Effect of Ginger, Curcumin and Their Mixture on Blood Glucose and Lipids in Diabetic Rats

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Abstract: The present study was conducted to evaluate the hypoglycemic, hypolipidemic and antioxidant effect of curcumin, ginger and their mixture in streptozotocin (STZ)-induced diabetic rats. **Material and Methods:** Male albino rats (n=35) weighing (180-195 g) were divided into two main groups; first group: negative control (n=7) fed standard diet and second group: diabetic rats (n=28), which divided equally to four subgroups as follows: diabetic untreated rats (positive control), diabetic rats treated with curcumin (0.5 % of diet), diabetic rats treated with ginger (3% of diet) and diabetic rats treated with their mixture. Diabetes was induced by a single intraperitonial injection of STZ (65 mg/kg body weight). **Results:** The results reported that the STZ-induced diabetic group exhibited very highly significant (p<0.001) hyperglycemia, hyperlipidemia, elevated in malondialdehyde (MDA) accompanied with weight loss and reduced in high density lipoprotein cholesterol (HDL-C) level, superoxide dismutase (SOD) and catalase (CAT) enzyme activities when compared with control negative group. Treatment with curcumin, ginger or their mixture reported very highly significant (p< 0.001) improvement in biological evaluation, glucose, insulin, lipid profile, lipid peroxidation and antioxidant enzymes activities when compared with untreated diabetic group. Histopathological investigation of liver and pancreatic tissues of diabetic rats represented the presence of sever changes, meanwhile treatment overcome this changes, the majority of the cells tend to be normal, this improvement in the cells may explain the antidiabetic effect of the plants under study especially in their mixture. **Conclusion:** This study demonstrates that the curcumin and ginger mixture possesses significant reduction in hyperglycemic and hyperlipidemic, as well as antioxidant effect in diabetic rats. Therefore, it recommends using mixture of curcumin and ginger to alleviate the oxidative stress caused by diabetes. Further research is required to find out the exact mechanisms of curcumin and ginger responsible for antidiabetic and antioxidant activities.
[Hala A. H. Khattab, Nadia S. Al-Amoudi and Al-Anood A. Al-Faleh. **Effect of Ginger, Curcumin and Their Mixture on Blood Glucose and Lipids in Diabetic Rats.** *Life Sci J* 2013;10(4):428-442] (ISSN: 1097-8135). http://www.lifesciencesite.com. 56

Key words: Curcumin, ginger, diabetic rats, hypoglycemic, hypolipidemic, antioxidant, histopathology.

1. Introduction

Diabetes mellitus (DM) is a heterogeneous metabolic disorder, a high occurrence of disease in noted and the numbers of diabetic patients are gradually increasing, thus the disease constitutes a major health concern **(Narendhirakannan *et al.*, 2005 and Etuk, 2010)**. According to the World Health Organization (WHO), there are approximately 347 million diabetics worldwide, the number of diabetics had been double in the last few years and WHO projects reported that, diabetes death will increase by two thirds between 2008 and 2030 **(WHO, 2012)**. Reasons for this rise include an increase in sedentary lifestyle, the consumption of energy-rich diet, obesity, etc… **(Yajmk, 2001)**. Diabetes is the fourth or fifth leading cause of death in most high-income countries and there are substantial evidences that it is epidemic in many economically developing and newly industrialized countries **(IDF, 2011)**. Due to its high prevalence and potential deleterious effect on a patient physical and psychological state, diabetes consider as a major medical concern **(Macedo *et al.*, 2005)**. Because of the importance of diabetes, its management considered a global problem and greatest expenses for health system in the whole world **(Martin *et al.*, 2007)**.

Effective control of hyperglycaemia in diabetic patients is critical for reducing the risk of micro- and macrovascular diseases **(Ismail *et al.*, 2010)**. Synthetic hypoglycemic agents that are capable of reducing blood sugar level possessed most worrying side effects, which have impeded their usefulness as antidiabetic agents **(Gandhi and Sasikumar, 2012)**. Therefore, finding other anti-diabetic agents especially those made from natural sources is desired **(Vishwakarma *et al.*, 2010).** Plants have always been an exemplary source of drugs, it's used in traditional medicine to treat diabetes mellitus represent a valuable alternative for the management of this disease, amongst such plants reported to have beneficial effects in the treatment of diabetes are spices such as curcumin and ginger **(Ugwuja *et al.*, 2010 and Gandhi and Sasikumar, 2012)**.

Curcumin (*diferuloylmethane*) [1,7-bis (4-hydroxy -3- methoxyphenyl) -1,6-heptadien -3,5 dione] is the active component in turmeric rhizomes (*Curcuma* Long Linn, *Zingiberaceae*), at a content of

3 to 5% **(Anand*et al*., 2007)**. Curcumin has been used as spice and colorant in Indian curries, as well as a component of Chinese medicines, and has been approved as a safe food in the US **(Shimatsu *et al*., 2012)**. In recent years, a number of studies have investigated the various biological effects of curcumin, attributed to polyphenol's potential to modulate multiple signaling molecules. Animal studies have suggested that curcumin may be active against a wide range of human diseases, including diabetes, obesity, neurologic, psychiatric disorders and cancer. Also, chronic illnesses affecting the eyes, lungs, liver, kidneys, gastrointestinal and cardiovascular systems, Curcumin plays a beneficial role in terms of being an antioxidant, anti-tumurogenic, and anti-inflammatory agent **(Sigrid, 2011 and Gupta *et al.*, 2012)**.

Ginger (*Zingiber officinale*, Roscoe Zingiberaceae) is one of the most widely consumed spices for the flavoring of food worldwide **(Li *et al*., 2012)**. Ginger rhizome has been used in traditional herbal medicine. Ginger has enormous health-promoting potential effects in number of ailments including degenerative disorders (arthritis and rheumatism), digestive health (indigestion and constipation), cardiovascular disorders (atherosclerosis and hypertension), diabetes mellitus and cancer. Also it has anti-inflammatory properties, which are beneficial in controlling the process of aging. Moreover, it has antimicrobial potential, which can help in treating infectious diseases and helminthiasis **(Jiang *et al*., 2006, White, 2007, Ali *et al*., 2008 and Nicoll and Henein, 2009)**. The health-promoting perspective of ginger is often attributed to its rich phytochemistry **(Park and Pizzuto, 2002 and Shukla and Singh, 2007)**. Therefore, the present study was conducted to evaluate the effect of curcumin, ginger or their combination on blood glucose, insulin and blood lipid levels, as well as, malondialdehyde and antioxidative enzyme activities in streptozotocin-induced diabetic rats.

2. Material and Methods

Material:

Plant material:

Fresh ginger rhizomes were purchased from the local market in Jeddah, KSA. Curcumin powder (95% curcuminoids) was purchased from GNC, Pittsburgh, PA.

