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RESEARCH ARTICLE

Modulatory effects of curcumin, aqueous ginger extract , and their mixture on hyperglycaemia, dyslipidaemia and oxidative stress in alloxan diabetic rats.

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Abstract

The present study was conducted to elucidate the hypoglycemic, hypolipidemic and antioxidant effect of curcumin, *Zingiber officinale* (ginger) extract and their mixture in alloxan induced diabetic rats. Male albino rats (n=72) weighing (225-250 g) were divided into two main groups; first group: negative control (n=7) fed standard diet and second group: diabetic rats (n=66),which divided equally to five subgroups as follows: diabetic untreated rats (positive control), diabetic rats treated with curcumin (10,25,50 mg/kg.body weight), diabetic rats treated with aqueous ginger extract (50,100,200mg/kg. body weight) ,diabetic rats treated with their mixture and finally diabetic rats treated with5 mg/kg.b.w of glibenelamide. Diabetes was induced by a single intraperitonial injection of alloxan (150 mg/kg body weight). All treatments were orally administered by intra-gastric intubations once a day for eight consecutive weeks to alloxan-induced diabetic rats. At the end of the experiment, blood samples were collected to measure fasting blood glucose, fasting insulin ,glycated hemoglobin (HbA_{1c}),serum lipid profile. Changes in serum enzymatic antioxidant activities such as superoxide dismutase (SOD),glutathione peroxidase (GPx) and catalase (CAT) ,non enzymatic antioxidants levels such as glutathione (GSH) and vitamin C ,vitamin E and lipid peroxidation marker malondialdehyde (MDA) were measured. The results reported that the alloxan-induced diabetic group exhibited very highly significant ($p<0.001$) hyperglycemia, hyperlipidemia, elevated in MDA accompanied with weight loss and reduced in high HDL-C level, SOD,GPx and CAT enzyme activities in addition low levels of glutathione ,vitamin E and C when compared with control negative group. Treatment either curcumin or aqueous ginger extract with different doses daily for 8 consecutively weeks modulated the above changes but their mixture reported very highly significant($p<0.001$)improvement in all biological evaluation, glucose, insulin,glycoslated hemoglobin (HbA_{1c}),lipid profile, lipid peroxidation and antioxidant enzymes activities when compared with untreated diabetic group. 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.

Introduction:-

Diabetes was a major threat to global public health, characterized by chronic hyperglycemia resulting from impaired insulin action/secretion or both and, it was classified into two major categories [(1)].The number of diabetic patients were rapidly in increasing all over the world, according to a projection of the International Diabetes Federation

(IDF),366 million people had diabetes in 2011, which will increase to 552 million by 2030 [2]. In Iraq, diabetes mellitus has become more evident in the last two decades as a result of dramatic change in life style and enivermental stress. Effective control of hyperglycemia in diabetic patients was critical for reducing the risk of micro and macro vascular diseases [3-5]. Side effects of the presently available hyperglycemic agents have impeded their usefulness as antidabetic agents. This led to continuous effort to explore effective agents for control of diabetes mellitus(DM). Plants used in traditional medicine to treat DM represent a valuable alternative for the management of this disease, amongst such plants reported to have beneficial effects in the treatment of diabetes were spices such as curcumin and ginger [6]. Previous scientific investigation and clinical studies had confirmed the efficacy of some medicinal plants and herbal preparations in the improvement of normal glucose homeostasis, thus several studies revealed the benefits of medical plants like curcumin or ginger which showed hypoglycemic effect and also delay in the development of DM [7-9].

Curcumin powder , an extract of turmeric rhizomes (*Curcuma longa*), was the most active component of spice turmeric (an essential component of curry powder) which makes 2-5% of turmeric spice [10]. Curcumin shown to have wide spectrum of biological action, these include its anti-inflammatory, antioxidant, antidiabetic and hypocholesteremic activities [11]. Ginger (*Zingiber officinale*) was one of the most widely consumed spices for the flavoring of food worldwide [12], It had several beneficial pharmacological effects such as hypoglycemic, insulinotropic and hypolipidemic activities in humans [13,14], and in experimental animals [15,16]. The major chemical constituents of ginger rhizome were essential volatile oil and non-volatile pungent compounds, such as gingerols, shogaols, paradols and zingerone[17] .Therefore, the present study is conducted to evaluate the effect of curcumin, ginger and their combination on blood glucose , lipid profile levels in addition to oxidative stress markers in alloxan induced diabetic rats, and the protective effect of this formulation .

