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Modulatory Effects (Anti-diabetic and Anti-oxidant) of Ginger, Garlic, and their Mix on Streptozotocin-Nicotinamide Induced Diabetic Rats

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Diabetes is a major challenge to healthcare systems all over the world. It is a metabolic disorder characterized by chronic hyperglycemia resulted from the disturbance of carbohydrate, fat and protein metabolisms due to the defects in insulin secretion, insulin action, or both. Oxidative stress caused by the effect of hyperglycemia result in many complications such as hepatopathy, nephropathy, retinopathy, neuropathy, and cardiovascular diseases. The present study was designed to clarify the anti-hyperglycemic and anti-oxidant effects of ginger, garlic, and their mix in comparison to the widely used hypoglycemic drug "metformin". For this purpose, 80 male Albino rats fed on standard laboratory diet were used and were divided randomly into 10 groups. The experiment continued for 42 days for studying clinico-pathological changes including hematological parameters, liver and kidney function tests, and some anti-oxidant enzymes activity. At the end of the experiment complete urine analysis was done and tissues of liver, kidney, and pancreas were collected for histopathological examination. Results of diabetic control group revealed presence of microcytic hypochromic anemia, neutrophilic leukocytosis, elevated HbA1c, hyperglycemia, liver and kidney functions impairment in association with reduction in anti-oxidant enzymes activity. Study results revealed the oral administration of ginger, garlic, and their mix improve the diabetic associated hematological, chemical, and histopathological changes when compared to that of diabetic control group. It clears that, ginger and garlic extracts had both anti-hyperglycemic and anti-oxidant effects on STZ-nicotinamide induced diabetic rats. Further studies are needed to determine its protective effects on the other diabetes complications.

Keywords: Diabetes mellitus, Anti-diabetic effect, Anti-oxidant effect, Hemato-biochemical studies, Ginger, Garlic, Metformin.

INTRODUCTION

Worldwide by the year 2025, the number of individuals suffering from diabetes is predicted to reach 325 million as a result of sedentary lifestyle, consumption of energy-rich diet, obesity, longer life span, etc. (Lefebvre, 2005). Diabetes is a metabolic disorder of multiple etiological agents, it is characterized by chronic hyperglycemia associated with disturbance in carbohydrate, fat, and protein metabolisms (Abeer, 2013). Diabetes mellitus has two types; type 1 and type 2. In type 1 diabetes mellitus or insulin dependent diabetes, pancreatic β-cells are progressively destroyed, and secrete little or no insulin. While, type 2 diabetes mellitus or non-insulin dependent diabetes is a heterogeneous disorder of insulin resistance, and pancreatic β-cell dysfunction (Lefebvre, 2005). The major causes of morbidity and mortality associated

with diabetic patients are resulted from clusters of pathological conditions including cardiovascular complications due to the metabolic syndrome, hepatic steatosis, obesity, dyslipidemia, and insulin resistance (Knuiman et al., 2009).

For experimental induction of type 1 diabetes in rats, streptozotocin (STZ) is widely used, as it causes severe degeneration of pancreatic β-cells (Merzouk et al., 2000). Recently, STZ can be used for experimental induction of type 2 diabetes in rats by the administration of a suitable dose of nicotinamide before STZ administration. Nicotinamide administration resulted in partial protection of pancreatic β-cells from necrosis resulted from STZ administration (Madkor et al., 2011). This model may give beneficial tool especially for pharmacological investigations of new insulinotropic agents (Madkor et al., 2011).

Nowadays, attentions have been concentrated on the relationship between the free radicals' production especially reactive oxygen species (ROS), and the pathogenesis as well as the progression of diabetes mellitus. In diabetes mellitus, production of free radicals may result in metabolic stress because of changing in energy metabolism, inflammatory mediators, and impairment of body anti-oxidant defense mechanisms (Anwar and Meki, 2003).

STZ induces oxidative stress and depletion of both blood and tissues anti-oxidant systems, resulting in membrane lipid peroxidation, and thence cellular injury. For this reason, uses of STZ-diabetic rat model may be suitable for the investigation of anti-oxidant properties of anti-hyperglycemic agents (Murugan and Pari, 2007).

Metformin is a widely known anti-hyperglycemic agent which has significant gastrointestinal side effects. It is a derivative of guanidine, and it has been prescribed in the treatment of hyperglycemia and type 2 diabetes mellitus (T2DM) for over 50 years (American Diabetes Association, 2014). Derivatives of guanidine include metformin, phenformin, and buformin, which were extracted from the plant isoamylene in the 1920s. As a result of the higher risk of cardiac mortality and lactic acidosis associated with the use of phenformin and buformin, they have been withdrawn in the early 1970s. Because of the superior safety profile of metformin, its use has been expanded from T2DM to include gestational diabetes, diabetic nephropathy, polycystic ovary disease and T2DM-associated cardiovascular complications (Viollet et al., 2012). Liver is the primary site of metformin pharmacodynamics and gut is also considered as an important site for its anti-diabetic effect (Duca et al., 2015; Paleari et al., 2018). Metformin precisely suppresses hepatic gluconeogenesis without increasing the effort on pancreatic β-cells to enhance insulin secretion or promoting adipocyte discrimination to induce weight gain. However, the exact molecular mechanisms of its glucose lowering effects remain unclear (Li et al., 2018).

Many spices have anti-hyperglycemic and anti-oxidant effects, and they are less toxic than anti-diabetic drugs (Eidi et al., 2006). Ginger (*Zingiber officinale*) and garlic (*Allium sativum*) have been widely used as dietary spices and for the treatment of different diseases in herbal medicine since ancient times (Suryanarayana et al., 2007). Worldwide, dried powders of ginger rhizome and garlic bulb, and their extracts are considered an important ingredient in many traditional and alternative medicines (Ali et al., 2008).

The present study aimed to compare the modulatory effects (anti-diabetic and anti-oxidant effects) of ginger rhizome as well as garlic bulb extracts separately and mixed together, with the effect of metformin on STZ-nicotinamide diabetic rats. Moreover, this study also examined any deleterious effects associated with their feeding on healthy rats. This comparison depends on studying their effects on hematological parameters [erythrogram with highlighting on glycated hemoglobin (HbA1c) estimation, and leukogram], biochemical parameters (liver and kidney function tests, and oxidative stress biomarkers focusing on activity of anti-oxidant enzymes including catalase, superoxide dismutase and glutathione peroxidase), and histopathological alterations of liver, kidney, and pancreas.

MATERIALS AND METHODS

Medicinal Plants and Drugs

Ginger powder (Rhizomes of *zingiber officinale*) was purchased from Imtenan Store from Egyptian local market. Fresh garlic bulbs (*Allium sativum*) were purchased from the local market, peeled, washed, and chopped into small pieces. Streptozotocin (STZ), nicotinamide and metformin were obtained from Sigma Chemicals Company.

Preparation of Ethanolic Extract of Ginger

Each 200 g of ginger fine powder was soaked in 1 L of 90% ethanol then kept in refrigerator with daily shaking for 3 days. Furthermore, filtrated using double layer of gauze and concentrated using rotatory evaporator connected with an

electric vacuum pump and metal water bath adjusted at 50°C (Abdulaziz-Bardi et al., 2013).

Preparation of Aqueous Extract of Garlic

Garlic as a whole plant was grinded and left to dry for 2-3 weeks. It was grinded into a fine powder; each 200 g of this powder was soaked in 1 L of distilled water then kept in refrigerator with daily shaking for 3 days. After that, it was filtrated using double layer of gauze and concentrated using rotatory evaporator connected with an electric vacuum pump and metal water bath adjusted at 50°C (Huzaifa et al., 2014).

Experimental Rats

Eighty Sprague-Dawely male Albino rats (150-200 g) were used in the experiment and were obtained from the Animal House, Faculty of Veterinary Medicine, Cairo University, Egypt. After the rats were allocated into the cages, they were acclimatized to the environment for 2 weeks prior to begin the experiment. They were fed on standard laboratory diet and allowed to drink water *ad libitum*. Ethical approval (CU II F 47 18) from the Institutional Animal Care and Use Committee (IACUC), Cairo University was taken.

Induction of Diabetes to Experimental Rats

Diabetes was induced by a single intra-peritoneal (I/P) injection of nicotinamide (100 mg/kg), 20 minutes before I/P injection of STZ at a dose of 50 mg/kg, that was dissolved in citrate buffer (0.1 M, PH 4.5). After 72 hours of STZ administration, the tail vein blood was collected to determine fasting blood glucose level. Only rats with fasting blood glucose over 250 mg/dl were considered diabetic and included in the experiment (Tahara et al., 2008). Ginger and garlic extracts were administered orally by stomach tube.

