Full Length Research Article

EFFECTS OF CLOVE OIL ON BLOOD GLUCOSE LEVEL, LIPID PROFILE, LIPID PEROXIDATION, AND KIDNEY FUNCTION ON DIABETIC RATS

*Hana M. Gashlan and Asma B. Al-Beladi

Department of Biochemistry, King Abdulaziz University, Faculty of Sciences, (Jeddah, Saudi Arabia)

ARTICLE INFO	ABSTRACT
	Objective: The aim of this study was to investigate the hypoglycemic and hypolipidemic effect of clove oil and whether treatment with different doses of it could improve the kidney function in streptozotocin-induced diabetic rats. **Materials and Methods:** Female albino rats (n=40) weighing (230-273 g) were divided into four groups. The first group (G1) served as control and consumed a standard diet. Diabetes was induced by injection of streptozotocin STZ (45 mg/kg body weight) to the other three groups. One diabetic group (G2) kept untreated and served as positive control. The other diabetic groups (G3 and G4) were treated with 300 mg/kg and 600 mg/kg of clove oil respectively. **Results:** STZ-induced diabetic group exhibited very highly significant increase in serum glucose level and lipid profile (p<0.001) as compared to the control group. The serum levels of creatinine and urea as well as the tissue levels of malondialdehyde and nitric oxide were significantly increase too. A reduction in the levels of high density lipoprotein cholesterol level was registered. Diabetic groups treated with different doses of clove oil showed an improvement in the levels of glucose, insulin, lipid profile, urea, creatinine and lipid peroxidation when compared with untreated diabetic group. Histopathological changes of rat kidney coincided with biochemical changes. **Conclusion:** This study demonstrated that clove oil produced significant reduction in glucose and lipid profile levels and shows remarkable ameliorates in kidney functions in diabetic rats. Therefor; the results of this study indicate that a dose of 600 mg/ kg of clove oil might be a beneficial adjuvant to oral hypolipidemic agents in diabetic patients.

INTRODUCTION

Diabetes mellitus (DM) is an endocrine disorder marked by hyperglycaemia and dyslipidaemia and cause many complications such as retinopathy, neuropathy, nephropathy, cardiovascular disorders and weight loss (Balogun *et al.*, 2016). It is known as a group of heterogeneous disorders with the common elements of hyperglycemia and glucose intolerance, due to insulin deficiency, impaired efficiency of insulin action, or both (Abed *et al.*, 2015). The International Diabetes Federation (IDF) reported that about 387 million of the global population suffers from diabetes and this number has been expected to be doubled in 2035 (IDF, 2014). Alanzi *et al.* (2014) stated that the Kingdom of Saudi Arabia (KSA) has the seventh highest incidence of diabetes in the world with estimates of 20% of the populations diagnosed with diabetes.

*Corresponding author: **Hana M. Gashlan***
Department of Biochemistry, King Abdulaziz University, Faculty of Sciences, (Jeddah, Saudi Arabia)

Streptozotocin (STZ) is used to induce diabetes in rats and causes hyperglycaemia. STZ is effective after intraperitoneal administration of single injection. After entering the cells, it leads to changes in the DNA of pancreatic beta cells leading to its fragmentation (Chaudhry *et al.*, 2013). The pathogenesis of DM is managed by insulin and oral administration of antihyperglycemic drugs such as sulfonylureas and biguanides (Tiwari and Madhusudanarao, 2002). Unfortunately, oral hypoglycemic drugs may be effective for glycemic control, but can cause some side effects such as liver disorders, renal tumors, abdominal pain, flatulence, hepatic injury (El-Kaissi and Sherbeeni, 2011). Insulin is effective in decreasing blood glucose and Glycosylated hemoglobin (HbA1C) but there are some disadvantages. Accordingly, there is an increase request to use natural products with antidiabetic activity due to the side effects associated with the use of insulin and oral hypoglycemic agents (Kim *et al.*, 2006). Many herbal medicines have been recommended for the treatment of diabetes which can also improve lipid metabolism, antioxidant

status and capillary function (Babu *et al*., 2007). Clove (*Syzygium aromaticum)* is an aromatic flower bud, commonly used in Asia, Africa and other parts of the world in preparation of spicy food. In addition, clove bud essential oil has many biological impacts, such as antimicrobial, insecticidal and antioxidant properties, antidiabetic, kidney reinforcement and the oil is used usually as flavoring agents in foods (Huang *et al*., 2002; Babu *et al*., 2007). This study aims to investigate the effect of different doses of clove oil on the levels of glucose, insulin, lipid profile, some oxidative stress markers, as well as on kidney functions and structure in streptozotocin-induced diabetic rats.

MATERIALS AND METHODS

Materials

Animals Forty female albino rats (Wistar strain), weighing 230-273 g, were obtained from The Animal Experimental Unit of King Fahad Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia. The rats were kept in special cages at (24± 3 °C) and humidity (60%) under 12 hour cycles of dark and light. Rats were supplied with standard pellet chow with free access to water for one week before the experiment for acclimatization. Animal handling will be performed in accordance with the guidelines provided and approved by the Experimental Animal Laboratory Committee of the King Abdulaziz University, Faculty of Science.