Chemicals and kits:

Streptozotocin, Zansor (STZ) was obtained from Sigma-Aldrich (St. Louis, MO) Chemical Co. Citric acid, trisodiumcitratedehydrate, glucose solution (5%) were purchased from Pharmaceutical Solutions Industry Ltd, Jeddah. Enzymatic glucose, total cholesterol, triglyceride and high density lipoprotein cholesterol kits were obtained from Human Gesellschaft for Biochemical, Germany. ALPCO immunoassay insulin ELISA kits, Cayman's kits for assays of thiobarbituric acid reactive substances (TBARS), catalase (CAT) and superoxide dismutase (SOD) were purchased from Cayman Chemical Company, Ann Arbor, MI, USA.

Experimental animals:

Male Wister albino rats (n=35 rats) weighing about (180-195 g) were obtained from the animal experimental unit of King Fahd Center for Medical Research, King Abdulaziz University. All animals were allowed to one week to acclimatize in animal housing conditions before being used for the study. The rats were housed in standard laboratory conditions at a temperature of (22 ± 3 °C), relative humidity (50-55%) and a 12 h light/dark cycle (2 rats / cage). All animals fed standard nutritionally balanced diet according to **(Reeves, 1997)** and drinking water *ad libitum*.

Methods:

Preparation of ginger powder:

Fresh ginger rhizomes 5 kg were washed and cut into small thin pieces, then lyophilized for 54 hrs, it yield 13.6% (w/w) of the fresh, the obtained powder were stored at -20ºC until further use according to **(Rai *et al*., 2010)**. Lyophilization was conducted by using Freeze-Dryer Lyophilizer Millorock Bench-Top Freeze Dryer, Germany.

Induction of diabetes mellitus:

Diabetes was induced by a single intraperitonial (i.p.) injection of freshly prepared STZ (at a dose level of 65 mg/kg of body weight dissolved in 0.02 ml of 0.05 M citrate buffer pH 4.5) according to **(Nafiu *et al*., 2011)**. 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 adjusted exactly at 4.5 by the use of pH meter according to **(Dawson *et al*., 1986).** After i.p. injection, the animals allowed to drink 5 % glucose solution overnight to overcome the death from hypoglycemia shock. Seventy-two hrs later, the blood samples obtained from orbital plexus vein of each injected rat by a fine capillary glass tube and the blood glucose concentration was determined to confirm induction of diabetes, the non-diabetic rats excluded from the study, and diabetes established with non-fasting blood glucose levels of ≥ 300 mg/dl.

Experimental design:

After the adaption period, animals divided into two main groups, as follows:

First group: Negative control group (non-diabetic), rats (n=7) received a single i.p. injection with 0.2 ml of 0.05 M citrate buffer pH: 4.5, and fed standard diet.

Second group: diabetic rats (n=28), which divided equally to four subgroups as follows: **subgroup (1): diabetic untreated rats** (positive control group),

animals fed standard diet, **subgroup (2): diabetic rats treated with curcumin**; animals fed on diet containing curcumin powder (0.5 g / 100 g diet) according to **(Manjunatha and Srinivasan, 2007), subgroup (3): diabetic rats treated with ginger**; animals fed on diet containing ginger powder (3g/100 g diet) according to **(Saraswat *et al*., 2010)** and **subgroup (4): diabetic rats treated with (curcumin + ginger)**; animals fed on diet containing curcumin and ginger powder (0.5% and 3%, respectively). During the experimental period food intake (FI) was recorded every second day per each group, and the animals were weighted twice weekly in all groups. The biological values of different diets were assessed by the determination of body weight gain% (BWG %) and feed efficiency ratio (FER) according to the method of **Chapman *et al*. (1959).**

Biochemical analysis:

At the end of the experimental period (8 weeks), rats were fasted overnight before scarification. Blood samples withdrawn by heparinized capillary tube from the retro orbital plexu of each rat under anesthesia with diethyl ether, then centrifuged at 3000 rpm for 15 min to separate serum,which stored at -20° C until biochemical analysis. Immediately after blood sampling, animals sacrificed and the liver and pancreas of each animal dissected out, and then fixed in 10% formalin for histopathological studies. Collected serum was used for determination of glucoseby enzymatic GOD / POD kits according to **(Trinder, 1969),** Insulin by enzyme linked immunosorbent assay ELISA method as described by **Clark and Hales (1994)**.

Enzymatic colorimetric kits used for determination of total cholesterol, triglyceride**,** high-density lipoprotein cholesterol (HDL-C) as described by **(Roeschlau*et al*., 1974, Fossati and Prenape, 1982 and Lopes-Virella *et al*., 1977**, respectively**)**, while low-density lipoprotein-cholesterol (LDL-C) and very low-density lipoprotein-cholesterol (VLDL-C) were calculated according to **(Friedwald *et al*., 1972).** Malondialdehyde (MDA) as a measure of lipid peroxidation was detertmined by Cayman's Thiobarbituric Acid Reactive Substances TBARS assay kits according to **(Yoshioka *et al*., 1979)**. Cayman's assay kits were used for determination of superoxide dismutase (SOD)and catalase (CAT) activities by the methods of **(Wheeler *et al*., 1990 and Sinha, 1972).**

Histopathological examination:

Specimens from the liver and pancreas were 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 3-5 µm 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. they were allowed to cool and stained with Hematoxylen and Eosin according to **(Bancroft and Cook, 1998)**, and examined microscopically.

Statistical analysis:

Results were expressed as a (mean ±SD). Data were analyzed statistically by analysis of variance, for statistical significance using L.S.D. test, one way ANOVA, post hoc multiple comparisons according to **Snedecor and Cochron (1989)**. DELL computer with a software system SPSS version 20 was used for these calculations.

3. Results:

Table (1) showed the effect of curcumin, ginger or their mixture on biological evaluation in STZ-diabetic rats. The results reported that diabetic group showed very highly significant differences ($p < 0.001$) in final body weight, BWG%, DFI and FER as compared with control (-ve) group.All diabetic treated groups demonstrated very highly significant differences in all biological parameters ($p < 0.001$) when compared with control (-ve) and untreated diabetic group, there were non-significant differences in between the three treated groups.

Table (2) showed the effect of curcumin, ginger or their mixture on serum glucose concentration and insulin level in STZ-diabetic rats. In diabetic rats there were a very highly significant ($p<0.001$) elevation in glucose concentration accompanied with a very highly significant ($p< 0.001$) reduction in insulin level as compared with control (-ve) group, with percentage (273.93% and -58.46%, respectively) as percent change from control group. Administration of curcumin, ginger or their mixture to diabetic rats showed remarkably ameliorated the elevation in glucose concentration and the reduction in insulin level, there were very highly significant ($p<0.001$) improvement in glucose concentration and insulin level as compared with diabetic untreated group. The results also demonstrated that serum glucose concentration and insulin level in diabetic group treated with curcumin recorded significant differences ($p< 0.05$) when compared with ginger treated group. Treatment with curcumin and ginger mixture showed non-significant differences when compared with curcumin treated group, while demonstrated highly significant differences ($p< 0.01$) with respect to ginger treated group in both glucose concentration and insulin levels.

Table (3) and showed the effect of curcumin, ginger or their mixture on serum lipid profile levels in STZ-diabetic male rats. In diabetic group there were

very highly significant (p<0.001) elevation in TC, TG, LDL-C VLDL-C, accompanied with a very highly significant (p < 0.001) reduction in HDL-C level as compared with control (-ve) group, with percentage (92.74%, 79.36%, 313.11%, 79.36% and -41.49%, respectively) as percent change from control group.