Experimental Design

Rats were randomly allocated into 10 groups, comprising 8 rats each as follow: Group (1); rats received distilled water for 42 days and served as non-diabetic non-treated control negative group. Group (2); served as STZ-diabetic group (Tahara et al., 2008). Group (3); rats were orally received ethanolic extract of ginger (200 mg/kg) (Ahmadi et al., 2013), and served as ginger treated group. Group (4); rats were orally received aqueous extract of garlic (400 mg/kg) (Johnson et al., 2015), and served as garlic treated group. Group (5); rats were orally received mix of ginger and garlic extracts at the same dose in groups 3 and 4, respectively, and served as mixed treated group. Group (6); STZ-diabetic rats treated with ginger extract (200 mg/kg). Group (7); STZ-diabetic rats treated with garlic extract (400 mg/ kg). Group (8); STZ-diabetic rats treated with mix of ginger and garlic extracts at dose of 200 and 400 mg/ kg, respectively. Group (9); rats were orally received metformin at dose of 250 mg/ kg (Okonkwo and Okoye, 2014). Group (10); STZ-diabetic rats treated with metformin at the same dose of group (9). All groups from 3 to 8 and 10 were treated after 72 hours of STZ administration. The experiment continued for 42 days.

Sample Collection and Methods

Blood Samples

Blood samples from each group were collected at 0, 14th, 28th and 42nd of treatments. The obtained blood sample from each rat (retro-orbital venous plexus) was divided into three parts. First part was anti-coagulated by di-potassium salt of ethylene diamine tetra-acetic acid (EDTA) and used for hemogram evaluation and glycated hemoglobin (HbA1c) estimation. Second part was anti-coagulated by sodium fluoride and used for glucose estimation. Third part was collected in a clean centrifuge tube and allowed to clot, then centrifuged at 3000 rpm for 10 minutes for serum separation. Clear non-hemolysed supernatant serum was harvested for biochemical studies. At the end of the experiment activity of anti-oxidant enzymes (catalase, superoxide dismutase and glutathione peroxidase), and level of uric acid were measured.

Urine Samples

At the end of the experiment (42nd day of treatment), all rats were kept inside specially designed metal containers. Owing to the special design of these metal containers, 24 hours urine sample from the rats was collected in a reservoir located below.

Tissue Specimens

At the end of the experiment (42nd day of treatment) and after blood sampling, rats of all groups were anaesthetized with diethyl ether and decapitated. For histopathological examination, small pieces of fresh tissues including liver, kidney and pancreas were collected and fixed in 10% buffered formalin, and then placed in fresh fixative solution and embedded in paraffin, sectioned at 5µm thick and stained with Hematoxylin and Eosin (H& E) stain for routine light microscope examination (Bancfort and Marilyn 2008).

Clinicopathological Studies

Hematological Studies

Red blood cells (RBCs) count, packed cell volume (PCV %), hemoglobin (Hb) concentration, mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC), total leukocytic count (TLC) and differential leukocytic count (DLC) on Giemsa stained blood smears were performed (Feldman et al., 2000).

Biochemical Studies

Serum samples were prepared to assay activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) enzymes (Reitman and Frankel, 1957). Concentrations of total proteins (Weichselbaun, 1946), albumin (Grant et al., 1987), total cholesterol (Allain et al., 1974), triglycerides, high density lipoprotein cholesterol (HDL-c) (Warnick et al., 1983), low density lipoprotein (LDL-c) (Friedewald et al., 1972), very low density lipoprotein (VLDL-c), blood urea nitrogen (BUN) (Searcy et al., 1967), creatinine (Fabiny and Ertingshausen, 1971), and uric acid (Zhao et al., 2008). Activities of anti-oxidant enzymes including catalase (Aebi, 1974), super oxide dismutase (SOD) (Mc Cord and Fridovich, 1969), glutathione peroxidase (GPx) (Hafemann et al., 1974). Levels of glucose (Howanitz, 1984), insulin (Korner et al., 2005), and HbA1c (Tahara et al., 1995).

STATISTICAL ANALYSIS

Data were analyzed using IBM SPSS statistics version 23. Two-way Analysis of Variance (ANOVA) was used to determine the differences among experimental groups followed by least significant difference (LSD) as post hock. Data was expressed as Mean ± Standard deviation (SD).

RESULTS

Hematological Results

Hemogram results of STZ-induced diabetic group (2) when compared to those of control negative group (1) showed the presence of microcytic hypochromic anemia which manifested by significant decreases in PCV %, Hb concentration, RBCs count, MCV and MCHC values. Leukocytosis with neutrophilia and lymphopenia from 14th day till the end of the experiment. All results from groups 3 to 10 were compared with those of group 2 showed the following; insignificant changes all over the experimental period in groups 3, 4, 5 and 9, while, microcytic hypochromic anemia and leukocytosis with neutrophilia and lymphopenia started from 14th day till the end of the experiment were observed in groups 6, 7, 8 and 10. These changes were more pronounced in group 2 than the other groups. (Tables 1-4)

Biochemical Results

By comparing results of STZ-induced diabetic group (2) by those of control negative group (1), liver function tests (activities of ALT, AST, and concentrations of total cholesterol, triglycerides, LDL-c and VLDL-c), kidney function tests (concentrations of BUN and creatinine) and blood glucose level were significantly elevated from 14th day till the end of the experiment. Results of groups 3 to 10 were compared with that of group 2 showed the following; insignificant changes all over the experimental period in groups 3, 4, 5 and 9, while, groups 6, 7, 8 and 10 revealed significant increase in the liver function tests (activities of ALT, AST, and concentrations of total cholesterol, triglycerides, LDL-c and VLDL-c), kidney function tests (concentrations of BUN and creatinine), and blood glucose level started from 14th day then decreased gradually till the end of the experiment. This elevation was more pronounced in group 2 than other groups. Group (2) showed decreased levels of total proteins, albumin, globulin, HDL-c and insulin from 14th day till the end of the experiment. Insignificant changes all over the experimental period in groups 3, 4, 5 and 9 were observed, while, significant increase in total proteins, albumin, globulins and HDL-c concentrations started from 14th day till the end of the experiment were recorded in groups 6, 7, 8 and 10. (Tables 5-10).

Anti-oxidant Enzymes Activities and Uric Acid Concentration

All the following mean values were compared to that of negative control group (1).

Table (1): Erythrogram of different experimental groups at zero and 14th day

Day	Zero day					14th day				
Parameter	RBCs (×10⁶/µl)	Hb conc. (g/dl)	PCV (%)	MCV (fl)	MCHC (%)	RBCs (×10⁶/µl)	Hb conc. (g/dl)	PCV (%)	MCV (fl)	MCHC (%)
G1	7.84±0.21[a]	14.96±0.59[a]	44.98±2.19[a]	57.18±1.5[a]	33.24±0.4[a]	7.82±0.27[a]	14.72±0.60[a]	44.26±2.05[a]	57.54±1.2[a]	33.18±0.3[a]
G2	7.86±0.23[a]	14.58±0.69[a]	44.84±1.95[a]	57.18±1.7[a]	33.04±0.5[a]	6.95±0.23[b]	12.66±0.75[b]	37.98±1.18[b]	53.44±1.5[b]	31.04±0.4[b]
G3	7.80±0.22[a]	14.90±0.58[a]	45.16±2.02[a]	57.12±1.4[a]	32.96±0.4[a]	7.82±0.23[a]	14.74±0.61[a]	43.70±2.05[a]	57.24±1.4[a]	33.10±0.5[a]
G4	7.87±0.21[a]	14.74±0.59[a]	43.92±2.28[a]	56.74±1.3[a]	33.30±0.3[a]	7.88±0.20[a]	14.72±0.52[a]	44.52±1.68[a]	57.14±1.3[a]	33.02±0.4[a]
G5	7.84±0.25[a]	14.86±0.66[a]	44.70±2.57[a]	56.78±1.4[a]	33.22±0.4[a]	7.89±0.19[a]	14.66±0.46[a]	43.98±1.81[a]	56.62±1.3[a]	32.82±0.6[a]
G6	7.91±0.20[a]	14.82±0.59[a]	44.30±2.14[a]	57.22±1.4[a]	33.18±0.4[a]	7.23±0.17[c]	13.62±0.33[c]	39.80±1.24[c]	54.20±1.6[c]	32.21±4.2[c]
G7	7.83±0.20[a]	14.60±0.71[a]	44.14±2.34[a]	57.04±1.5[a]	33.06±0.6[a]	7.18±0.11[c]	13.50±0.43[c]	39.54±1.33[c]	54.66±1.7[c]	32.10±0.4[c]
G8	7.78±0.22[a]	14.72±0.58[a]	44.16±2.28[a]	56.78±1.4[a]	32.92±0.5[a]	7.19±0.08[c]	13.48±0.38[c]	39.50±1.36[c]	54.46±1.5[c]	32.08±0.4[c]
G9	7.85±0.17[a]	14.78±0.57[a]	44.84±1.87[a]	57.14±1.2[a]	33.01±0.5[a]	7.86±0.23[a]	14.90±0.55[a]	44.66±1.93[a]	57.34±1.4[a]	32.96±0.6[a]
G10	7.87±0.21[a]	14.84±0.51[a]	44.28±2.19[a]	56.60±1.4[a]	33.06±0.5[a]	7.41±0.16[c]	13.78±0.40[c]	40.04±1.56[c]	54.74±1.2[c]	32.01±0.3[c]