Chemicals

Streptozotocin was purchased from Sigma-Aldrich (St. Louis, MO) Chemical Co. Insulin ELISA kit was obtained from Bertin Pharma Company, Montigny-le-Bretonneux, France. Cholesterol, triacylglycerol (TAG), high density lipoprotein (HDL), low density lipoprotein (LDL), creatinine and urea were purchased from Dimension (DAD BEHRING Company, USA). Clove oil was purchased from local market in Jeddah.

Methods

Induction of Diabetes: The experimental animals were fasted for 10 hours and then diabetes was induced by a single intraperitoneal injection of STZ, dissolved in 50 mM citrate buffer (pH 4.5) at a dose of 45 mg/kg body weight according to Al-Attar (2010). While normal rats received only equal volume of vehicle (normal saline or physiological saline). The citrate buffer was prepared by adding 47 ml of 0.05 M citric acid to 53 ml of 0.05 M trisodium citrate dehydrate, pH of citrate buffer was adjusted at 4.5 by HCL using pH meter. All animals were returned to their cages after injection and given free access to food and water. After 4 days, the fasting blood glucose levels were measured by using a portable glucometer and the blood samples were taken from tail (Accu-Chek, Roch, Germany). Animals with blood glucose levels more than 250 mg/dl were considered diabetic and used for the experiment according to Almohammadi *et al.* (2013).

Experimental design This study included four groups consisting each of ten rats divided as follow:

    Group one (G1): normal control rats. Rats of this group will receive normal commercial chow diet.

Group two (G2): non treated diabetic rats. Rats of this group will receive 45mg/kg body weight of STZ as a single intraperitoneal injection.

Group three (G3): diabetic rats treated with low doses of clove oil. Rats of this group will receive 300mg/kg of clove oil daily for six weeks.

Group four (G4): diabetic rats treated with high doses of clove oil. Rats of this group will receive 600mg/kg of clove oil daily for six weeks. Rats were weighed at the start and the end of the experimental period using a digital balance. At the end of the experimental period, all rats were fasted for 10 hours, water was not constrained, and then blood samples were drawn under diethyl ether anesthesia retro-orbitally from the inner canthus of eye using capillary tubes (Micro Hematocrit Capillaries, Mucaps). The blood was collected into EDTA coated test tubes for serum separation. Serum was obtained after the blood was allowed to clot at room temperature and centrifuged at 3000 rpm for 15 minutes. Serum was transferred into sterile Eppendorf tubes and stored at -80° C until biochemical analysis. Serum was used for the measurement of glucose, insulin, lipid profile, creatinine and urea. After samples collection, all rats of each group were sacrificed under ether anesthesia and the organs (the liver and the kidney) were removed immediately. The liver for the homogenate and the kidney for histological study. 0.5g of liver was weighed then homogenized in 5ml of ice cold of 10% TCA using an electrical homogenizer. The homogenates were centrifuged at 3000 rpm for 15 minutes at 4ºC using cold centrifuge. The supernatants were collected and aliquoted in Eppendorf tubes and stored at -20°C and used to detect the level of lipid peroxidation (MDA) and nitric oxide (NO) in the tissue (Fayed, 2013). MDA was determined in tissue liver homogenate by colorimetric assay according to RuizLarrea *et al*. (1994). MDA level was calculated using coefficient of MDA 1.56×105 / M / cm according to Buege and Aust (1978). Nitric oxide was determined in tissue liver homogenate by colorimetric assay according to Moshage *et al*. (1995).

Histopathological Examination

The kidney was excised and placed in 10% neutral buffered formalin. The fixed tissues were then trimmed, washed with ice saline and dehydrated in ascending grades of isopropyl alcohol and cleared in xylene. The wax impregnated tissues were embedded in paraffin blocks using the same grade wax, the paraffin blocks were cut with rotary microtome at 3-5μ thickness. The sections were floated on a tissue floatation bath at 40°C and taken on glass slides. The sections were then melted in an incubator at 60°C and after 5 min. the sections were allowed to cool and stained with Hematoxylin and Eosin according to (Bancroft and Cook, 1998), and examined microscopically.

Statistical analysis

All values were expressed as mean ± standard error (X ± SE). Statistical analyses were performed with one-way analysis of variance (ANOVA) test and independent sample *t*-test using MegaStat Excel (version 10.3, Butler University). Differences were considered significant when P < 0.05.

RESULTS

Effect of clove oil on body weights Table 1 showed that initial body weights did not significantly differ among the groups. During the experiment at period, the body weights were increase in all groups. At the end of the experiment, there was a very highly significant decrease (P<0.001) in the body weight of diabetic group as compared to the control group (G1). While, there were highly significant increase (P<0.01) in the body weight of treated diabetic group (G3) and a very highly significant increase (P<0.001) in treated diabetic group (G4) as compared to untreated diabetic group (G2).

Effect of clove oil on serum levels of glucose and insulin

The results of serum insulin and glucose levels are shown in Table 2. There was a very highly significant increase (P<0.001) by 358.67% in glucose level accompanied with a very highly significant decrease (P<0.001) by -41.67% in insulin levels in diabetic group (G2) as compared to control group (G1). While there were very highly significant decrease (P<0.001) by 83.65% in glucose levels accompanied with highly significant increase (P<0.01) by 17.26% in insulin level in diabetic group treated with 300mg/kg (G3) of clove oil as compared to (G2). A very highly significant decrease (P<0.001) by 86.19% was noticed in the level of glucose in diabetic group treated with 600mg/kg (G4) of clove oil accompanied with a very highly significant increase (P<0.001) by 7.74% in insulin level as compared to (G2).

