a]±0.91
DG gp	4.72[b]±0.44	40.51[b]±1.53
DSA gp	6.91[a]±0.15	33.48[a]±1.24

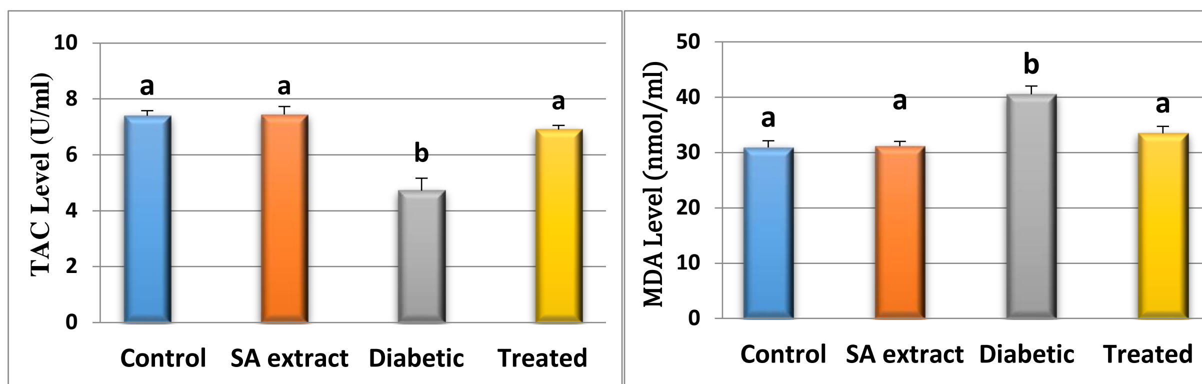

Fig (6A): Statistical comparison of serum TAC Level in different groups. Values are expressed as mean ± SE. Groups sharing the same letters are not significantly different.

Fig (6B): Statistical comparison of serum MDA level in different groups. Values are expressed as mean ± SE. Groups sharing the same letters are not significantly different.

3.2. Serum albumin and total protein levels

Results represented in Table (6) and Figures (7A&7B) illustrate that serum albumin and total protein levels not significantly changed in rats treated with SA compared to the normal controls. Induction of diabetes in rats by STZ (DG group) resulted in a significant reduction in albumin and total protein levels, compared to the controls. Meanwhile, serum levels of albumin and total protein remarkably increased ($P<0.05$) in DSA group compared to the DG rats.

Table (6): Levels of albumin and total protein in different groups.

Groups	albumin (g/dl)	Total protein (g/dl)
CG gp	$4.46^{a}\pm0.11$	$7.97^{a}\pm0.20$
SAG gp	$4.27^{a}\pm0.08$	$7.75^{a}\pm0.13$
DG gp	$3.74^{b}\pm0.09$	$5.55^{b}\pm0.09$
DSA gp	$4.19^{a}\pm0.10$	$7.20^{a}\pm0.28$

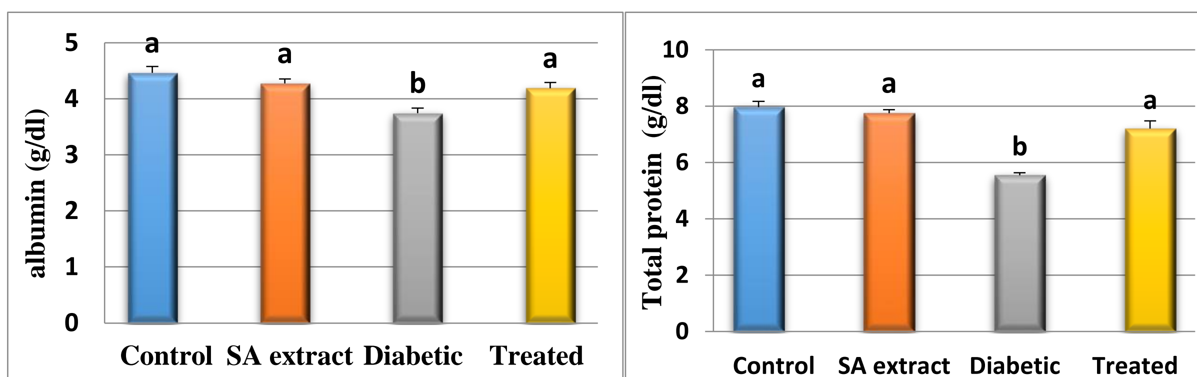

Fig (7A): Statistical comparison of serum albumin level in different groups. Values are expressed as mean ± SE. Groups sharing the same letters are not significantly different.