G1: Non-diabetic non-treated rats.
G2: STZ-induced diabetic rats.
G3: Non-diabetic rats treated with ginger.
G4: Non-diabetic rats treated with garlic.
G5: Non-diabetic rats treated with ginger and garlic.
G6: Diabetic rats treated with ginger.
G7: Diabetic rats treated with garlic.
G8: Diabetic rats treated with ginger and garlic.
G9: Non-diabetic rats treated with metformin.
G10: Diabetic rats treated with metformin.

Table (2): Erythrogram of different experimental groups at 28th and 42nd day

Day	28th day					42nd day				
Parameter	RBCs (×10⁶/µl)	Hb conc. (g/dl)	PCV (%)	MCV (fl)	MCHC (%)	RBCs (×10⁶/µl)	Hb conc. (g/dl)	PCV (%)	MCV (fl)	MCHC (%)
G1	7.89±0.2[a]	14.78±0.66[a]	43.80±2.42[a]	57.78±1.7[a]	33.14±0.3[a]	7.82±0.22[a]	14.72±0.52[a]	45.64±0.98[a]	57.22±1.6[a]	32.80±0.6[a]
G2	6.29±0.5[b]	11.68±0.61[b]	33.74±0.62[b]	52.98±1.0[b]	30.12±0.5[b]	6.14±0.42[b]	11.48±0.32[b]	37.26±0.84[b]	50.64±0.5[b]	30.36±0.9[b]
G3	7.84±0.2[a]	14.74±0.53[a]	43.76±1.99[a]	57.20±1.4[a]	32.82±0.4[a]	7.80±0.21[a]	14.72±0.52[a]	44.10±1.68[a]	57.08±1.21[a]	33.01±0.5[a]
G4	7.84±0.2[a]	14.78±0.56[a]	44.60±1.89[a]	57.24±1.3[a]	33.06±0.4[a]	7.79±0.23[a]	14.80±0.58[a]	44.58±1.89[a]	57.32±1.3[a]	32.82±0.6[a]
G5	7.85±0.2[a]	14.90±0.54[a]	45.08±2.05[a]	57.54±1.2[a]	32.90±0.5[a]	7.81±0.22[a]	14.82±0.53[a]	44.78±1.86[a]	57.22±1.4[a]	32.80±0.4[a]
G6	7.63±0.3[c]	13.84±0.57[c]	40.22±1.65[c]	55.76±0.8[c]	32.40±0.3[c]	7.66±0.29[c]	13.86±0.53[c]	40.98±1.62[c]	55.72±1.6[c]	33.02±0.4[a]
G7	7.65±0.2[c]	13.84±0.62[c]	41.01±2.64[c]	55.68±1.4[c]	32.34±0.4[c]	7.62±0.29[c]	13.78±0.47[c]	40.42±1.45[c]	55.62±1.5[c]	33.40±0.4[a]
G8	7.66±0.4[c]	13.94±0.50[c]	41.06±2.02[c]	56.24±0.5[c]	32.44±0.3[c]	7.77±0.26[a]	13.48±0.41[c]	40.26±0.99[c]	56.44±1.3[c]	33.34±0.3[a]
G9	7.88±0.2[a]	14.78±0.61[a]	44.56±1.96[a]	57.26±1.3[a]	32.94±0.4[a]	7.86±0.19[a]	14.68±0.54[a]	43.86±2.20[a]	57.34±1.3[a]	32.94±0.7[a]
G10	7.71±0.2[c]	13.96±0.68[c]	40.68±2.49[c]	54.18±0.9[c]	32.38±0.5[a]	7.75±0.26[c]	13.96±0.35[c]	40.80±1.24[c]	55.72±1.2[c]	33.44±0.4[a]

Table (3): Leukogram of different experimental groups at zero and 14th day

Day	Zero day					14th day				
Parameter	TLC (×10³/µl)	Neutrophil (×10³/µl)	Lymphocyte (×10³/µl)	Monocyte (×10³/µl)	Eosinophil (×10³/µl)	TLC (×10³/µl)	Neutrophil (×10³/µl)	Lymphocyte (×10³/µl)	Monocyte (×10³/µl)	Eosinophil (×10³/µl)
G1	8.20±0.95^{a}	3.80±0.56^{a}	2.56±0.43^{a}	0.324±0.07^{a}	0.160±0.03^{a}	8.24±0.92^{a}	3.84±0.54^{a}	2.52±0.33^{a}	0.328±0.07^{a}	0.174±0.04^{a}
G2	8.24±1.10^{a}	3.86±0.49^{a}	2.74±0.40^{a}	0.342±0.09^{a}	0.172±0.04^{a}	9.82±0.70^{b}	4.86±0.30^{b}	1.96±0.31^{b}	0.324±0.07^{a}	0.176±0.04^{a}
G3	8.44±0.98^{a}	3.82±0.53^{a}	2.56±0.42^{a}	0.314±0.08^{a}	0.166±0.04^{a}	8.26±1.09^{a}	3.74±0.55^{a}	2.64±0.46^{a}	0.324±0.08^{a}	0.162±0.04^{a}
G4	8.28±0.89^{a}	3.86±0.49^{a}	2.60±0.39^{a}	0.336±0.08^{a}	0.164±0.04^{a}	8.36±0.92^{a}	3.96±0.52^{a}	2.60±0.38^{a}	0.354±0.08^{a}	0.148±0.04^{a}
G5	8.30±0.97^{a}	3.94±0.53^{a}	2.64±0.40^{a}	0.328±0.07^{a}	0.158±0.04^{a}	8.52±1.04^{a}	3.84±0.49^{a}	2.58±0.40^{a}	0.334±0.08^{a}	0.162±0.04^{a}
G6	8.20±1.06^{a}	3.88±0.55^{a}	2.48±0.36^{a}	0.338±0.09^{a}	0.156±0.04^{a}	9.08±0.73^{c}	4.66±0.26^{c}	2.16±0.23^{b}	0.330±0.08^{a}	0.166±0.04^{a}
G7	8.28±0.97^{a}	3.88±0.48^{a}	2.78±0.31^{a}	0.330±0.09^{a}	0.164±0.04^{a}	9.34±0.59^{c}	4.64±0.35^{c}	2.14±0.60^{b}	0.324±0.08^{a}	0.16±0.041^{a}
G8	8.24±0.92^{a}	3.76±0.53^{a}	2.60±0.43^{a}	0.296±0.08^{a}	0.166±0.04^{a}	9.42±0.43^{c}	4.44±0.49^{a}	2.10±0.44^{b}	0.342±0.09^{a}	0.167±0.03^{a}
G9	8.24±0.94^{a}	3.94±0.50^{a}	2.80±0.29^{a}	0.338±0.12^{a}	0.164±0.04^{a}	8.24±0.93^{a}	3.82±0.51^{a}	2.64±0.32^{a}	0.332±0.08^{a}	0.158±0.04^{a}
G10	8.14±0.94^{a}	3.80±0.56^{a}	2.56±0.46^{a}	0.444±0.24^{a}	0.166±0.04^{a}	9.32±0.63^{c}	3.84±0.54^{a}	2.09±0.36^{b}	0.322±0.08^{a}	0.172±0.03^{a}