Effect of clove oil on serum levels of lipid profile

The serum levels of cholesterol, TAG, HDL and LDL are shown in Table 2. There was a very highly significant increase (P<0.001) by 53.43% in diabetic group (G2) as compared to control group (G1). Diabetic group treated with 300 mg\kg of clove oil (G3) showed significant decrease (P<0.05) by 33.4% in cholesterol levels, while there was a very highly significant decrease (P<0.001) by 16.6% in diabetic group treated with 600 mg\kg of clove oil (G4) in cholesterol levels as compared to (G2).

diabetic group treated with 600mg\kg of clove oil (G4) as compared to G2. Highly significant decrease (P<0.01) by -32.92% was found in HDL levels in diabetic group (G2) as compared to control group. While, a very highly significant increase (P<0.001) in HDL levels in treated diabetic groups (G3 and G4) as compared to untreated diabetic group (G2) by 1.25% and 8.13% respectively. There was a very highly significant increase (P<0.001) by 124.14% in LDL levels in diabetic group (G2) as compared to control group. While no significant change was registered in treated diabetic groups (G3 and G4) as compared with untreated diabetic group (G2).

Effect of Clove Oil on Malondialdehyde (MAD) and Nitric Oxide (NO)

Table 3 shows the effect of clove oil on malondialdehyde (MAD) and nitric oxide (NO). There was a very highly significant increase (p<0.001) by 184.71% in MAD level in diabetic group (G2) ascompared to control group. Clove oil cause a very highly significant decrease (p<0.001) in MAD levels in treated diabetic groups (G3 and G4) as compared to untreated diabetic group by 10.04% and 1.10% respectively as percent change from diabetic group. There was a very highly significant increase (p<0.001) by 77.25% in NO level in diabetic group (G2) as compared to control group. There was no significant change in diabetic group treated with 300mg\kg of clove oil (G3) and significant decrease (p<0.05) by 49.70% in diabetic group treated with 600mg\kg of clove oil (G4) as compared to untreated diabetic group.

Effect of clove oil on serum levels of creatinine and urea

The level of serum creatinine and urea are represented in Table 4. There was a highly significant increase (P<0.001) by 42.55% in the level of creatinine in diabetic group (G2) as compared to control group (G1). While there were a highly significant decrease (P<0.01) by 10.64% in diabetic groups treated with clove oil (G3 and G4) as compared to untreated diabetic group. There was a very highly significant increase (P<0.001) by 56.10% in diabetic group (G2) as compared to control group.

Table 1. Effect of Clove Oil on Body Weight

Groups Parameters	G1	G2	G3	G4
Initial body weight (g)	244± 2.89	246.20 ±1.79	246.30 ± 2.9	245.70 ± 2.01
P-value		NS	NS	NS
Final body weight (g)	287.10 ±2.88	253.0 ±1.13	265.30 ±2.61	268.90 ±2.61
P-value		a	b	b
		***	**	***

± SE: ± standard error of the mean.
G1= control group.
G2= diabetic group.
G3= diabetic group treated with 300mg\kg of clove oil.
G4= diabetic group treated with 600mg\kg of clove oil.
NS= No significant.
*0.01   P-value   0.05, the difference between samples is significant.
**0.001   P-value   0.01, the difference between samples is highly significant.
*** P-value   0.001, the difference between samples is very highly significant.
[a]: Significant difference between G2 and G1.
[b]: Significant difference between diabetic treated groups (G3&G4) and G2.

In diabetic group (G2) there was a very highly significant increase (P<0.001) by 101.32% in TAG level as compared to control group (G1). While, no significant change was found in TAG level in diabetic group treated with 300 mg\kg of clove oil (G3) as compared to (G2). There was a very highly significant decrease (P<0.001) by 16.29% in TAG level in

There was significant decrease (P<0.05) by 34.15% in diabetic group treated with 300mg\kg of clove oil (G3) while a highly significant decrease (P<0.01) by 18.29% was noted in diabetic group treated with 600mg\kg of clove oil (G4) as compared to untreated diabetic group (G2).

Histopathological investigation of kidney tissues

Histological study of the normal kidneys of the control group, displayed in Figure 1(A), revealed normal glomeruli surrounded by the Bowman's capsule, distal and proximal convoluted tubules without any changes.

The tissue of the diabetic group, exhibited in Figure 1(B), demonstrated damage of brush border and increase lumen of some tubules. Kidney tissues in diabetic groups treated with (300 and 600 mg/kg) of clove oil and normal groups treated with (300 and 600 mg/kg) of clove oil showed no alterations in morphology as shown in Figures 2(A) and 2(B), respectively.