Diabetic groups treated with curcumin, ginger or their mixture showed improvement in lipid profile levels, but curcumin group and ginger group showed very highly significant differences (p < 0.001) in lipid profile levels comparing with control group, while their mixture group recorded that TC and HDL-C values showed highly significant differences (p < 0.01) and TG, LDL-C and VLDL-C recorded very highly significant differences (p< 0.001) comparing with control group (-ve).

Administration of curcumin, ginger or their mixture to diabetic rats showed remarkably amelioration in the elevation in TC, TG, LDL-C, VLDL-C and the reduction in HDL-C levels, there were very highly significant (p< 0.001) improvement for lipid profile levels as compared with diabetic untreated group. Also, the data demonstrated that serum TC, TG, VLDL-C levels recorded highly significant differences (p < 0.01), but LDL-C level showed a very highly significant difference (p < 0.001) and HDL-C level recorded a significant difference (p< 0.05) in diabetic group treated with curcumin when compared with ginger treated group. Treatment with curcumin and ginger mixture showed non-significant difference when compared with curcumin treated group, while demonstrated very highly significant differences (p < 0.001) with respect to ginger treated group in lipid profile levels.

Table1. Biological evauationin diabetic rats treated with curcumin, ginger or their mixture after 8 weeks of treatment.

Experimental groups	Initial body weight (g)	Final body weight (g)	BWG %	DFI (g/rat/day)	FER
Control (- ve)	188.96 ± 4.04	354.59 ± 8.97	87.65 ± 4.96	19.41± 1.71	0.152 ± 0.012
Diabetic (+ ve)	189.16 ± 2.84	a # 250.94 ± 10.85	a # 32.66 ± 2.68	a # 27.11±1.69	a # 0.041 ± 0.003
Diabetic + Curcumin	186.26 ± 2.61	a # b # 307.75± 7.02	a # b # 65.23 ± 3.78	a # b # 22.79 ± 1.01	a # b # 0.095 ± 0.009
Diabetic + Ginger	188.16 ± 3.16	a # b # 302.00 ± 11.02	a # b # 60.50 ± 3.73	a # b # 23.60 ± 0.83	a # b # 0.086 ± 0.008
Diabetic + (Curcumin & Ginger)	188.67 ± 3.47	a # b # 310.89 ± 8.57	a # b # 64.78 ± 2.67	a # b # 23.39 ± 0.97	a # b # 0.093 ± 0.007

BWG%: Body weight gain, **DFI:** Daily food intake and **FER**: Feed efficiency ratio.
Each value represents the mean of 7 rats ± SD.
[a]: Significant difference between control and diabetic groups.
[b]: Significant difference between diabetic untreated and diabetic treated groups.
[c]: Significant difference between diabetic treated with curcumin and diabetic treated with ginger.
[d]: Significant difference between diabetic treated with curcumin or ginger and diabetic treated their mixture.
($^{*}p$< 0.05, ^{+}p< 0.01 and $^{\#}p$< 0.001)

Table 2. Serum glucose (mg/dl) and insulin (µU/ml) levels in diabetic rats treated with curcumin, ginger or their mixture after 8 weeks of treatment.

Experimental groups	Glucose (mg/dl)	Insulin (µU/ml)
Control (- ve)	99.73±7.13	68.13±3.70
Diabetic (+ ve)	a # 372.92±18.03	a # 28.30±2.11
Diabetic + Curcumin	a # b # 193.27±3.79	a # b 47.61±3.16
Diabetic + Ginger	a # b # c *d + 204.54±8.69	a # b #c * d + 43.56±2.55
Diabetic + (Curcumin& Ginger)	a # b # 186.96±7.27	a # b # 50.16±2.16

Each value represents the mean of 7 rats ± SD.
[a]: Significant difference between control and diabetic groups.
[b]: Significant difference between diabetic untreated and diabetic treated groups.
[c]: Significant difference between diabetic treated with curcumin and diabetic treated with ginger.
[d]: Significant difference between diabetic treated with curcumin or ginger and diabetic treated their mixture.
($^{*}p$< 0.05, ^{+}p< 0.01 and $^{\#}p$< 0.001)

Table 3. Serum lipid profile levels (mg/dl) in diabetic rats treated with curcumin, ginger or their mixture after 8 weeks of treatment.

Experimental groups	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
Control (- ve)	90.16 ± 5.95	80.33± 5.19	45.49 ± 2.68	28.60 ± 1.50	16.07 ± 1.04
Diabetic (+ ve)	a # 173.77 ± 4.23	a # 144.08 ±7.52	a # 26.80 ± 1.89	a # 118.15 ± 3.25	a # 28.82 ± 1.50
Diabetic + Curcumin	a # b # 108.80 ± 8.25	a # b # 100.49 ± 7.25	a # b # 38.23 ± 2.88	a # b # d # 50.47 ± 1.10	a # b # 20.10 ± 1.45
Diabetic + Ginger	a # b # c + d # 118.75± 6.43	a # b # c+ d# 112.16 ±7.38	a # b # c*d# 35.33 ± 2.59	a # b # c # d # 60.99 ± 3.89	a # b # c + d# 22.43 ± 1.48
Diabetic + (Curcumin&Ginger)	a + b # 101.80 ±7.26	a # b # 93.22 ± 5.87	a + b # 40.91 ±2.38	a # b # 42.25 ± 2.59	a # b # 18.64 ± 1.17

TC: Total cholesterol, **TG:** Triglycerides, **HDL-C:** Highdensity lipoprotein cholesterol, **LDL-C:** Low density lipoprotein cholesterol and **VLDL-C:** Very low density lipoproteincholesterol.
Each value represents the mean of 7 rats ± SD.
[a]: Significant difference between control and diabetic groups.
[b]: Significant difference between diabetic untreated and diabetic treated groups.
[c]: Significant difference between diabetic treated with curcumin and diabetic treated with ginger.
[d]: Significant difference between diabetic treated with curcumin or ginger and diabetic treated their mixture.
($^{*}p< 0.05$, $^{+}p< 0.01$ and $^{\#}p< 0.001$)

Table (4) showed the effect of curcumin, ginger or their mixture on serum MDA level and some antioxidant enzymes activity SOD, CAT in STZ-diabetic rats. In diabetic group there were very highly significant ($p< 0.001$) elevation in MDA levels accompanied with very highly significant ($p< 0.001$) reduction in SOD and CAT enzymes activity as compared with control (-ve) group, with percentage (289.42%, -58.87% and -60.34, respectively) as percent change from control group. Diabetic groups treated with curcumin, ginger or their mixture showed improvement in MDA level, SOD and CAT enzymes activity, but there values showed significant differences ($p < 0.001$) with respect to control negative group. Administration of curcumin, ginger or their mixture to diabetic rats showed remarkably amelioration the elevation of MDA level and the reduction in SOD, CAT enzymes activity. There were very highly significant ($p < 0.001$) improvement in MDA level and SOD, CAT enzyme activities as compare with diabetic untreated group. The data also demonstrated that serum MDA level and SOD, CAT enzymes activity in diabetic group treated with curcumin recorded significant differences ($p < 0.05$) when compared with ginger treated group. Treatment with curcumin and ginger mixture showed non-significant difference when compared with curcumin treated group, while demonstrated highly significant improvement ($p< 0.01$) as compared with ginger treated group in MDA level, SOD and CAT enzyme activities.