Table (4): Leukogram of different experimental groups at 28th and 42nd day

Day	28th day					42nd day				
Parameter	TLC (×10³/µl)	Neutrophil (×10³/µl)	Lymphocyte (×10³/µl)	Monocyte (×10³/µl)	Eosinophil (×10³/µl)	TLC (×10³/µl)	Neutrophil (×10³/µl)	Lymphocyte (×10³/µl)	Monocyte (×10³/µl)	Eosinophil (×10³/µl)
G1	8.36±1.09^{a}	3.78±0.52^{a}	2.52±0.40^{a}	0.34±0.087^{a}	0.182±0.04^{a}	8.42±1.02^{a}	4.04±0.37^{a}	2.50±0.40^{a}	0.328±0.08^{a}	0.172±0.05^{a}
G2	10.02±0.46^{b}	4.94±0.45^{b}	2.08±0.39^{b}	0.348±0.08^{a}	0.172±0.04^{a}	10.56±1.18^{a}	4.82±0.43^{b}	2.06±0.37^{b}	0.312±0.06^{a}	0.174±0.05^{a}
G3	8.28±1.03^{a}	3.82±0.51^{a}	2.64±0.42^{a}	0.334±0.09^{a}	0.184±0.04^{a}	8.26±0.97^{a}	3.82±0.54^{a}	2.44±0.40^{a}	0.334±0.08^{a}	0.152±0.04^{a}
G4	8.18±0.95^{a}	3.78±0.59^{a}	2.52±0.34^{a}	0.328±0.08^{a}	0.180±0.04^{a}	8.44±1.09^{a}	3.76±0.55^{a}	2.68±0.42^{a}	0.338±0.08^{a}	0.190±0.03^{a}
G5	8.18±0.99^{a}	3.86±0.56^{a}	2.54±0.42^{a}	0.302±0.04^{a}	0.170±0.04^{a}	8.54±0.94^{a}	4.02±0.58^{a}	2.60±0.38^{a}	0.332±0.09^{a}	0.166±0.04^{a}
G6	9.10±1.01^{c}	4.41±0.60^{c}	2.25±0.36^{b}	0.340±0.08^{a}	0.162±0.03^{a}	8.42±0.97^{a}	3.94±0.56^{a}	2.52±0.40^{a}	0.342±0.08^{a}	0.162±0.04^{a}
G7	9.08±0.92^{c}	4.58±0.41^{c}	2.22±0.45^{b}	0.308±0.09^{a}	0.168±0.05^{a}	8.46±1.04^{a}	4.02±0.49^{a}	2.74±0.23^{a}	0.324±0.07^{a}	0.168±0.04^{a}
G8	8.48±1.10^{a}	4.28±0.45^{a}	2.27±0.36^{b}	0.322±0.08^{a}	0.148±0.03^{a}	8.38±1.01^{a}	3.98±0.59^{a}	2.64±0.37^{a}	0.324±0.07^{a}	0.168±0.03^{a}
G9	8.24±0.92^{a}	3.80±0.60^{a}	2.56±0.37^{a}	0.348±0.08^{a}	0.172±0.05^{a}	8.14±0.96^{a}	4.02±0.52^{a}	2.72±0.21^{a}	0.310±0.08^{a}	0.164±0.04^{a}
G10	8.46±0.85^{a}	3.78±0.52^{a}	2.26±0.36^{b}	0.326±0.07^{a}	0.154±0.04^{a}	8.36±0.96^{a}	4.04±0.37^{a}	2.66±1.34^{a}	0.320±0.09^{a}	0.176±0.05^{a}

Table (5): Liver function tests of different experimental groups at zero and 14th day

Day	Zero day						14th day					
Parameter	ALT (IU/L)	AST (IU/L)	T.P (g/dl)	Albumin (g/dl)	Globulins (g/dl)	A/G ratio	ALT (IU/L)	AST (IU/L)	T.P (g/dl)	Albumin (g/dl)	Globulins (g/dl)	A/G ratio
G1	36.8±7.33^{a}	38.2±6.9^{a}	6.12±0.29^{a}	3.68±0.28^{a}	2.82±0.24^{a}	1.34±0.11^{a}	37.2±6.98^{a}	40.6±8.2^{a}	6.18±0.26^{a}	3.78±0.24^{a}	2.82±0.22^{a}	1.36±0.11^{a}
G2	35.8±9.20^{a}	37.8±7.2^{a}	6.18±0.26^{a}	3.70±0.37^{a}	2.72±0.23^{a}	1.36±0.13^{a}	77.4±7.80^{b}	82.2±7.2^{b}	5.22±0.19^{b}	3.06±0.21^{b}	2.16±0.11^{b}	1.20±0.10^{b}
G3	36.0±7.42^{a}	39.0±7.1^{a}	6.08±0.33^{a}	3.70±0.25^{a}	2.88±0.23^{a}	1.32±0.13^{a}	37.2±7.60^{a}	39.4±6.9^{a}	6.24±0.30^{a}	3.82±0.26^{a}	2.80±0.35^{a}	1.36±0.11^{a}
G4	36.4±7.83^{a}	38.4±6.7^{a}	6.16±0.30^{a}	3.80±0.25^{a}	2.64±0.17^{a}	1.34±0.11^{a}	34.8±7.22^{a}	36.0±7.2^{a}	6.22±0.36^{a}	3.76±0.24^{a}	2.82±0.22^{a}	1.34±0.11^{a}
G5	38.2±7.60^{a}	38.4±6.7^{a}	6.16±0.29^{a}	3.76±0.24^{a}	2.82±0.28^{a}	1.32±0.13^{a}	35.2±7.56^{a}	38.4±6.7^{a}	6.18±0.31^{a}	3.82±0.23^{a}	2.82±0.24^{a}	1.36±0.11^{a}
G6	37.8±8.14^{a}	38.0±7.1^{a}	6.14±0.36^{a}	3.76±0.24^{a}	2.78±0.24^{a}	1.36±0.11^{a}	59.4±5.55^{c}	59.4±6.8^{c}	5.76±0.22^{c}	3.21±0.19^{c}	2.32±0.19^{c}	1.29±0.08^{c}
G7	39.0±7.68^{a}	37.0±7.7^{a}	6.12±0.29^{a}	3.70±0.25^{a}	2.82±0.26^{a}	1.38±0.13^{a}	61.2±6.50^{c}	57.6±5.3^{c}	5.68±0.33^{c}	3.20±0.19^{c}	2.26±0.18^{c}	1.30±0.12^{c}
G8	37.0±7.78^{a}	38.0±7.1^{a}	6.30±0.29^{a}	3.78±0.26^{a}	2.94±0.43^{a}	1.34±0.11^{a}	58.6±7.23^{c}	56.2±9.1^{c}	5.88±0.20^{c}	3.10±0.16^{c}	2.24±0.11^{c}	1.28±0.07^{c}
G9	39.2±7.79^{a}	38.2±7.9^{a}	6.22±0.28^{a}	3.76±0.27^{a}	2.84±0.24^{a}	1.32±0.13^{a}	37.6±7.67^{a}	37.4±6.9^{a}	6.22±0.26^{a}	3.78±0.24^{a}	2.78±0.26^{a}	1.27±0.11^{c}
G10	36.0±8.34^{a}	38.4±7.3^{a}	6.24±0.27^{a}	3.80±0.25^{a}	2.76±0.36^{a}	1.36±0.11^{a}	59.8±6.38^{c}	56.0±9.6^{c}	5.54±0.35^{c}	3.16±0.18^{c}	2.26±0.05^{c}	1.29±0.07^{c}

Table (6): Liver function tests of different experimental groups at 28th and 42nd day