Table 2. Effect of Clove Oil on Serum Glucose, Insulin, Cholesterol, TAG, HDL and LDL levels

Groups / Parameters	G1	G2	G3	G4
Glucose mg\dl	90.5±3	415.10±16.07	166.2±8.21	168.5±4.34
P-Value		••• a	••• b	••• b
Insulin ng\ml	1.68± 0.07	0.98±0.05	1.39±0.08	1.55±0.12
P-Value		••• a	•• b	••• b
Cholesterol mg\dl	53.9±2.61	82.7±3.23	71.9±4.62	62.9±2.97
P-Value		••• a	• b	••• b
Triacylglycerol mg\dl	75.5±8.80	152±13.18	132.5±8.52	87.8±7.93
P-Value		••• a	NS	••• b
HDL mg\dl	48±2.84	32.2±2.53	48.6±3.16	51.9±2.58
P-Value		•• a	••• b	••• b
LDL mg\dl	11.6±1.69	26±2.29	22.3±2.42	21.6±1.71
P-Value		••• a	NS	NS

± SE: ± standard error of the mean.
G1= control group.
G2= diabetic group.
G3= diabetic group treated with 300mg\kg of clove oil.
G4= diabetic group treated with 600mg\kg of clove oil.
NS= No significant.
*0.01 P-value 0.05, the difference between samples is significant.
**0.001 P-value 0.01, the difference between samples is highly significant.
*** P-value 0.001, the difference between samples is very highly significant.
a: Significant difference between G2 and G1.
b: Significant difference between diabetic treated groups (G3&G4) and G2.

Table 3. Effect of Clove Oil on MAD and NO

Groups / Parameters	G1	G2	G3	G4
MAD (Nmoles\ g of tissue)	72.58±1.42	206.64±5.05	79.87±4.45	71.78±3.26
P-Value		*** a	*** b	*** b
NO (ug\ g of tissue)	1.67±0.17	2.96±0.13	2.70±0.24	2.50±0.12
P-Value		*** a	NS	* b

± SE: ± standard error of the mean.
G1= control group.
G2= diabetic group.
G3= diabetic group treated with 300mg\kg of clove oil.
G4= diabetic group treated with 600mg\kg of clove oil.
NS= No significant.
*0.01 P-value 0.05, the difference between samples is significant.
**0.001 P-value 0.01, the difference between samples is highly significant.
*** P-value 0.001, the difference between samples is very highly significant.
a: Significant difference between G2 and G1.
b: Significant difference between diabetic treated groups (G3&G4) and G2

Table 4. Effect of Clove Oil on Urea and Creatinine Levels

Groups / Parameters	G1	G2	G3	G4
Creatinine mg\dl	0.47±0.021	0.67±0.050	0.52±0.029	0.52±0.049
P-Value		***[a]	**[b]	**[b]
Urea mg\dl	16.4+0.60	25.6+1.77	22+4.88	19.4+4.84
P-Value		***[a]	*[b]	**[b]

± SE: ± standard error of the mean.
G1= control group.
G2= diabetic group.
G3= diabetic group treated with 300mg\kg of clove oil.
G4= diabetic group treated with 600mg\kg of clove oil. NS= No significant. *0.01 P-value 0.05, the difference between samples is significant.
**0.001 P-value 0.01, the difference between samples is highly significant. *** P-value 0.001, the difference between samples is very highly significant.
a: Significant difference between G2 and G1.
b: Significant difference between diabetic treated groups (G3&G4) and G2.
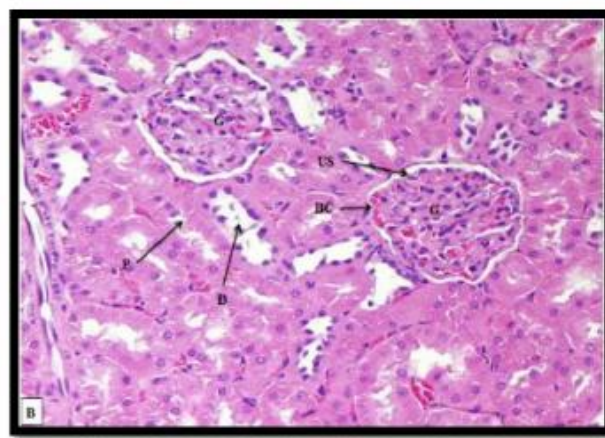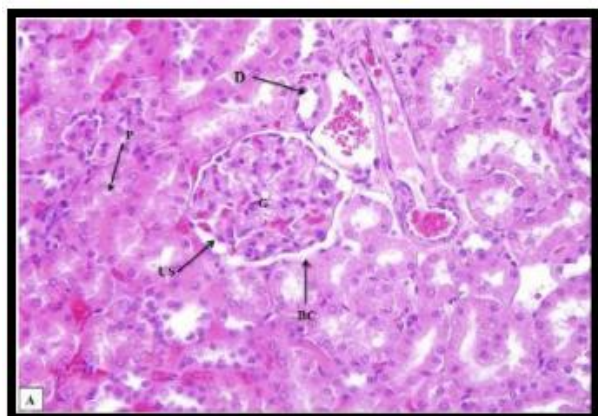