Table 4. Serum malondialdehyd (MDA) (nmol/ml) and some antioxidant enzymes activity in diabetic rats treated with curcumin, ginger or their mixture after 8 weeks of treatment.

Experimental groups	MDA (nmol/ml)	SOD (U/ml)	CAT (nmol/ml)
Control (- ve)	10.21±0.88	0.231±0.021	32.53±1.87
Diabetic (+ve)	a # 39.76± 1.49	a # 0.095±0.008	a # 12.90±1.10
Diabetic + Curcumin	a # b # 20.80±1.76	a # b # 0.194±0.016	a # b # 23.63±2.01
Diabetic + Ginger	a # b # c* d+ 22.67±1.64	a # b # c* d+ 0.179±0.005	a # b # c* d+ 21.70±1.51
Diabetic + (Curcumin& Ginger)	a # b # 19.85±1.64	a # b # 0.201±0.010	a # b # 24.83±1.10

MDA: Malondialdehyde, **SOD**: Superoxide dismutases and **CAT**: Catalase.
Each value represents the mean of 7 rats ± SD.
[a]: Significant difference between control and diabetic groups.
[b]: Significant difference between diabetic untreated and diabetic treated groups.
[c]: Significant difference between diabetic treated with curcumin and diabetic treated with ginger.
[d]: Significant difference between diabetic treated with curcumin or ginger and diabetic treated their mixture.
($^{*}p< 0.05$, $^{+}p< 0.01$ and $^{\#}p< 0.001$).

Histopathological investigation:

Microscopically, liver from control rat group showed the normal histological structure of hepatic lobule and portal vein without alterations (Fig. 1), the figure showed that hepatocytes were arranged in the form of branching cords, separated by blood sinusoids and radiated from the central vein, the hepatocytes appeared polyhedral in shape and containing basophilic granules and central rounded vesicular nuclei. Liver tissues in diabetic rats showing activation of kupffer cells, sinusoidal leucocytosis, and apoptosis of hepatocytes (Fig. 2), marked dilatation and congestion of central vein with necrosis of sporadic hepatocytes (Fig. 3), as well as congestion of central vein and focal hepatic necrosis replaced by mononuclear infiltration (Fig. 4).

Liver tissues in diabetic rat treated with curcumin showed apparent normal histological structure (Fig. 5), except slight kupffer cells activation (Fig. 6). While, liver tissues in diabetic rats treated with ginger showed kupffer cells activation (Fig. 7), slight congestion of hepatic sinusoids with binucleation of hepatocytes (Fig.8). Meanwhile, in diabetic rats treated with both curcumin and ginger examined liver sections showed kupffer cells activation (Fig. 9), and other sections of diabetic treated rats revealed no histopathological alteration (Fig. 10).

The histological appearance of the pancreatic islet cells of normal control rats showed no histopathological changes (Fig. 11). Microscopic examination of the pancreatic sections of diabetic untreated group revealed necrosis of β-cells of islets of langerhan's (Fig. 12), and atrophy of islets of langerhan's (Fig. 13), as well as cystic dilatation of pancreatic duct and congestion of pancreatic blood vessel (Fig. 14).

However, examined sections of diabetic rats treated with curcumin showed no histopathological changes (Fig. 15), and other sections revealed slight congestion of blood vessel with normal pancreatic acini and normal β- cells of islet of langerhan's (Fig. 16). While some examined sections of diabetic rats treated with ginger showed no histopathological changes (Fig. 17), and other sections revealed slight vacuolation of sporadic β-cells of islets of langerhan's (Fig. 18). On the other hand, examined pancreatic islet tissues in diabetic rats treated with both curcumin and ginger revealed no histological changes (Fig. 19), except few leucocytic cells infiltration in some sections (Fig. 20).

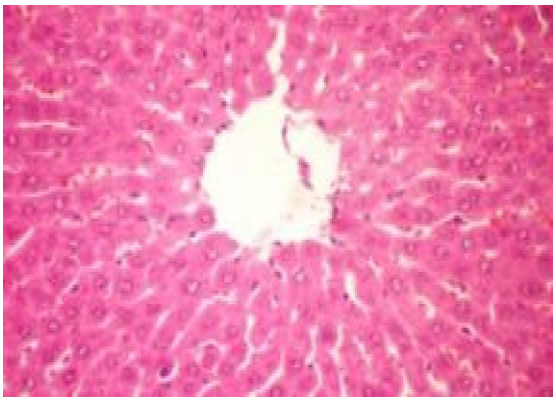

Fig 1. Liver of control negative (-ve) showing no histopathological alteration. (H&E stain x 400)

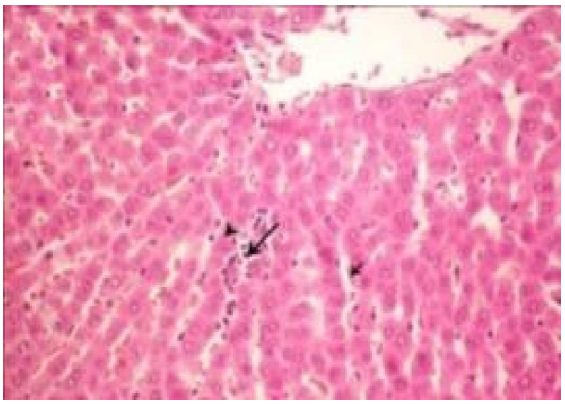

Fig 2. Liver of diabetic rats showing activation of kupffer cells (small arrow), sinusoidal leucocytosis (large arrow), and apoptosis of hepatocytes (arrow head). (H&E stain x 400)

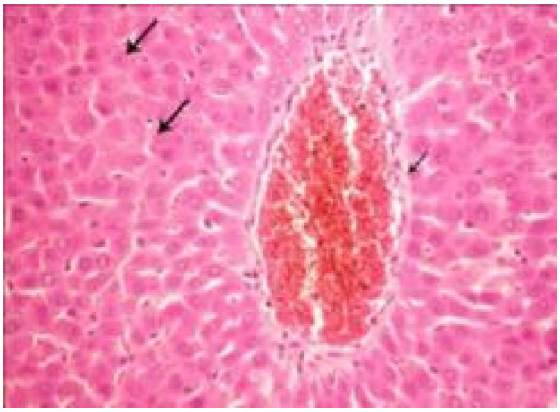

Fig 3. Liver of diabetic rats showing marked dilatation and congestion of central vein (small arrow), with necrosis of sporadic hepatocytes (large arrow). (H&E stain x 400)