Day	28th day						42nd day					
Parameter	ALT (IU/L)	AST (IU/L)	T.P (g/dl)	Albumin (g/dl)	Globulins (g/dl)	A/G ratio	ALT (IU/L)	AST (IU/L)	T.P (g/dl)	Albumin (g/dl)	Globulins (g/dl)	A/G ratio
G1	38.2±6.83^{a}	37.4±6.7^{a}	6.24±0.27^{a}	3.78±0.26^{a}	2.80±0.25^{a}	1.32±0.13^{a}	34.8±7.29^{a}	37.8±7.5^{a}	6.16±0.27^{a}	3.82±0.24^{a}	2.72±0.25^{a}	1.34±0.11^{a}
G2	92.4±5.32^{b}	101.6±12.3^{b}	5.20±0.16^{b}	2.94±0.11^{b}	2.01±0.16^{b}	1.19±0.11^{b}	97.8±8.70^{b}	134.8±8.4^{b}	5.16±0.11^{b}	3.04±0.35^{b}	2.04±0.29^{b}	1.24±0.16^{b}
G3	37.0±8.37^{a}	38.2±7.2^{a}	6.30±0.29^{a}	3.80±0.25^{a}	2.80±0.25^{a}	1.32±0.13^{a}	38.0±7.07^{a}	38.8±7.1^{a}	6.14±0.30^{a}	3.78±0.24^{a}	2.82±0.22^{a}	1.36±0.11^{a}
G4	38.4±6.58^{a}	38.6±7.3^{a}	6.18±0.29^{a}	3.74±0.34^{a}	2.82±0.19^{a}	1.36±0.11^{a}	37.4±7.44^{a}	39.0±7.3^{a}	6.16±0.26^{a}	3.80±0.23^{a}	2.92±0.24^{a}	1.38±0.13^{a}
G5	37.4±6.95^{a}	39.6±7.4^{a}	6.26±0.27^{a}	3.76±0.24^{a}	2.72±0.23^{a}	1.36±0.11^{a}	36.6±7.47^{a}	40.2±6.1^{a}	6.24±0.27^{a}	3.78±0.36^{a}	2.78±0.24^{a}	1.34±0.11^{a}
G6	57.4±3.51^{c}	54.0±5.2^{c}	5.86±0.34^{c}	3.46±0.21^{c}	2.46±0.21^{c}	1.34±0.13^{a}	44.4±6.43^{c}	40.2±7.3^{a}	6.22±0.28^{a}	3.60±0.16^{a}	2.58±0.16^{a}	1.32±0.14^{a}
G7	55.2±5.97^{c}	54.8±7.8^{c}	6.01±0.29^{c}	3.36±0.21^{c}	2.36±0.21^{c}	1.34±0.11^{a}	45.2±6.98^{c}	41.4±7.7^{a}	6.26±0.27^{a}	3.66±0.32^{a}	2.64±0.34^{a}	1.36±0.11^{a}
G8	56.8±7.09^{c}	54.2±4.3^{c}	5.78±0.38^{c}	3.42±0.26^{c}	2.46±0.19^{c}	1.34±0.11^{a}	40.4±7.44^{c}	41.4±6.9^{a}	6.24±0.27^{a}	3.60±0.16^{a}	2.60±0.16^{a}	1.36±0.11^{a}
G9	36.8±7.40^{a}	37.0±8.1^{a}	6.12±0.25^{a}	3.74±0.23^{a}	2.70±0.58^{a}	1.38±0.13^{a}	39.2±5.07^{a}	39.8±7.0^{a}	6.22±0.27^{a}	3.86±0.21^{a}	2.76±0.24^{a}	1.41±0.11^{a}
G10	51.8±6.76^{c}	57.8±7.1^{c}	5.86±0.48^{c}	3.40±0.27^{c}	2.40±0.21^{c}	1.38±0.13^{a}	41.4±6.88^{c}	41.0±7.6^{a}	6.24±0.24^{a}	3.66±0.24^{a}	2.70±0.22^{c}	1.36±0.11^{a}

Table (7): Lipid Profile of different experimental groups at Zero and 14th day

Day	Zero day					14th day				
Parameter	T. cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)	T. cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)
G1	93.8±7.5[a]	67.0±4.6[a]	57.8±3.5[a]	39.6±2.1[a]	13.40±0.92[a]	96.0±7.7[a]	62.4±6.7[a]	58.2±3.6[a]	38.8±1.9[a]	12.48±1.33[a]
G2	92.8±7.7[a]	65.2±4.4[a]	58.0±3.4[a]	39.4±2.1[a]	13.04±0.89[a]	120.8±7.9[b]	90.2±4.8[b]	44.8±4.9[b]	48.8±1.9[b]	18.04±0.95[b]
G3	95.0±6.6[a]	65.2±6.1[a]	57.8±3.8a	39.2±1.9[a]	13.04±1.21[a]	95.8±8.1[a]	65.6±4.9[a]	58.6±4.8a	37.8±3.7[a]	13.12±0.99[a]
G4	94.2±7.6[a]	63.0±5.5[a]	57.8±3.4[a]	38.4±2.1[a]	12.60±1.10[a]	98.4±7.5[a]	65.4±5.4[a]	56.8±3.4[a]	37.8±2.3[a]	13.08±1.08[a]
G5	93.2±7.9[a]	64.0±5.2[a]	58.2±3.4[a]	38.8±1.9[a]	12.80±1.03[a]	96.6±6.5[a]	64.4±4.3[a]	57.4±3.8[a]	39.2±2.6[a]	13.48±0.88[a]
G6	95.2±7.5[a]	66.6±4.9[a]	57.6±5.3[a]	37.8±3.8[a]	13.32±1.00[a]	105.0±7.3[c]	76.4±4.7[c]	50.8±2.5[c]	44.0±3.4[c]	15.28±0.95[c]
G7	95.4±6.9[a]	65.4±6.0[a]	58.0±3.2[a]	39.4±2.0[a]	13.08±1.20[a]	107.4±6.2[c]	77.2±5.7[c]	51.6±3.7[c]	45.8±3.4[c]	15.44±1.15[c]
G8	96.8±7.0[a]	65.0±4.6[a]	57.0±3.3a	39.8±1.9[a]	13.01±0.92[a]	109.2±9.2[c]	77.4±5.2[c]	51.2±4.1c	44.8±5.3[c]	15.48±1.04[c]
G9	96.8±8.9[a]	66.0±6.1[a]	57.6±3.9[a]	39.2±1.9[a]	13.20±1.22[a]	94.2±7.3[a]	64.0±5.2[a]	55.6±5.3[a]	38.8±2.6[a]	12.80±1.05[a]
G10	95.0±6.8[a]	64.2±4.7[a]	56.2±3.6[a]	39.0±2.4[a]	12.84±0.95[a]	106.2±9.3[c]	76.8±6.0[c]	52.4±5.9[c]	44.2±2.7[c]	15.36±1.20[c]

Table (8): Lipid Profile of different experimental groups at 28th and 42nd day

Day	28th day					42nd day				
Parameter	T. cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)	T. cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)
G1	93.2±7.3[a]	62.4±6.7[a]	56.8±4.3[a]	38.8±1.9[a]	12.48±1.33[a]	97.8±9.7[a]	64.2±5.3[a]	58.2±4.6[a]	38.8±1.9[a]	12.84±1.06[a]
G2	149.8±6.5[b]	99.8±8.2[b]	41.4±2.1[b]	53.4±3.9[b]	19.96±1.65[b]	174.5±8.6[b]	96.8±7.4[b]	40.4±3.6[b]	53.0±2.7[b]	20.36±1.49[b]
G3	95.2±7.3[a]	65.0±4.9[a]	57.4±3.4a	39.4±2.4[a]	13.01±0.99[a]	96.4±6.3[a]	65.8±4.7[a]	57.0±3.9a	37.6±2.1[a]	13.16±0.94[a]
G4	93.2±4.9[a]	64.8±5.4[a]	58.6±3.6[a]	38.8±1.9[a]	12.96±1.07[a]	93.6±9.9[a]	65.4±6.2[a]	56.2±4.2[a]	38.4±2.1[a]	13.08±1.23[a]
G5	98.8±8.9[a]	64.0±4.9[a]	57.8±2.8[a]	39.6±2.1[a]	12.80±0.99[a]	95.0±6.2[a]	62.8±6.5[a]	56.6±3.5[a]	38.2±1.9[a]	12.56±1.31[a]
G6	101.6±6.9[c]	75.0±5.3[c]	51.0±3.4[c]	44.8±2.5[c]	14.01±1.08[c]	98.75±4.4[a]	66.6±5.4[a]	56.0±5.3[a]	40.6±2.1[c]	13.32±1.09[a]
G7	105.0±8.9[c]	75.2±4.8[c]	51.4±5.7[c]	43.6±3.0[c]	14.04±0.96[c]	97.4±15.2[a]	67.4±5.3[a]	58.2±2.9[a]	41.4±2.1[c]	13.48±1.06[a]
G8	99.8±8.2[c]	71.2±4.3[c]	50.4±4.7c	44.2±5.4[c]	14.24±0.86[c]	94.4±9.4[a]	66.2±5.6[a]	57.4±2.9a	39.8±1.9[a]	13.24±1.13[a]
G9	93.2±4.7[a]	65.2±4.6[a]	58.0±3.7[a]	39.8±1.9[a]	13.04±0.93[a]	96.8±6.5[a]	65.2±5.2[a]	58.0±3.9[a]	37.4±2.1[a]	13.04±1.05[a]
G10	100.8±7.9[c]	70.4±4.9[c]	53.0±3.1[c]	44.4±4.2[c]	14.88±0.99[c]	94.6±5.5[a]	66.2±5.4[a]	56.4±3.0[a]	39.4±2.3[a]	13.24±1.09[a]

Table (9): Kidney function tests and anti-oxidant enzymes of different experimental groups