Figure (1.A). Photomicrographs of a section in the kidney tissue of the control group showing normal Bowman's capsule (BC), urinary space (US), glomeruls (G), proximal (P) and distal (D) tubules, and (1.B) diabetic group showing damage of brush border and increase lumen of some tubules (H&E X 400)
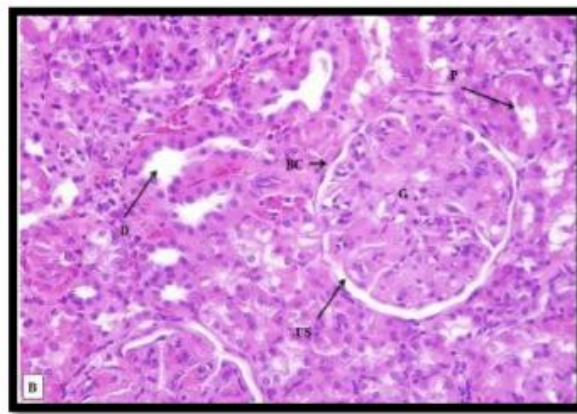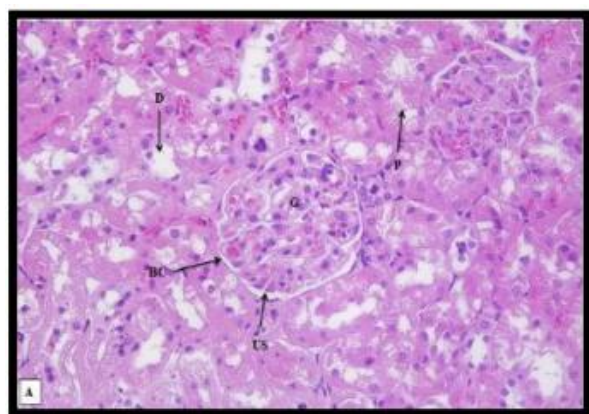

Figure (2.A) Photomicrographs of a section in the kidney tissue of the diabetic group treated with 300mg/kg of clove oil, and (2.B) diabetic group treated with 600mg/kg of clove oil showing no histopathological changes (H&EX 400)

DISCUSSION

In the current study, untreated diabetic rats showed very highly significant decrease in final body weight as compared to control group.

The obtained results were in agreement with Narasimhulu *et al*. (2014) who reported that, significant decrease in body weight was observed in diabetic rats when compared to control rats. Kota *et al*. (2012) found that there were an association between hyperglycemia and decrease body weight of diabetic animals, diabetes induced

reduction in body weight, and the body's inability to store or use glucose causes hunger and weight loss. The damage of -cells and disorder of insulin secretion in the diabetic state causes physio metabolic abnormalities such as a reduction in body weight gain and increase in food and water intake and urine volume. The diabetic rats induced by STZ also showed these changes (Kang et al., 2006). STZ-induced diabetes is characterized by sever weight loss which might be the result of protein wasting due to absence of carbohydrate as an energy source (Al-Attar, 2010). Diabetic rats treated with clove oil (300 and 600 mg/kg) showed significant increase in final body weight as compared to untreated diabetic rats. Moreover, Al-Attar and Zari (2007b) reported that diabetic rats giving diets containing clove oil had higher body weight change than diabetic rats giving control diets. This increase in weight may be due to the insulin like action of clove on muscle, adipose tissue and hepatocytes (Chaudhry et al., 2013). Srinivasan et al. (2014) found that administration of eugenol for 30 days significantly improved glycemic control which prevented the loss of body weight and excess of food and fluid intake on diabetic control rats in dose dependent manner.

In the present study, STZ- induced diabetic rats showed very highly significantly increase in serum glucose concentration accompanied by a very highly significantly decrease in serum insulin level as compared to control group. A similar result was reported by Basha and Sankaranarayanan (2015) who reported that STZ- diabetic rats in a dose of 40 mg/kg and had a negative effect in glucose concentration and insulin levels. In addition, this finding is in agreement with the report of Daniel et al. (2015) who reported that rat injected with STZ had shown a marked raise in glucose level and decrease in insulin level. STZ destroys the insulin producing -cells which is accompanied by characteristic variations in blood insulin and glucose concentrations. Intracellular action of STZ results in changes of DNA in pancreatic -cells including its fragmentation. This results to impaired glucose oxidation and decreases insulin biosynthesis and secretion (Daniel et al., 2015). Treatment of diabetic rats with different doses (300 and 600 mg/kg) of clove oil exhibited remarkably ameliorated effect. There were very highly significant improvement in glucose concentration and insulin levels when compared with untreated diabetic group. Hassanen (2010) shown that administration of clove and clove oil to diabetic rat significantly reduce the level of blood glucose and significantly elevate the level of insulin as compared to untreated diabetic group. Glucose lowering effect of clove could be due to stimulation of functioning pancreatic -cells, to increase the release of insulin, or may be due to regeneration of -cells (Chaudhry et al., 2013). Toda et al. (2000) reported that anti hyperglycemic effect of clove may be due to its inhibitory action on alpha-glucosidase. The ability of eugenol to significantly decrease fasting plasma glucose levels in diabetic rats is due to its possibility to secrete insulin from existing islet -cells and increases the intake of glucose by the tissues. Gallic acid, a phenolic compound increases insulin release in experimental diabetic rats (Srinivasan et al., 2014).