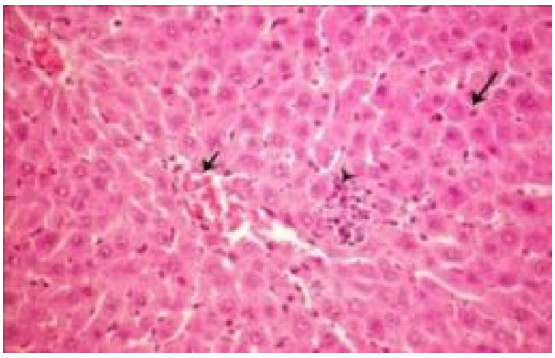

Fig 4. Liver of diabetic rats showing congestion of central vein (small arow), kupffer cells activation (large arrow), necrosis of sporadic hepatocytes and focal hepatic necrosis replaced by mononuclear infiltration (arrow head). (H&E stain x 400)

Fig 5. Liver of diabetic rats treated with curcumin showing no histopathological changes. (H&E stain x 400)

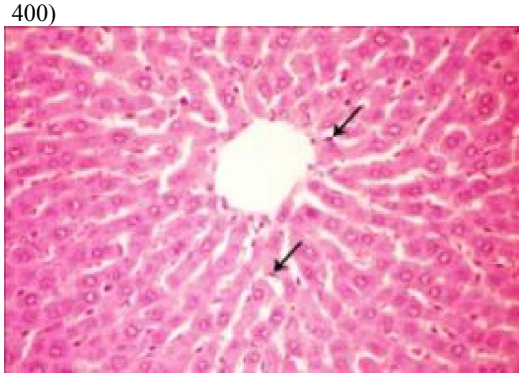

Fig 6. Liver of diabetic rats treated with curcumin showing kupffer cells activation. (H&E stain x 400)

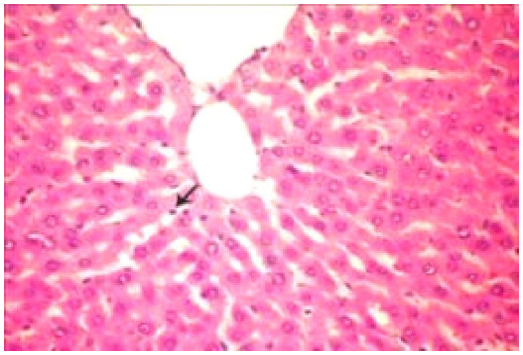

Fig 7. Liver of diabetic rats treated with ginger showing kupffer cells activation. (H&E stain x 400)

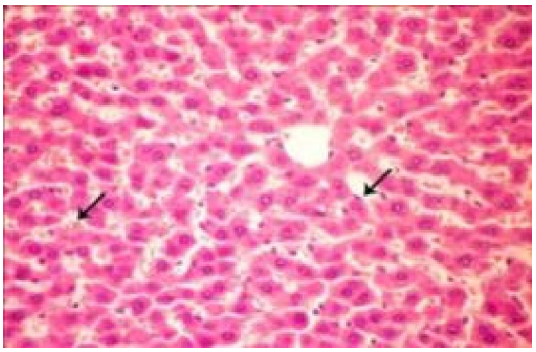

Fig 8. Liver of diabetic rats treated with ginger showing kupffer cells activation and slight congestion of hepatic sinusoids. (H&E stain x 400)

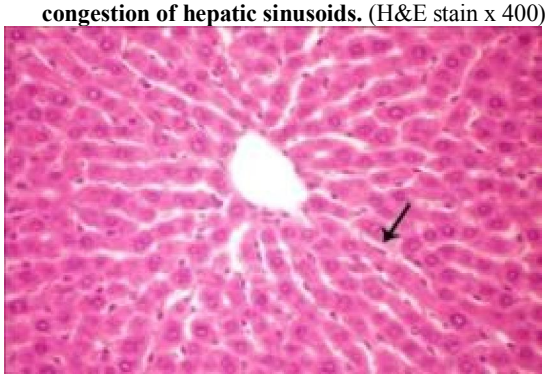

Fig 9. Liver of diabetic rats treated with curcumin and ginger showing mixturekupffer cells activation (large arrow). (H&E stain x 400)

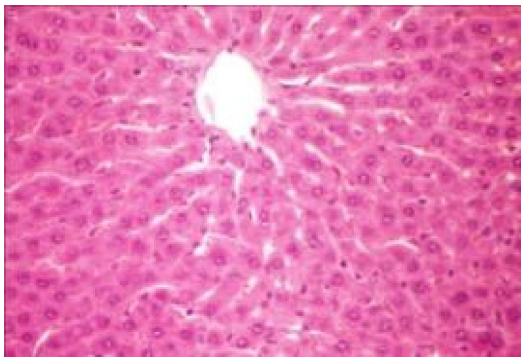

Fig 10. Liver of diabetic rats treated with curcumin and ginger mixture showing no histopathological alteration. (H&E stain x 400)

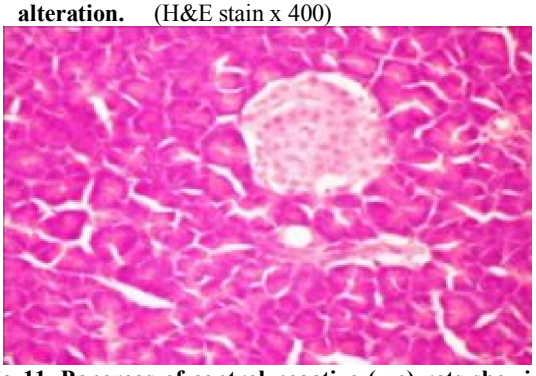

Fig 11. Pancreas of control negative (-ve) rats showing no histopathological change. (H&E stain x 400)

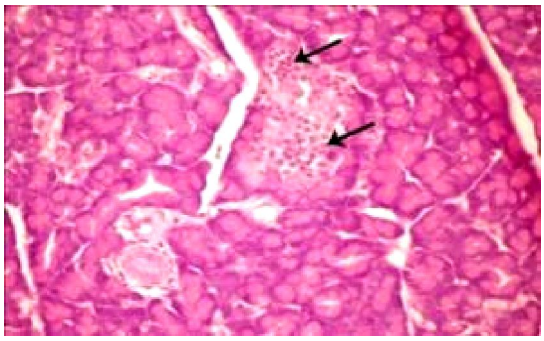

Fig 12. Pancreas of diabetic rats showing necrosis of *β*-cells of islets of langerhans (large arrow). (H & E stain x 400)

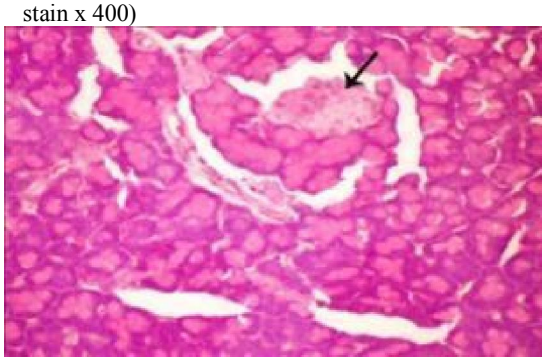

Fig 13. Pancreas of diabetic rats showing necrosis and atrophy of islets of langerhans (large arrow). (H & E stain x 400)