Day	Zero day		14th day		28th day		42nd day		42nd day			
Parameter	BUN (mg/dl)	Creatinine (mg/dl)	BUN (mg/dl)	Creatinine (mg/dl)	BUN (mg/dl)	Creatinine (mg/dl)	BUN (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)	Catalase (IU)	SOD (IU)	GPX (IU)
G1	12.6±1.5^{a}	0.48±0.045^{a}	12.6±1.8^{a}	0.46±0.055^{a}	13.4±1.1^{a}	0.46±0.055^{a}	13.4±1.6^{a}	0.46±0.055^{a}	2.66±0.46^{a}	118.94±10.0^{a}	3.56±0.34^{a}	54.98±3.17^{a}
G2	13.0±1.6^{a}	0.46±0.055^{a}	25.2±2.4^{b}	1.10±0.122^{b}	30.0±2.8^{b}	1.32±0.084^{b}	32.6±4.4^{b}	1.24±0.114^{b}	4.52±0.43^{b}	46.96±5.32^{b}	1.58±0.55^{b}	16.56±0.73^{b}
G3	12.6±1.5^{a}	0.48±0.045^{a}	13.0±1.5^{a}	0.46±0.055^{a}	14.1±1.4^{a}	0.48±0.045^{a}	14.0±1.5^{a}	0.48±0.045^{a}	2.65±0.46^{a}	120.12±8.55^{a}	3.54±0.32^{a}	55.44±3.34^{a}
G4	12.6±1.5^{a}	0.48±0.045^{a}	12.6±1.8^{a}	0.48±0.084^{a}	13.2±1.4^{a}	0.48±0.045^{a}	13.4±1.5^{a}	0.48±0.045^{a}	2.50±0.41^{a}	117.10±9.91^{a}	3.52±0.29^{a}	55.16±3.21^{a}
G5	12.6±1.8^{a}	0.48±0.045^{a}	12.6±1.1^{a}	0.48±0.084^{a}	12.8±1.3^{a}	0.48±0.045^{a}	14.6±1.7^{a}	0.46±0.055^{a}	2.51±0.41^{a}	121.24±9.13^{a}	3.56±0.31^{a}	54.24±3.25^{a}
G6	13.0±1.5^{a}	0.46±0.055^{a}	19.2±2.2^{c}	0.62±0.100^{c}	16.2±2.4^{c}	0.56±0.055^{c}	15.0±1.5^{c}	0.56±0.055^{c}	3.08±0.33^{c}	102.12±3.19^{c}	2.62±0.22^{c}	40.14±5.70^{c}
G7	13.0±1.5^{a}	0.48±0.045^{a}	20.4±2.0^{c}	0.66±0.089^{c}	17.6±2.7^{c}	0.54±0.055^{c}	15.6±2.0^{c}	0.54±0.055^{c}	3.04±0.18^{c}	99.44±3.20^{c}	2.44±0.36^{c}	44.66±2.69^{c}
G8	12.4±1.7^{a}	0.46±0.055^{a}	19.4±1.9^{c}	0.66±0.089^{c}	17.4±1.1^{c}	0.54±0.055^{c}	14.8±2.7^{c}	0.56±0.055^{c}	2.98±0.56^{a}	101.40±1.92^{c}	2.56±0.19^{c}	41.50±6.00^{c}
G9	12.8±2.1^{a}	0.44±0.055^{a}	12.8±1.8^{a}	0.47±0.084^{a}	13.4±1.8^{a}	0.46±0.055^{a}	13.2±1.7^{a}	0.46±0.055^{a}	2.56±0.31^{a}	118.34±9.17^{a}	3.42±0.31^{a}	55.54±3.44^{a}
G10	12.6±1.82^{a}	0.48±0.045^{a}	20.2±1.79^{c}	0.64±0.055^{c}	16.8±2.28^{c}	0.58±0.045^{c}	15.6±2.70^{c}	0.62±0.084^{d}	2.86±0.60^{a}	99.02±4.22^{c}	2.32±0.25^{c}	44.44±0.87^{c}

Table (10): Glucose concentration, HbA1c % and insulin level of different experimental groups

Days	Zero day		14th day		28th day		42nd day		Zero day	42nd day
Parameter	Glucose (mg/dl)	Insuline (µU/ml)	Glucose (mg/dl)	Insuline (µU/ml)	Glucose (mg/dl)	Insuline (µU/ml)	Glucose (mg/dl)	Insuline (µU/ml)	HbA1c (%)	HbA1c (%)
G1	87.8±14.1^{a}	0.34±0.05^{a}	83.6±16.7^{a}	0.36±0.05^{a}	94.4±15.1^{a}	0.34±0.05^{a}	90.2±12.5^{a}	0.34±0.05^{a}	2.54±0.36^{a}	2.56±0.36^{a}
G2	91.0±14.1^{a}	0.36±0.05^{a}	296.8±27.1^{b}	0.20±0.01^{b}	294.0±22.6^{b}	0.24±0.09^{b}	290.2±17.3^{b}	0.24±0.09^{b}	2.64±0.36^{a}	3.16±0.28^{b}
G3	92.2±13.8^{a}	0.34±0.05^{a}	84.6±14.2^{a}	0.36±0.05^{a}	85.0±14.2^{a}	0.32±0.08^{a}	91.4±13.1^{a}	0.32±0.08^{a}	2.58±0.34^{a}	2.52±0.35^{a}
G4	92.2±13.8^{a}	0.34±0.05^{a}	89.8±09.7^{a}	0.36±0.05^{a}	89.2±11.8^{a}	0.36±0.05^{a}	88.0±15.3^{a}	0.36±0.05^{a}	2.58±0.34^{a}	2.32±0.18^{a}
G5	91.6±14.7^{a}	0.34±0.05^{a}	93.0±13.3^{a}	0.36±0.05^{a}	91.6±13.2^{a}	0.36±0.05^{a}	87.0±14.6^{a}	0.36±0.05^{a}	2.58±0.36^{a}	2.44±0.40^{a}
G6	92.6±13.8^{a}	0.34±0.05^{a}	176.6±15.5^{c}	0.32±0.04^{a}	136.8±6.9^{c}	0.32±0.04^{a}	130.2±3.9^{c}	0.32±0.04^{a}	2.50±0.39^{a}	2.74±0.35^{a}
G7	92.4±14.4^{a}	0.34±0.05^{a}	169.8±24.6^{c}	0.32±0.05^{a}	131.6±10.5^{c}	0.34±0.05^{a}	125.2±15.5^{c}	0.34±0.05^{a}	2.48±0.36^{a}	2.78±0.44^{a}
G8	95.0±13.8^{a}	0.36±0.05^{a}	174.0±14.2^{c}	0.33±0.05^{a}	131.0±08.5^{c}	0.36±0.05^{a}	118.0±17.7^{c}	0.36±0.05^{a}	2.68±0.43^{a}	2.52±0.33^{a}
G9	89.2±13.4^{a}	0.34±0.05^{a}	91.8±13.1^{a}	0.36±0.05^{a}	87.8±12.9^{a}	0.36±0.05^{a}	96.2±06.7^{a}	0.36±0.05^{a}	2.68±0.35^{a}	2.48±0.16^{a}
G10	90.0±14.8^{a}	0.34±0.05^{a}	171.6±18.4^{c}	0.34±0.05^{a}	122.0±09.4^{c}	0.34±0.05^{a}	118.2±07.8^{c}	0.34±0.05^{a}	2.58±0.37^{a}	2.60±0.37^{a}

Activities of anti-oxidant enzymes including catalase, SOD and GPx were significantly decreased in groups 2, 6, 7, 8 and 10, this decrease was more pronounced in group STZ-induced diabetic group (2) than other groups. Groups 3, 4, 5 and 10 showed insignificant changes. Uric acid level in group (2), diabetic treated groups with ginger (6) and garlic (7) was significantly increased. (Table, 9)

HbA1c Concentration

By comparing concentration of HbA1c at 42nd day with that concentration at zero day, STZ-induced diabetic group (2) showed significant increase while, other groups showed insignificant changes. (Table, 10)

Urine Analysis

At the end of the experiment, 24 hours urine samples were collected for complete urine analysis. Insignificant changes including physical, chemical, and microscopical examinations were observed in all groups except STZ-induced diabetic group (2) showed presence of glucose, uric acid, and amorphous urate crystals.

Histopathological Examination

Pancreatic histopathological examination of groups 1, 3, 4, 5 and 9 showed no alterations, and the normal histological structure of islands of Langerhans cells as endocrine portion, and the surrounding acini and ducts system as exocrine one was recorded in Figure, 1A. STZ-induced diabetic group (2) showed degeneration and nuclear pyknosis in most of the islands of Langerhans cells (Figure, 1B), while, groups 6, 7, 8 and 10 showed degenerative changes in some cells and mild atrophy in other cells in the islands of Langerhans (Figure, 1C).