In the present study, STZ-induced diabetic rats showed a very highly significant increase in serum cholesterol, TAG and LDL and a very highly significant decrease in HDL when compared with control group. Rajasekaran et al. (2006) reported that in the STZ-diabetic rats, the levels of cholesterol, TAG and LDL activities were increase, while the levels of HDL were markedly decrease. Also, a similar result was

reported by Kota et al. (2012) who found that STZ-induced diabetic rat in a dose of 30 mg/kg had a negative effect in lipid profile levels when compared with non diabetic rats. The abnormal high concentrations of serum lipids in diabetic rats are mainly due to an increase in the mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone-sensitive lipase. In the serum of diabetic rats, excess fatty acids are converted into phospholipids and cholesterol along with excess triglycerides formed at the same time in the liver may be discharged into the blood in the form of lipoproteins (Al Logmani and Zari, 2011). In this study, when the diabetic rats treated with different doses (300 and 600 mg/kg) of clove oil exhibited remarkably ameliorated effects, there were an improvement in cholesterol, TAG, LDL and HDL levels in both doses when compared with untreated diabetic group. On the other hand the mean values of lipid profile decrease gradually by increasing the doses of clove oil. The high dose of clove oil (600mg) is more effective in reducing the lipid levels than the dose level (300mg). Hassanen (2010) stated that STZ-induced diabetic rats decrease the lipid profiles by oral administration of clove oil and clove powder once daily for 60 days when compared with untreated diabetic rats. The hypolipidemic effect can be explained as a direct reduction in blood glucose level, lowering cholesterol and TAG could also be due to regenerate pancreatic -cells and a suppression of the hepatic fatty acid synthase, glucose-6-phosphate dehydrogenase (Srinivasan *et al.,* 2005; Aboelnaga, 2015). Hyperglycemia may be associated with increase lipid peroxidation caused by oxidative stress and may also affect the development of diabetic complications (Kota et al., 2012) and leads to the formation of lipid products such as MDA and 4-HNE (4-hydroxynonenal), which then cause damages to the membrane components of the cell, cell necrosis and inflammation (Stark, 2005). In the present study, STZ-induced diabetic rats showed very highly significant increase in the levels of MDA and NO in liver tissue.

A similar results was reported by Kanter et al. (2004) who reported that STZ- diabetic rats in a dose level of 40 mg/kg had increase in MDA and NO level, this may be attributed to increase in ROS which is involved in the development and progression of diabetes mellitus (Suryanarayana et al., 2007). In addition, Yilmaz et al. (2004) exhibited that rat injected with STZ has been shown a marked raise in MDA levels in the hepatic tissues. The increase MDA level in diabetes mellitus suggested that hyperglycemia induces the peroxidative reactions in lipids and oxidative stress in the liver. After treating the diabetic rats with (300 and 600 mg/kg) of clove oil, the results of this study showed decrease in MDA and NO in treated diabetic rats. Shukri et al. (2010) reported that dietary supplementation with clove significantly reduced blood sugar and lipid peroxidation in diabetic rats. This may be due to the presence of phenolic compounds and flavonoids that present in clove extract and clove essential oil which cause scavenging of free radicals (Dorman et al., 2000; Gulcin et al., 2004; Abdel-Wahhab and Aly, 2005). In the kidney, creatinine is the major waste product of creatinine metabolism which filtered by the glomerulus and actively excreted by the tubules (Myers et al., 2006).

Creatinine concentration is used to assess impairment of kidney function and to detect treatment related toxic effects of compound on the kidney in experimental animals. Urea is the main end product of protein catabolism (Narasimhul et al.,

2012). The data of the present study revealed a very highly significant elevation in serum creatinine and urea concentrations in STZ induced diabetic rats.This results were in agreement with the results of Al-Attar (2010) and Al-Logmani and Zari (2011). In experimental diabetes, accumulation of urea nitrogen may be due to the enhanced breakdown of both liver and plasma proteins. Alterations in nitrogen homeostasis lead to increase hepatic elimination of urea nitrogen and increase peripheral release of nitrogenous substances due to changes occurring within the hepatocytes. An elevation in creatinine usually occurs simultaneously with an increase in urea nitrogen (Narasimhul *et al.*, 2012). Narasimhul *et al.* (2014) stated that hyperglycemia causes renal dysfunction such as acute glomerulonephritis, nephronsclerosis and even tubular necrosis by elevating serum urea nitrogen and creatinine. In the current study, treatment of the diabetic rats with both 300 and 600 mg/kg of clove oil showed significant decrease in the levels of serum creatinine and urea as compared to untreated diabetic rats. The mean values of serum creatinine and urea were decrease gradually with increasing the dose of clove oil. A previous study conducted on diabetic rats revealed that the oral administration of clove oil or clove powder cause an improvement in kidney function of diabetic rats (Hassanen, 2010).

In the present study, it was reported that microscopic examination of the kidney sections of untreated diabetic group revealed damage of brush border and increase lumen of some tubules. Several study reported that the histological architecture of kidney sections of untreated diabetic rats showed some changes (Hussein and Abu-Zinadah, 2010; Abed *et al.* 2015). Leegwates and Kuper (1984) demonstrated two types of effects of STZ on the kidneys of rats. The primary effect, the diabetes factor was associated with hyperglycemia and was responsible for dilatation of proximal and distal tubules in the cortex. The secondary effect, named the individual response factor, was associated with inflammatory processes. In the current study, the examined sections of the kidney of diabetic rats treated with (300 and 600 mg/kg) of clove oil showed no histopathological changes. Hassanen (2010) reported that the kidney of diabetic rats administrated with clove oil showed no changes with apparent normal histological structure. Treatment with clove oil reversed changes to near normal, which could be associated with decrease membrane damage as evidenced by improved antioxidant status and reversed fatty acid changes as evidenced by improved insulin level.