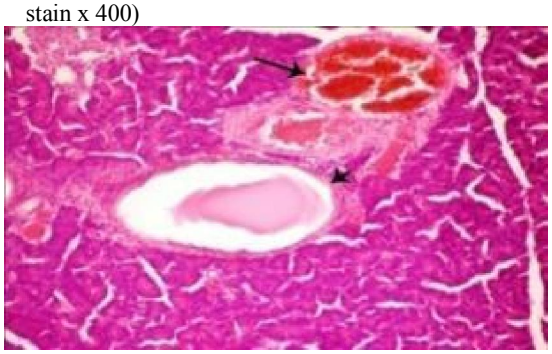

Fig 14. Pancreas of diabetic rats showing cystic dilatation of pancreatic duct (small arrow) and congestion of pancreatic blood vessel (large arrow). (H & E stain X 400)

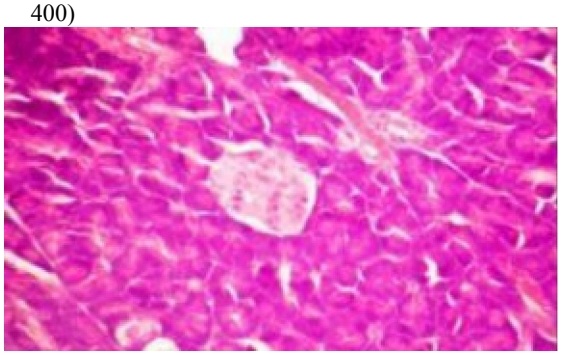

Fig 15. Pancreas of diabetic rats treated with curcumin showing no histopathological changes. (H&E stain X 400)

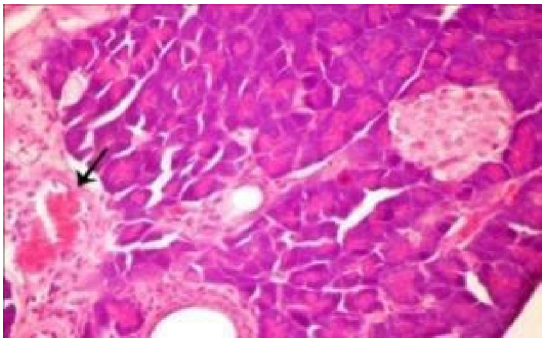

Fig 16. Pancreas of diabetic rats treated with curcumin showing slight congestion of blood vessel. Note normal pancreatic acini and normal *β* -cells of islet of langerhan's (large arrow). (H&E stain x 400)

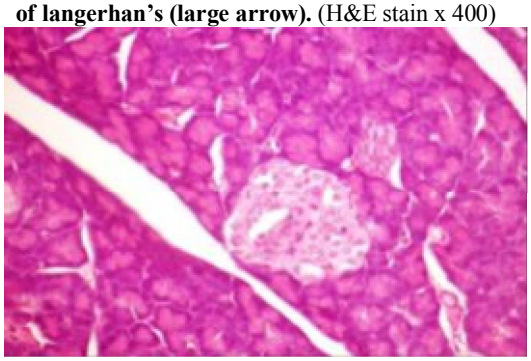

Fig 17. Pancreas of diabetic rats treated with ginger showing no histopathological changes. (H&E stainx 400)

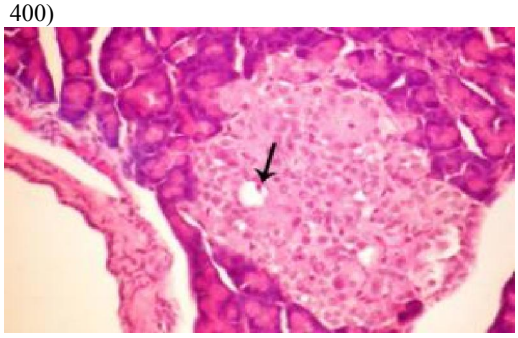

Fig 18. Pancreas of diabetic rats treated with ginger showing slight vacuolation of sporadic *β*- cells of islets of langerhan's (large arrow). (H&E stain x 400)

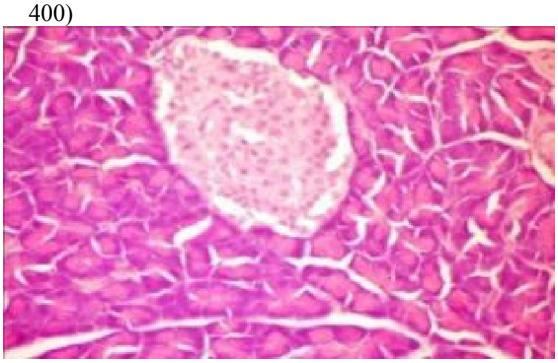

Fig19. Pancreas of diabetic rats treated with both curcumin and ginger showing no histological changes. (H&E stainx 400)

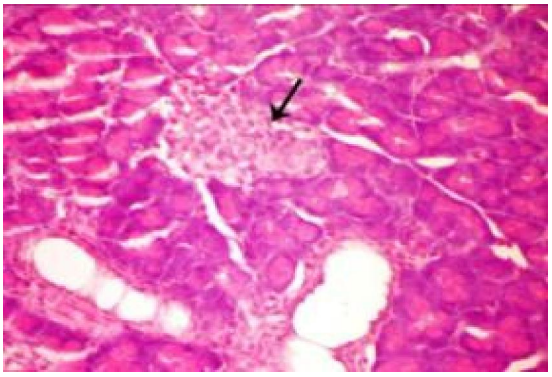

Fig 20. Pancreas of diabetic rats treated with both curcumin and ginger showing few leucocytic cells infiltration (large arrow). (H&E stain x 400)

4. Discussion

Diabetes is one of the leading causes of morbidity and mortality in the world **(Abo *et al*., 2008)**. Recent scientific investigation and clinical studies had confirmed the efficacy of some medicinal plants and herbal preparations in the improvement of normal glucose homeostasis **(Rafiq *et al*., 2009)**.The herbal drugs have been prescribed widely because of their effectiveness, fewer side effects and relatively low cost **(Venkatesh *et al*., 2003)**. Several studies revealed the benefits of medical plants like curcumin or ginger which showed hypoglycemic effect and also delay in the development of DM **(Sawatpanich *et al*., 2010 and El-Moselhy *et al*., 2011).**

In the current study, STZ- induced diabetic rats showed very highly significantly decrease in the final body weight which also accompanied with increased on DFIand accordingly decreased in BWG%, as well as FER when compared with control group (-ve). Diabetic rats treated with curcumin, ginger or their mixture showed significantly improvement in all biological evaluation, where there were ameliorated reduction in final body weight, BWG%, FER and increased in DFI when compared with untreated diabetic rats. The obtained results were in agreement with **(Gupta *et al*., 2012 and Kota *et al*., 2012)** who found that there were an association between hyperglycemia and decreased body weight of diabetic animals, DM induced reduction in body weight, and the body's inability to store or use glucose causes hunger and weight loss.