Hepatic histopathological examination of groups 1, 3, 4, 5 and 9 showed normal histological structure of central vein and surrounding hepatocytes (Figure, 2A). Group (2) showed vacuolar degeneration in diffuse manner all over the hepatocytes associated with congestion and dilatation in the central and portal veins as well as inflammatory cells infiltration surrounding bile ducts in the portal area (Figure, 2B, C). Groups 6, 7, 8 and 10 showed congestion in the portal veins with degeneration in some of the hepatocytes (Figure, 2D).

Renal histopathological examination of groups 1, 3, 4, 5 and 9 showed normal histological structure of glomeruli and tubules at the cortex (Figure, 3A) while, group (2) showed severe congestion in cortical blood vessels and glomeruli with degeneration and coagulative necrosis in the lining epithelium of some tubules (Figure, 3B). Focal hemorrhage was detected in between the degenerated tubules at the cortico-medullary junction (Figure, 3C). Groups 6, 7, 8 and 10 showed no histopathological changes as recorded in Figure, 3D.

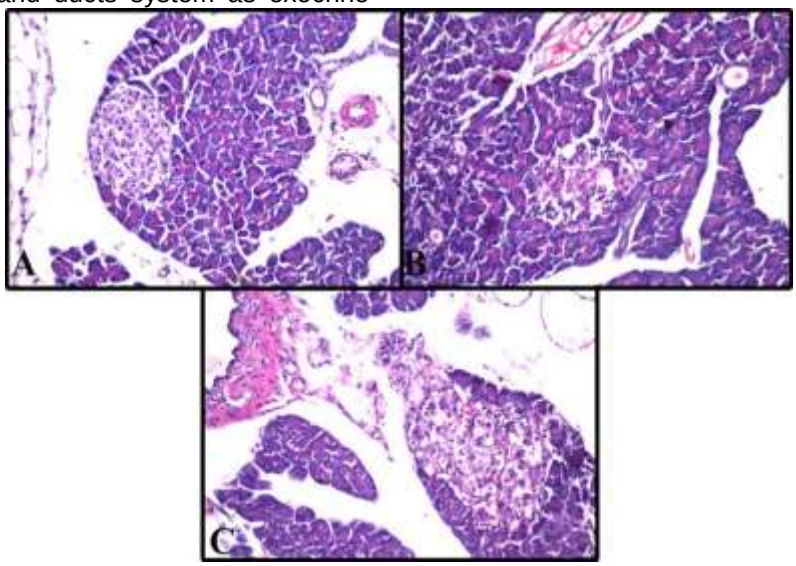

Figure (1): Pancreas of different experimental groups

A. Normal histopathological structure of islands of Langerhans in groups 1,3,4,5 and 9 (H& E, x40).
B. Degeneration and necrosis in some cells of the islands of Langerhans with pyknotic nuclei in group 2 (H& E, x40).
C. Degeneration in some cells of islands of Langerhans in groups 6,7,8 and 10 (H& E, x40).

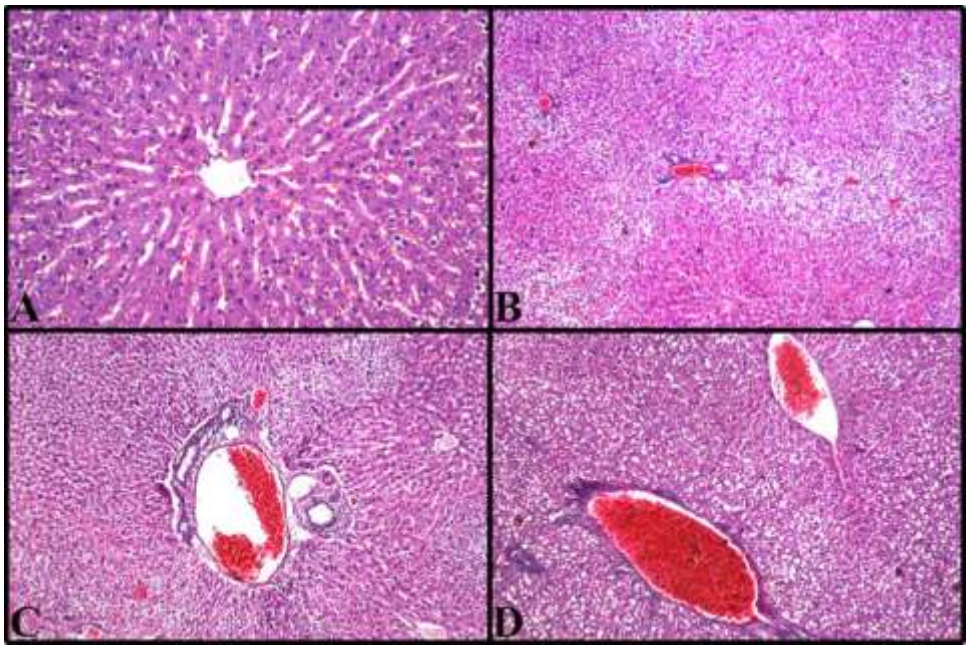

Figure (2): Liver of different experimental groups

A. Normal histological structure of central vein and surrounding hepatocytes in hepatic parenchyma in groups 1,3,4,5 and 9 (H& E, x40).
B. Vacuolar degeneration in diffuse manner all over the hepatocytes in group 2 (H& E, x16).
C. Congestion and dilatation in central and portal veins as well as inflammatory cells infiltration surrounding the bile ducts in the portal area in group 2 (H& E, x16).
D. Congestion in both portal and central veins with degeneration in some of the hepatocytes in groups 6,7,8 and 10 (H& E, x16).

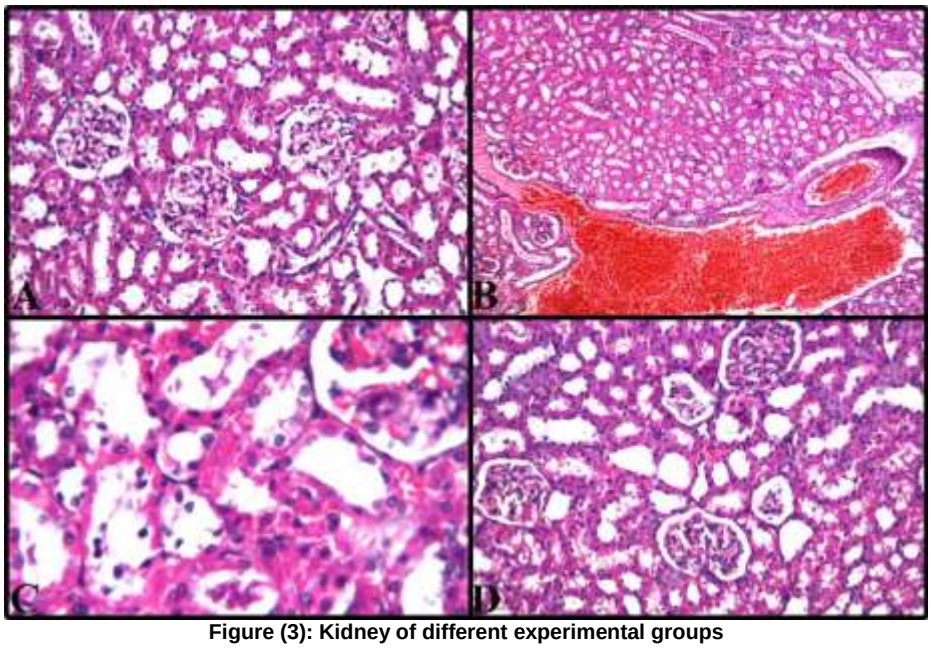

Figure (3): Kidney of different experimental groups

A. Normal histological structure of the glomeruli and tubules at the cortex in group 1 (H& E, x40).
B. Congestion in cortical blood vessels and glomeruli with degeneration and coagulative necrosis in lining epithelium of some of cortical tubules in group 2 (H& E, x16).
C. Focal hemorrhage detected in between the degenerated tubules at the cortico-medullary junction in group 2 (H& E, x16).
D. No histopathological alteration in the structure of glomeruli and tubules at the cortex in groups from 3 to 10 (H& E, x40).

DISCUSSION

Diabetes is a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbance of carbohydrate, fat and protein metabolisms. Diabetes results from defects in insulin secretion, insulin action, or both (Abeer, 2013). Oxidative stress and high production of reactive oxygen species (ROS) in pancreatic islets considered the most important adverse effects associated with diabetes, thus deplete the activity of anti-oxidant defense system and promote free radical generation (Savu et al., 2012). Streptozotocin (STZ) is commonly used for induction of diabetes in experimental animals as a potential inducer of oxidative stress (Shafik, 2012), and as the best model for screening of anti-hyperglycemic activities. Significant adverse effects are associated with many oral anti-hyperglycemic agents, in addition to some of them are ineffective in chronic diabetic patients (Pari and Saravanan, 2004). Respecting the high incidence of diabetes worldwide, there is a need for new therapies with less side effects and more efficacy. Many medicinal plants are used for decreasing diabetic symptoms (Waltner-Law et al., 2002). Ginger (*Zingiber officinale*) and garlic (*Allium sativum*) extracts are considered an important ingredient in different traditional and alternative medicines. The present study designed to compare anti-diabetic and anti-oxidant effects of ginger rhizome as well as garlic bulb extracts separately and mixed together, with the effect of metformin as a widely used oral anti-hyperglycemic drug on STZ-nicotinamide diabetic rats.