Conclusion

This study together with existing reports demonstrates that the administration of clove oil possesses anti-hyperglycemic and anti-hyperlipidemic activity in diabetic rats. Also it shows a remarkable ameliorates in kidney functions. In addition, the dose of 600 mg/kg of clove oil might be use as a valuable agent in the therapy of diabetes mellitus and its complications.

Acknowledgements

REFERENCES

Abdel-Wahhab, M., Aly, S. 2005. Antioxidant property of nigella sativa (black cumin) and Syzygium aromaticum (clove) in rats during aflatoxicosis. *Journal of Applied Toxicology.* 25(3):218-223.

Abed, S., El-Shazely, M., Ahmed, K., Abdel-mawla, E., Ibrahim, A. 2015. Pathological, immunohistochemical and biochemical studies on the therapeutic effect of raphanus sativus oil on streptozotocin Induced Diabetic Rats. Egypt. *J. Comp. Path &Clinic Path*. 28(1):1-17.

Aboelnaga, S. 2015. Effect of some levels of cardamom, clove and anise on hepatotoxicity in rats caused by CCL4. *World Applied Sciences Journal.* 33(6):854-865.

Alanzi, T., Istepanian, R., Philip, N., Sungoor, A. 2014. A study on perception of managing diabetes mellitus through social networking in the Kingdom of Saudi Arabia. XIII *Mediterranean Conference on Medical and Biological Engineering and Computing*. 2013: 1907-1910.

Al-Attar, A, Zari, T. 2007a. Modulatory effects of ginger and clove oils on physiological responses in streptozotocin-induced diabetic rats. *Int. J. Pharmacol., 3*;34-40.

Al-Attar, A. 2010. Physiological effects of some plant oils supplementation on streptozotocin-induced diabetic rats. *Research Journal of Medicine and Medical Sciences.* 5(1):55-71.

Al-Attar, A., Zari, T. 2007b. Effects of ginger and clove oils on some physiological parameters in Ssreptozotocin-diabetic and non-diabetic rats. *Journal of Medical Sciences (Faisalabad).* 7(2):267-275.

Al-Logmani, A., Zari, T. 2011. Long-term effects of Nigella sativa L. oil on some physiological parameters in normal and streptozotocin-induced diabetic rats. *Journal of Diabetes Mellitus.* 1(3):46-53.

Almohammadi, S., Hobbenaghi, R., Javanbakht, J., Kheradmand, D., Mortezaee, R., Tavakoli, M., Khadivar, F., Akbari, H. 2013. Protective and antidiabetic effects of extract from Nigella sativa on blood glucose concentrations against streptozotocin (STZ)-induced diabetic in rats: an experimental study with histopathological evaluation. Diagn Pathol. 8(1):137.

Babu, P., Prabuseenivasan, S., Ignacimuthu, S. 2007. Cinnamaldehyde—a potential antidiabetic agent. *Phytomedicine*. 14(1):15-22.

Balogun FO, Ashafa AO (2016). Antidiabetic medicinal plants used by the Basotho tribe of eastern Free State: A review. Journal of Diabetes Research. 2016:1-13.

Basha, R. and Sankaranarayanan, C. 2015. Protective role of caryophyllene, a sesquiterpene lactone on plasma and tissue glycoprotein components in streptozotocin-induced hyperglycemic rats. *Journal of Acute Medicine.* 5(1):9-14.

Buege, J., Aust, S. 1978. Microsomal lipid peroxidation. Methods in enzymology. 52:302-310.

Chaudhry, Z., Chaudhry, S., Naseer, A., Chaudhry, F. 2013. Effect of Syzygium aromaticum (clove) extract on blood glucose level in streptozotocin induced diabetic rats. *Pakistan Armed Forces Medical Journal.* 63(3):323-328.

Chaudhry, Z., Naseer, A., Chaudhry, E., Chaudhry, S. 2015. Effect of aqueous and ethanolic extract of syzygium aromaticum on blood glucose in diabetic rats. *Journal of Islamic International Medical College.* 10(3): 214-218.

Daniel, E., Mohammed, A., Tanko, Y., Ahmed, A., Adams, M, Atsukwei, D. 2015. Effects of lycopene on thyroid profile in streptozotocin-induced diabetic Wistar rats. *European Journal of Biotechnology and Bioscience.* 3(1):21-28.

Dorman, H., Figueiredo, A., Barroso, J., Deans, S. 2000. In vitro evaluation of antioxidant activity of essential oils and their components. *Flavour and Fragrance Journal.* 15(1):12-16.

El-Kaissi, S., Sherbeeni, S. 2011. Pharmacological management of type 2 diabetes mellitus: an update. *Current Diabetes Rreviews.* 7(6):392-405.

Fayed, D. 2013. Improvement of the efficiency of praziquantel by Citharexylum quadrangular extract and micronutrients in murine schistosomiasis. PH.D, Ain Shams University, Egypt.