Previous studies have reported significantly improvement in body weight and feed intake in diabetic rats treated with curcumin compared with diabetic untreated rats **(Soetikno *et al*., 2012 and Hussein and Abd El-Maksoud, 2013)**, the effect of curcumin treatment may be explained by its ability to inhibit angiogenesis in adipose tissue and decrease differentiation of preadipocytes**(Ejaz *et al.,* 2009)**. In addition, **Nasri *et al.* (2012)** and **Waer and Helmy (2012)** suggested that the gradual increase in the body weight was observed in the STZ diabetic rats treated with curcumin may be due to the retained levels of glucose and insulin because of the antioxidant effects of curcumin. Moreover, curcumin control the leptin signaling in diabetic mice by reducing the phosphorylation levels of the leptin receptor and the induction of adiponectin, which improves body weight and related metabolic disease **(Tang *et al.,* 2009 and Weisberg *et al.,* 2009).**

In addition, the present results in agree with the previous studies, which found that STZ diabetic rats treated with ginger induced significant improvement in body weight and feed intake when compared with STZ diabetic untreated rats **(Madkor *et al.,* 2010 and Al-Aassaf, 2012)**, this is probably due to ginger contains over 20 phenolic compounds, which have been reported to display diverse biological activities such as antidiabetic, hypoglycemic and antioxidant **(White, 2007, Ali *et al.,* 2008, Islam and Choi, 2008 and Saraswat *et al.,* 2010).**

Diabetes syndromes characterized by increased blood glucose, altered lipids, carbohydrate and an increased risk of diabetic complications and oxidative stress **(Davis, 2006 and Al-Assaf, 2012)**. In the present study, diabetic rats showed very highly significantly increase in serum glucose concentration accompanied by a very highly significantly decrease in serum insulin level when compared with control group (-ve). Diabetic treated groups showed significantly improvement in glucose and insulin levels, where there were ameliorated in diabetes syndromes, the mixture of curcumin and ginger was more effective, it exhibits remarkable glycemic control in diabetic group.

A similar result was reported by **Yaghmoor and Khoja (2010)** who reported that STZ-induced diabetic rats had a negative effect in glucose concentration and insulin levels.It may be explained by STZinduced destruction of β-cells of islets of Langerhans and causing degranulation and reduction of insulin secretion as proposed by **Zhang and Tan (2002)**.This result agreement with **Seo *et al.* (2008)** who revealed that curcumin improved homeostasis model assessment of insulin resistance and glucose tolerance, and elevated the plasma insulin level in mice. Moreover, **Gupta *et al*. (2012)** showed that curcumin revealed an anti-hyperglycemic effect and improved insulin sensitivity. On the other hand, **Saraswat *et al*. (2010)** reported that dietary ginger (3%) in the diet for 8 weeks in STZ- diabetic rat induced decrease in blood glucose levels while insulin was unaffected by ginger.

The hypoglycemic effect of curcumin may be attributed to curcumin induces electrical activity in

rat pancreatic β-cells by activating volume-regulated anion channel, this effect led to depolarization of cell membrane potential, generation of electrical activity, and enhanced insulin release **(Best *et al*., 2007)**. Furthermore, curcumin protected islets against STZ-induced oxidative stress and corresponding islet damage and dysfunction by scavenging free radicals **(Pari and Murugan, 2007)**. The hypoglycemic effect of ginger in diabetes may be attributed to the bioactive and pharmacological compounds of ginger they may help in suppressing the free radicals **(Ramudu *et al.*, 2011 a). Chakraborty *et al.* (2012)** revealed that ginger has been shown to modulate insulin release in rat pancreatic β-cells, thus enhanced plasma insulin levels in conjunction with lowered blood glucose, this may be due to 6-gingerol, which is active component in ginger, which showed a protective effect on pancreatic β-cells and restored the plasma insulin level.

In the present study, STZ- induced diabetic rats showed very highly significantly increase in serum TC, TG, LDL-C and VLDL-C levels accompanied by a very highly significantly decrease in serum HDL-C level when compared with control group (-ve). A similar result reported that STZ-induced diabetic rat in a dose of 30 mg/kg had a negative effect in lipid profile levels when compared with normal rats (**Kota *et al*., 2012**). Insulin affects many sites of mammalian lipid metabolism, it stimulates synthesis of fatty acids in liver, adipose tissues and in the intestine, insulin deficiency has also been reported to increase the cholesterol synthesis and increase the activity of lipoprotein lipase in white adipose **(Suryawanshi *et al*., 2006)**.

Treatment of diabetic rats with curcumin, ginger or their mixture exhibited remarkably ameliorated effects in all lipid profile parameters, and their mixture was more effective. These results are conforming by**Rai *et al.* (2010)** who revealed that curcumin significantly lower TC, TG, LDL-C, VLDL-C levels and improved HDL-C level as compared with diabetic untreated rats. Moreover, **Madkor *et al.* (2010) and Al-Assaf (2012)** who reported improvement effect of ginger in TC and TG levels in STZ-induced diabetic rats when compared with untreated diabetic rats.

Curcumin is effective in inhibiting lipid synthesis, storage, and stimulating fatty acids degradation,these effects mediated by regulating the activities of several key enzymes and the expression of transcription factors that regulate lipid metabolism **(Alappat and Awad, 2010)**. Curcuminhypolipidemic activities could be mediated through cholesterol catabolism by the stimulation of hepatic cholesterol-7α-hydroxylase activity, and this step converts cholesterol to bile acid, which is important pathway in the degradation of cholesterol **(Wongeakin *et al.*, 2009)**, or might be due to its alkaloid components of curcumin **(Halim and Hussain, 2002)**.The hypolipidemic activities of ginger may be explained by **Han *et al*. (2005)** who found that *Z. officinale* increased the faecal excretion of cholesterol, suggesting that ginger may block absorption of cholesterol in the gut. Moreover, **Nammi *et al*. (2009)** and **Ramudu *et al*. (2011a)** mentioned that the hypocholesterolemic effect of ginger may be attributed to inhibition of cellular cholesterol synthesis, results in augmenting the LDL receptor activity, leading to the elimination of LDL from plasma thus modifying lipoprotein metabolism.

In the present study, STZ- induced diabetic rats showed very highly significant increase in MDA levels accompanied by a very highly significant decrease in serum SOD and CAT enzymes activities when compared with control group (-ve). While treatment of diabetic rats with curcumin, ginger or their mixture exhibited remarkably ameliorated effects, there were very highly significant improvement in MDA, SOD and CAT enzyme activities levels when compared with untreated diabetic group, and the mixture of both curcumin and ginger was more effective than curcumin or ginger alone, it exhibits remarkable oxidative stress control in diabetic group.A similar results was reported by **(Suryanarayana *et al.*, 2007 and Morakinyo *et al*., 2011)** who reported that STZ- diabetic rats had a negative effect in MDA level and SOD and CAT enzyme activities, this may be attributed to increase in reactive oxygen species (ROS) which is involved in the development and progression of DM.

These results are conforming to the results of **(Hussein and Abu-Zinadah, 2010 and Hussein and Abd El-Maksoud, 2013)** whose reported diabetic rats treated with curcumin revealed an improvement in TBARS and antioxidant enzyme activities compared with untreated group. This may be attributed tocurcumin normalizes erythrocyte and hepatic antioxidant enzyme activities (SOD, CAT and GP_X) **Seo *et al.* (2008)**.