Hematological parameters usually reverse the animal physiological responsiveness to its internal and external surroundings, and these parameters being an actual tool for monitoring the animal physiological or pathophysiological status (Abeer et al., 2017). In the present study, erythrogram results of STZ-induced diabetic group (2) revealed the presence of microcytic hypochromic anemia which was similar to that results obtained by Nasirian et al., (2017). This anemia may be attributed to the failure of erythropoietin production associated with the affected kidney; this affection was documented in this group by the recorded significant increases in BUN and creatinine concentrations in association with the observed renal histopathological changes including degenerative and coagulative necrosis of lining epithelium of some renal tubules. Another explanation of this anemia may be the raises of non-enzymatic glycosylation of membrane proteins of red blood cells (Arun and Ramesh, 2002). Stress leukocytosis associated with group (2) was similar to that obtained by Elkind et al., (2001). Significant changes in hemogram (erythrogram and leukogram) of groups 6, 7, 8 and 10 revealed the improving effect of ginger, garlic, their mix, and metformin administrations, respectively (Gloria et al., 2015). This positive effect of extract administration of ginger (Bairwa et al., 2012) and garlic (Hamlaoui et al., 2012) may be related to their anti-oxidant activities in scavenging free radicals.

Hyperglycemia observed in group (2) agreed with Ahmadi et al., (2013). This hyperglycemia was resulted from the cytotoxic effect of STZ on pancreatic β-cells leading to insulin deficiency (Akbarzadeh et al., 2007) also group (2) showed an elevation in HbA1c level which was agreed with Al Hroob et al., (2018). Improving effect of ginger, garlic, their mix, and metformin administration on groups 6, 7, 8 and 10, respectively, was cleared by decreasing the levels of both blood glucose and HbA1c, this result was agreed with Ahmadi et al., (2013); El-Aarag et al., (2017) and Al Hroob et al., (2018). Possible mechanisms of hypoglycemic effect of ginger and garlic may be due to their anti-oxidant, their anti-glycation properties, and their ability to express the glucose transporter Glut 4 (Nasri et al., 2015). Glucose transporter Glut 4 is a carrier protein that facilitates glucose movement across cell membranes, and it is the main transporter for glucose transferring between liver and blood, and for glucose reabsorption from kidneys (Freitas et al., 2005). Another hypoglycemic effect of ginger and garlic may be due to inhibition of key enzymes controlling carbohydrate metabolism and increasing insulin release/sensitivity, resulting in enhanced glucose uptake in peripheral adipose and skeletal muscle tissues (Li et al., 2012). Metformin reduces blood glucose levels by inhibiting hepatic glucose production and reducing insulin resistance, particularly in liver and skeletal muscles. Also, metformin reduces blood glucose levels by decreasing intestinal absorption of glucose and increasing insulin sensitivity via enhancement of glucose uptake and utilization in peripheral tissues (Marić, 2010).

Significant increases in hepatic enzymes activity including ALT and AST in group (2) were like to that recorded by Abd El-Azim et al., (2013), these increases related to the hepatic dysfunction or the destructive changes in the hepatocytes because of toxemia resulted from diabetes mellitus (Kim et al., 2006). Histopathological findings were confirmed these changes by observing of the

congestion of central vein, degeneration of sporadic hepatocytes and hemorrhage. Our histopathological findings were agreed with that noticed by Fatemeh et al., (2013). Gradual decreases in the activities of ALT and AST which observed in the treated groups 6, 7, 8 and 10 may confirm the hepato-protective effect of ginger, garlic, their mix, and metformin, respectively (Emily et al., 2014). Hepato-protective effect of ginger (Darbar et al., 2010) and garlic (Rahman, 2003) could be attributed to their anti-oxidant activity in scavenging free radicals. These anti-oxidant properties may be resulted in acceleration the regenerative capacity of hepatocytes, stabilizing of hepatocyte cell membranes, and protecting hepatocytes from free radicals mediated toxic damages.

Recorded hypoproteinemia, hypoalbuminemia and hypoglobulinemia in group (2) were similar to that reported by Zafar et al., (2009) who suggested that, these findings associated with diabetic rats may be resulted from decreased hepatic protein synthesis, increased protein degradation, and increased their urinary excretion. Observed improvement in protein profile of treated groups 6, 7, 8 and 10 confirm the potential hepato-protective effect of administration of ginger, garlic, their mix, and metformin, respectively (Gehan et al., 2014).

Regarding the results of lipid profile, group (2) exhibited significant increases in total cholesterol, triglycerides, and LDL-c levels with significant decrease in HDL-c level, our results were similar to Ebuehi et al., (2010) who explained that, oxidative stress associated with diabetes mellitus could affect lipid level and metabolism resulting in these changes. Diabetic groups 6, 7, 8 and 10 treated with ginger, garlic, their mix, and metformin, respectively, showed significant decreased levels of total cholesterol, triglycerides and LDL-c and significant increased level of HDL-c which confirm the hypo-lipidemic effect of both ginger, garlic, and their mix these results agreed with Abd El-Azim et al., (2013) and Amna et al., (2017). Hypo-lipidemic effect of ginger could result from its inhibitory effect on cellular cholesterol biosynthesis after its consumption. Furthermore, this inhibition associated with increased activity of LDL-c receptor resulting in increasing LDL-c removal and reducing cholesterol level (Fuhrman et al., 2000). Garlic hypo-lipidemic effect may be attributed to the ability of its active substance allicin to limit hepatic cholesterol biosynthesis, or to enhance cholesterol turn over to bile acids and its excretion through gastrointestinal tract, or by inhibiting cholesterol absorption from intestinal lumen without changing HDL-c level (Singh and Porter, 2006; Ugwu and Omale, 2011). Hypo-lipidemic effect of metformin may be attributed to its ability to stimulate insulin secretion which increases production of LDL-c receptor and decreases LDL-c level (Kasim et al., 1986; Monnier et al., 1995 and Yassin and Mwafy 2007).

Concerning the results of kidney function tests, group (2) showed significant increase in the concentrations of BUN, creatinine and uric acid. Our results matched with those obtained by Rahman et al., (2012). These results were confirmed by 24 hours urine analysis which revealed the presence of both uric acid and amorphous urate crystals. Additionally, renal histopathological findings of this group including severe congestion in cortical blood vessels and glomeruli with degeneration and coagulative necrosis in lining epithelium of some tubules, confirm the impairment of kidney function of this group. These histopathological findings are similar to those noticed by Adeyi et al., (2012). Renoprotective effect of ginger, garlic, their mix, and metformin were cleared by significant decreases in BUN, creatinine, and uric acid concentrations in groups 6, 7, 8 and 10, respectively. Our results agreed with results of Elshater et al., (2009); Erejuwa et al., (2011) and Rotimi et al., (2015).

Results of anti-oxidant markers including activity of catalase, SOD and GPx enzymes revealed significant reduction in groups 2, 6, 7, 8 and 10, these reductions might be due to rises in ROS production because of diabetes induced by STZ (Vincent et al., 2004 and Lee et al., 2006). Anti-oxidant effects of ginger, garlic, their mix, and metformin administration were observed by increasing the activity of these anti-oxidant enzymes toward that of negative control, in groups 3, 4, 5 and 10, respectively, similar findings were obtained by Madkor et al., (2011) and Sani et al., (2014). Anti-oxidant potency of ginger was attributed to its content of gingerols (Ali et al., 2008 and Lee et al., 2009), and that for garlic was related to its content of organosulphur (Rahman et al., 2012).

CONCLUSION

The present study concluded that, both ginger and garlic have anti-hyperglycemic and anti-oxidant effects on STZ-nicotinamide diabetic rats, which were documented in this study by their improvement of hematological changes, hepatic and renal function impairments associated with diabetes. So that, they may be considered a

promising medicinal plant alternative for hypoglycemic drugs in the management of diabetes.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

AUTHOR CONTRIBUTIONS

AAA and SY designed and performed the experiments. AAA wrote the manuscript. AMS and ES performed animal treatments, flow experiments, and tissue collection. KMAM and AAA performed data analysis and reviewed the manuscript. All authors read and approved the final version.

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