Gulcin, I., Sat, I., Beydemir, S., Elmastas, M., Kufrevioglu, O. 2004. Comparison of antioxidant activity of clove (Eugenia caryophylata Thunb) buds and lavender (Lavandula stoechas L.). Food Chemistry. 87(3):393-400.

Hassanen, N. 2010. Protective effect of cinnamon, clove and ginger spicces or their essential oils on oxidative stress of streptozotocin-induced diabetic rats. Arab Universities *Journal of Agricultural Sciences.* 18(1):137-154.

Huang, Y., Ho, S., Lee, H., Yap, Y. 2002. Insecticidal properties of eugenol, isoeugenol and methyleugenol and their effects on nutrition of Sitophilus zeamais Motsch.(Coleoptera:Curculionidae) and Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae). Journal of Stored Products Research. 38(5):403-412.

International Diabetes Federation, 2014. IDF Diabetes Atlas, 6th edition.

Kang, K., Kim, H, Yamabe, N., Nagai, R., Yokozawa, T. 2006. Protective effect of sun Ginseng against diabetic renal damage. *Biological and Pharmaceutical Bulletin.* 29(8):1678-1684.

Kanter, M., Coskun, O., Korkmaz, A, Oter, S. 2004. Effects of Nigella sativa on oxidative stress and -cell damage in streptozotocin-induced diabetic rats. The Anatomical Record Part A: Discoveries in Molecular, Cellular, and Evolutionary Biology. 279(1):685-691.

Kim, S., Hyun, S., Choung, S. 2006. Anti-diabetic effect of cinnamon extract on blood glucosein db/db mice. *Journal of Ethnopharmacology*. 104(1):119-123.

Kota, N., Panpatil, V., Kaleb, R., Varanasi, B., Polasa, K. 2012. Dose-dependent effect in the inhibition of oxidative stress and anticlastogenic potential of ginger in STZ induced diabetic rats. *Food Chemistry.* 135(4):2954-2959.

Moshage, H., Kok, B., Huizenga, J.R., Jansen, P.L. 1995. Nitrite and nitrate determinations in plasma: a critical evaluation. *Clinical Chemistry*. 41(6):892-896.

Myers, G., Miller, W., Coresh, J., Fleming, J., Greenberg, N., Greene, T., Hostetter, T., Levey, A., Panteghini, M., Welch, M., Eckfeldt, J. 2006. Recommendations for improving serum creatinine measurement: a report from

the Laboratory Working Group of the National Kidney Disease Education Program. *Clinical Chemistry*. 52(1):5-18.

Narasimhulu, G., Mohamed, J., Reddy, K. 2014. Antihyperglycemic effect of pimpinella tirupatiensis leaves in streptozotocin-induced diabetic rats. *Bulletin of Environment, Pharmacology and Life Sciences.* 3(2):5-13.

Rajasekaran, S, Ravi, K., Sivagnanam, K, Subramanian, S. 2006. Beneficial effects of Aloe vera leaf gel extract on lipid profile status in rats with streptozotocin diabetes. *Clinical and Experimental Pharmacology and Physiology*. 33(3):232-237.

Ruiz-Larrea, M.B., Leal, A.M., Liza, M., Lacort, M., De Groot, H. 1994. Antioxidant effects of estradiol and 2-hydroxyestradiol on iron-induced lipid peroxidation of rat liver microsomes. *Steroids*. 59(6):383-388.

Shukri, R., Mohamed, S., Mustapha, N. 2010. Cloves protect the heart, liver and lens of diabetic rats. *Food Chemistry.* 122(4):1116-1121.

Srinivasan, K., Viswanad, B., Asrat, L., Kaul, C., Ramarao, P. 2005. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: A model for type 2 diabetes and pharmacological screening. *Pharmacological Research*. 52(4):313-320.

Srinivasan, S., Sathish, G., Jayanthi, M., Muthukumaran, J., Muruganathan, U., Ramach, A., Ran, V. 2014. Ameliorating effect of eugenol on hyperglycemia by attenuating the key enzymes of glucose metabolism in streptozotocin-induced diabetic rats. *Molecular and Cellular Biochemistry.* 385(1-2):159-168.

Stark, G. 2005. Functional consequences of oxidative membrane damage. *The Journal of membrane biology.* 205(1):1-16.

Suryanarayana, P., Satyanarayana, A., Balakrishna, N., Kumar, P.U., Reddy, G.B. 2007. Effect of turmeric and curcumin on oxidative stress and antioxidant enzymes in streptozotocin-induced diabetic rat. Medical Science Monitor. 13(12):286-292.

Tiwari, A., Madhusudanarao, J. 2002. Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. *Current Science*. 83(1):30-38.

Toda, M., Kawabata, J., Kasai, T. 2000. Glucosidase inhibitors from Clove (Syzgium aromaticum). *Bioscience, Biotechnology and Biochemistry*. 64(2):294-298.

Yilmaz, H., Uz, E., Yucel, N., Altuntas, I., Ozcelik, N. 2004. Protective effect of caffeic acid phenethyl ester (CAPE) on lipid peroxidation and antioxidant enzymes in diabetic rat liver. *Journal of Biochemical and Molecular Toxicology*. 18(4):234-238.

****