Fig (7B): Statistical comparison of serum total protein level in different groups. Values are expressed as mean ± SE. Groups sharing the same letters are not significantly different.

4. Discussion

Novel, safer and affordable therapeutic tools that prevent and treat diabetes are instantly needed **[25]**.

Herbal medicine, a carefully selected formula of plant products (leaves, stems, flowers, roots, and seeds) with numerous active components maximize therapeutic effects and reduce toxicity, nowadays it recognized as an alternate therapy for diabetic patients **[5,26]**. Diabetes causes elevation in food, water intake and urine volume and decline in body weight gain **[27]**. These results recorded a significant reduction in final body weight of untreated diabetic group compared to normal group because of hyperglycemia. Similar observations have also been reported as the body couldn't store or use glucose that causes starvation and weight loss of diabetics **[28, 29].** In addition, absence of carbohydrate as an energy source resulted in wasting of protein **[30]**. In contrast, treatment of diabetic rats with clove extract causes higher body weight change due to clove insulin like action on muscle, hepatocytes and adipose tissue. The present results are in agreement with that of **Chaudhry *et al.* [31]** and **Srinivasan *et al.* [32]** who reported significant improvement in the glycemic control of diabetic rats that prevented body weight loss and extra intake of food and fluids.

In this study, DG rats showed high significant increase in serum glucose concentrations in consistent with those of **Daniel *et al.* [33]** and **Rabeh *et al.* [19]** who reported a marked raise in glucose level and decrease in insulin level in diabetic rats. It has been proposed that STZ destroys the insulin producing β-cells, causes alterations of DNA and this causes impairment of glucose oxidation and decreases insulin biosynthesis and secretion **[33]**. Clove extract exhibited remarkably ameliorated effect in treatment of diabetic rats as there was a significant improvement in glucose concentration compared to DG. **Gashlan and Al-Beladi [29]** revealed a significant reduction in blood glucose and a significant elevation in insulin levels as a result of clove and clove oil administration to diabetic rats. Clove could lower glucose level owing to stimulation of functioning pancreatic β-cells to increase the insulin release, or may be due to regeneration of β-cells **[26].** Moreover, **Rabeh *et al.* [19]** stated that inhibitory action of clove on alpha-glucosidase resulted in its anti-hyperglycemic effect.

This study demonstrated that the activities of liver enzymes were significantly increased in diabetic rats due to the release of hepatic enzymes into the bloodstream from the cytosol **[34]** as an evidence of hepatocellular injury and liver damage **[3, 35]**. Meanwhile, ALT, AST and ALP concentrations were significantly decreased in DSA group, this point to the fact that clove is actually rich in phenolic compounds and has great potential to alter or reduce the effects of drug-induced hepatotoxicity and oxidative stress **[36]**. In consistent with **Abozid and EL-Sayed [37]** and **Sultana *et al.* [38]** who confirmed the clove hepatoprotective action due to its constituents of polyphenolic compounds and flavonoids **[39]**.

Amylases, hydrolysis carbohydrates into small particles of glucose **[40]**. The alpha amylases act on alpha 1-4, 1-6 links of glucose residues **[41]**, hydrolysis starch into small sugar molecules, produced in exocrine pancreatic cells and may be recognized as an adequate indicator of organ activity in physiological and pathological states **[42]**. Serum amylase activity was significantly reduced in the diabetic group since STZ interfere with calcium and magnesium homeostasis and amylase gene expression **[40]**. Administration of *Syzygium aromaticum* ethanolic extract to DSA rats significantly increased serum amylase activity due to clove stimulant effect on this enzyme and other pancreatic hydrolases **[40]**.

Treated diabetic rats (DSA group) showed a significant reduction in serum urea and creatinine levels. Hyperglycemia gives rise to redox imbalance which damages renal tissue and promotes tissue injury. The epithelial cells undergo structural changes or cell death following injury promoting infiltration of cells releasing inflammatory mediators and endothelial activation **[25]**, this explains the increase in urea and creatinine levels in the positive control rats (DG). The significance decrease in renal parameter, urea and creatinine was brought about by treatment with clove extract which inhibit free radical production due to its antioxidant contents of the active ingredient eugenol. This active ingredient eugenol and β-caryophyllene also possess a scavenging function initiating radicals, chelating the transition metal catalyst and breaking chain reactions that may results in oxidative stress **[25, 43]**.