Several studies have shown that consumption of nutrient-rich antioxidant such as curcumin and ginger decreased diabetic complications and improves the antioxidant system **(Shanmugam *et al.*, 2011 and Hussein and Abd El-Maksoud, 2013)**. Curcumin activity as an antioxidant and free-radical scavenger has been demonstrated from several studies, it prevents the oxidation of hemoglobin and inhibits lipid peroxidation, this activity can arise either from the phenolic hydroxyl group or the methylene group of the β-diketone (heptadiene-dione) moiety **(Chen *et al*., 2006, Anand *et al*., 2008 and Gupta *et al*., 2012).** Further, ginger has an

ability to increase the intracellular activities of SOD, CAT and GSH enzymes and has synergistically combats oxidative stress by scavenging free radicals and/or augmenting endogenous antioxidant activities **(Shobana and Akhilender, 2000)**. This may be due to ginger contain many antioxidant compounds that may modulate the antioxidant enzymes in diabetic rats, especially gingerol and hyxahydrocurcumin which are responsible for significant inhibition of lipid peroxidation. Preliminary clinical trials showed that the antioxidant effect of ginger was potent when it was used in combination with some herbs, significantly physiological improvement accompanied with reduction of serum triglyceride and cholesterol in diabetic and hyperlipidemic patients have been recorded **(Kamal and Aleem, 2009 and Ugwuja *et al.*, 2010).**

In the current study, the results showed that, liver tissues in STZ-induced diabetic rats showing apoptosis of hepatocytes, marked dilatation and congestion of central vein with necrosis of sporadic hepatocytes, as well as congestion of central vein and focal hepatic necrosis replaced by mononuclear infiltration when compared with control (-ve) group. A similar result reported by **Hussein and Abu-Zinadah (2010)** who reported that, liver sections of STZ diabetic rats showed massive fatty changes, necrosis and broad infiltration of lymphocytes. Moreover, **Hashemnia *et al.* (2012)** reported that liver sections of untreated diabetic rats showed degenerative changes in the hepatocytes represented by disorganization of the hepatic cords, congestion of the central veins with mild hepatocellular necrosis and the sinusoids were infiltrated by mild nonspecific inflammatory cells, and the hepatocytes showed pyknosis, karyorrehexis, chromatolysis and cytoplasmic vacuolization.The damage effect of STZ could be attributed to STZ stimulates H_2O_2 generation, which cause DNA fragmentation and increase oxidative stress in liver and pancreas cells **(Bolkent *et al.*, 2008 and Nirmala *et al.*, 2009).**

In the present study, liver tissues in diabetic rat treated with curcumin showed apparent normal histological structure, except slight kupffer cells activation, as well as liver tissues in diabetic rats treated with ginger showed kupffer cells activation, slight congestion of hepatic sinusoids with binucleation of hepatocytes. Meanwhile, in diabetic rats treated with both curcumin and ginger examined liver sections showed no histopathological alteration except kupffer cells activation in few sections.The present findings confirmed by **Murugan and Pari (2006)** who reported that curcumin improve the liver pathological changes and reduced the congestion and inflammation when compared with diabetic untreated rats. In addition, curcumin found to prevent liver lipid peroxidation in rats with STZ-induced diabetes and showed graduate restoration of hepatocytes and blood sinusoids, also most of the hepatocytes showed normal and regular pattern **(Soetikno *et al*., 2012 and Waer and Helmy, 2012)**. **Ramudu *et al.* (2011b)** reported that ginger protected the liver tissues from STZ-induced oxidative damage.The protective effect of curcumin may be attributed to, curcumin is an unique antioxidant, which contains a variety of functional groups **(Wright, 2002)**,and its ability to inhibit cyclooxygenase-2 (COX-2), lipoxygenase (LOX), and inducible nitric oxide synthase (iNOS) **(Venugopal and Adluri, 2007**), it prevented the activation of NF-$_K$B (nuclear factor kappa-light-chain-enhancer of activated B cells) in rats **(Nanji *et al.*, 2003)**, thus explained its ability to inhibit lipid peroxidation and liver injury.The hepatoprotective activity of ginger extract may be due to its direct radical scavenging activity **(Ajith *et al*., 2007).**

In the present study, it was reported that microscopic examination of the pancreatic sections of diabetic untreated group revealed necrosis and atrophy of β-cells of islets of Langerhan's, as well as cystic dilatation of pancreatic duct and congestion of pancreatic blood vessels. This results are in accordance with the findings of **Qadori (2011**) who reported that diabetic pancreatic tissue showed shrinkage of islets Langerhans in size, signs of necrosis of β-cells destruction and reduction in number of islets, and significant reduction was in β-cells diameter.This effect may be explained by STZ stimulates H_2O_2 generationin pancreatic β -cells which causes DNA fragmentation **(Kaneto *et al*., 1996)**. In addition, DM cause disturbs and imbalance between oxygen frees radicals (OFR_S) production and cellular defense mechanisms, this imbalance can result in cell dysfunction and destruction in pancreas tissues **(Waer and Helmy, 2012).**

In the present study, the examined sections of pancreatic of diabetic rats treated with curcumin showed no histopathological changes, and other sections revealed slight congestion of blood vessel with normal pancreatic acini and normal β- cells of islet of Langerhan's. Also some examined sections of diabetic rats treated with ginger showed no histopathological changes, while other sections revealed slight vacuolation of sporadic β-cells of islets of Langerhan's. On the other hand, examined pancreatic islet tissues in diabetic rats treated with both curcumin and ginger revealed no histological changes, except few leucocytic cells infiltration in some sections.

Clear evidence of pancreatic islets showed that curcumin induced electrical activity in rats pancreatic β-cells by activating the volume-regulated anion channel, this effect were accompanied by

potential depolarization of the cell membrane and enhanced insulin release **(Best *et al.*, 2007)**. Moreover, curcumin retarded islet ROS generation and inhibited apoptosis, indicating that it protects islets against STZ-induced oxidative stress by scavenging free radicals **(Meghana *et al.*, 2007 and Waer and Helmy, 2012)**.Ginger is a good source of antioxidant and therefore may be capable of preventing tissue damage by ROS, it can protect the liver and pancreas tissues from lipid peroxidation on STZ diabetic rats **(Usha and Saroja, 2000 and Bhandari *et al*., 2005).** The present results also agree with **Aggarwal (2010)** and **Chakraborty *et al*. (2012)** who revealed that ginger has been shown to modulate insulin release in rats pancreatic β-cells, the effect of ginger may be explained by 6-gingerol, which is active component in ginger, it showed a protective effect on pancreatic β-cells, inhibit and intervene cytodegeneration of pancreatic β-cells and hepatocytes and helped in scavenging the free radicals.

Conclusion

This study demonstrated that curcumin and ginger mixture possesses significantly reduction in hyperglycemic, hyperlipidemic and antioxidant effect in diabetic rats, as well as overcome most of the histopathology changes in liver and pancreas tissues, the majority of the cells tend to be normal. Therefore, it recommended that dietary curcumin and ginger or their mixture could be excellent adjuvant support in the therapy of DM and prevent its complications.

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10/6/2013