Malondialdehyde (MDA), degradative product in the cells membrane of polyunsaturated fatty acids (PUPA) peroxidation. High levels of MDA is an indicator of oxidative damage and lipid peroxidation, it was reported as one of the underlying cause of diabetes **[44,45]**. The antioxidant activity of *Syzygium aromaticum* may be due to the presence of phenolic compounds as thymol, eugenol and eugenol acetate **[17, 46]**. Our observations showed that TAC level in the DG-group were significantly lower than those in healthy control rats; while, its level in DSA group was significantly increased. In the diabetic group, serum level of MDA was increased significantly. On the other hand, MDA significantly reduced in groups treated with clove extract compared to control diabetic group. The increase in

the level of reactive oxygen species causes a decrease in the activity of antioxidant enzymes. Hyperglycemia in diabetes is associated with increased levels of MDA and increased oxidative stress in diabetic rats **[47]**. Increased lipid peroxidation leads to damage of tissue and the inability of defense mechanisms against antioxidant to prevent the free radical attack, which may lead to leakage of metabolites and enzymes into the bloodstream **[48]**. Clove extract by chelating temporary metal ions, scavenging free radicals, inhibiting oxidant enzymes, or by α-tocopherol repairing from the α-tocofoxyl radical can prevent cell damage **[49,50]**. Also, flavonoids can scavenge $OH^{\bullet}$, O_2, and peroxyl radicals and inhibit LPO activity **[51]**. Clove ethanolic extract can increase the antioxidant system enzymes and reduce oxidative stress **[52]**.

Moreover, serum total protein (TP) level was significantly diminished in diabetic rats. Chronically elevated blood glucose enhances the formation of advanced glycosylated products with hyper filtration and glomerular hypertrophy **[53]** and that is agree with **Evans and Capell [54]** and **Hosseini *et al.*** **[55]**. Furthermore, **Changrani *et al.* [56]** and **Abo El-Magd *et al.*** **[57]** reported that decrease in protein secretion and its content may be due to disturbance in the parotid and pancreatic atrophy, $[Ca^{2+}]_I$ and $[Mg^{2+}]_I$ homeostasis, deranged protein synthesis release and breakdown, altered intracellular signaling and gene regulation, and a derangement in gene expression for protein synthesis.

Alteration of albumin concentration in serum of diabetic rats has been associated with faster kidney disease progression. Our studies showed a significant decrease in serum albumin of diabetic rats. This most likely suggests that diabetes mellitus resulted in renal failure that led to loss of serum albumin gradually because negatively charged glycosaminoglycan were lost in the cellular basement membrane and the consequent enlargement of basement membrane pore size thus leading to albuminuria **[58]**. Interestingly, treatment of diabetic rats with clove extract normalized serum albumin; this revealed the therapeutic potency of clove ethanolic extract in ameliorating kidney function **[37, 59]**.

5. Conclusion

Our study showed that clove (*Syzygium aromaticum*) is a source of natural antioxidants that could be efficiently extracted with ethanol. Clove ethanol extract could protect biomolecules against oxidation and peroxidation and demonstrated high free radical scavenging property due to its content of phenolic compounds. Thus, because of clove beneficial effects, it can be considered as a promising therapeutic agent in reducing and treating the complications of diabetes.

6. Abbreviations

DM *Diabetes Mellitus*
ROS *Reactive oxygen species*
HPLC *High-performanceliquid chromatography*
STZ *Streptozotocin*
AST *Aspartate aminotransferase*
ALT *Alanine aminotransferase*
ALP *Alkaline phosphatase*
TAC *Total antioxidant capacity*
MDA *Malondialdehyde*
IDDM *Insulin-dependent diabetes mellitus*
NIDDM *Non-insulin-dependent diabetes mellitus*
TP *Total protein*
ESL *Endothelial surface layer*
CI-AK *Contrast-induced acute kidney injury*

7. Declarations

6.1. Acknowledgements

Not applicable.

7.2. Consent for publication

Not applicable.

7.3. Competing interests

The authors declare no competing interests.

8. References

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