Materials and methods:-

The experiment was conducted at the department of Biotechnology, University of Baghdad. Seventy two male Albino rats (n=72) were included in the study and divided into 12 groups of 6 rats each, with a live-weight ranging from225-250g. The rats were acclimatized under standard rat house conditions for 14 days before the trial was initiated. These rats were housed in steel wire cages and maintained in controlled temperature at 27^{o}C with light cycle of 12h light and 12h dark. Aqueous ginger extract was prepared from locally available ginger roots. Ginger roots (500g) were peeled on crushed ice and was cut in to small pieces and homogenized in 750ml cold, sterile 0.9% NaCl solution and 250ml ice cold water to make the volume 1000ml.The homogenization was carried out in a blender for 12 minutes. The homogenized mixture was filtered three times through cheese cloth. The filtrate was centrifuged at 2000rpm for 10 min and the clear supernatant fraction was separated and volume made up to 1000ml with normal saline. The concentration of this ginger preparation was considered to have 500mg/ml on the basis of the weight of the starting material according to the formula of Majeed [18] . The extract was stored in sample tubes at -20°C until fed to rats. After overnight fasting, diabetes was induced in each rat by intra-peritoneal injection of Alloxan (Sigma-Aldrich, Cat # A7413, USA) prepared one hour before injection dissolved in 1ml distilled water at a dose of 150mg/ kg .b.w [19]. After three days of alloxan injection, blood was collected from of each rat to estimate glucose level of rats by enzymatic kits (Merck, Germany) . The rats having serum glucose above 200mg/dl were considered diabetic. The treatment with *Zingiber officinale* extract or curcumin and their mixture was started on 3th day after alloxan injection and this was considered as the first day of treatment because the serum glucose level increased much above normal limits. The treatment continued for 8 weeks All the rats received ginger extract (50,100 and 200mg/kg b.w/day orally for eight weeks as this duration was tested to be effective as hypoglycemic effect as reported earlier [18].The second group treated with pure curcumin(Sigma) at 10,25 and 50 mg/kg.b.w daily for 8 consecutive weeks ,while the third group of animals treated with a mixture of aqueous ginger extract and curcumin compared with the last group of animals treated with 5 mg of drug.. Serum samples were also collected from each rat on 1st, 4 and 8 weeks of the experiment for the determination of serum glucose. Lipid profile ,insulin, SOD,GPx and catalase , vitamin C and E were measured using ELISA kits in addition GSH was determined according to [19]procedure reported by and MDA was calculated by method[20].The data was subjected to statistical analysis using Analysis of Variance (ANOVA) to check over all significance and individual variations and least significant difference (LSD) test for finding out differences between treatments.

Results and discussion:-

Effect of oral administration of Curcumin , and aqueous ginger extract and their mixture on body weight, water intake ,and serum blood glucose in diabetic rats

Results from table (1a,b&c) showed that the control rats (group I) gained weight over the eight weeks of the experimental period, with the mean body weight increasing Moreover, the untreated diabetic rats (group II) lost an average of 37 grams of their weight after eight weeks ($P \leq 0.05$)compared with control undiabetic group. When these diabetic rats treated with curcumin alone, they did not lost their weight but gained 25 grams of body weight which represented 41.7% of weight gained by normal non diabetic control rats. Also, diabetic rats treated with aqueous ginger extract did not lost their weight but gained 20 grams of body weight which represented 33.3% of weight gained by normal non diabetic control rats. Loss of body weight observed in non-treated diabetic rats was improved by 60-70% when they treated with aqueous ginger extract or curcumin respectively alone while when treated with mixture of aqueous ginger extract and curcumin the improvement was 95 %.In the present study, alloxan induced diabetic rats showed very highly significantly decrease in final body weight which also accompanied with increased fluid intake , when compared with control group (-ve). Data summarized in Table 1(a,b,c) revealed that, fluid intake in untreated diabetic groups (Group II) were significantly ($P \leq 0.05$) increased by 100% than non-diabetic normal rats (group I).When these diabetic rats treated with curcumin or aqueous ginger extract alone, this increase in water intake was decreased significantly ($P \leq 0.05$). However, 90% of the increase in water intake in diabetic rats was decreased ($P \leq 0.05$) whenever treated with mixture of both curcumin and aqueous ginger extract ginger extract. On the other hand obtained results demonstrated that, ginger diabetic treated group showed significantly improvement and ameliorated reduction in final body weight. The present results in agree with the previous studies, which reported that alloxan diabetic rats treated with ginger induced significant improvement in body weight and feed intake when compared with alloxan diabetic untreated rats [21,22]. Furthermore, alloxan induced diabetic rats treated with ginger in diverse doses of reported that ginger is effective in reversing the weight loss observed in diabetic rats [23,24], 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.[25-28]Treatment of diabetic rats with curcumin, aqueous ginger extract or their mixture exhibited remarkably ameliorated effect, there were very highly significant ($P<0.01$) improvement in glucose concentration and insulin levels when compared with untreated diabetic group, and the mixture of both curcumin and ginger extract was more effective than curcumin and ginger extract alone, it exhibits remarkable glycemic control in diabetic group. This result agreement with many previously studies[29].Seo who revealed that curcumin improved homeostasis model assessment of insulin resistance and glucose tolerance, and elevated the plasma insulin level in db/db mice. Moreover, Gupta showed that curcumin revealed an anti-hyperglycemic effect which improved insulin sensitivity[30]

Effect of oral administration of Curcumin , aqueous ginger extract and their mixture on serum insulin and glycoslated hemoglobin in diabetic rats

Table (2) showed the effect of oral intake of each curcumin, aqueous ginger extract and their mixture on serum insulin concentration in alloxan diabetic male rats. In diabetic rat there were a very highly significant ($p<0.001$) elevation in glucose concentration accompanied with a very highly significant reduction in insulin level ($p < 0.001$) in addition increased in HbA1c as compared with control group (G_1) with percentage (273.93% and -58.46%, respectively) as percent change from control group when compared with control group (-ve).

Administration of curcumin, ginger or their mixture to diabetic rats showed remarkably ameliorated the elevation in glucose concentration and the reduction in insulin level, and there were very highly significant ($p< 0.001$) improvement in glucose concentration and insulin level as compared with diabetic untreated group when the diabetic rats intake a mixture of both curcumin and aqueous ginger extract compared with diabetic rats treated with either curcumin or aqueous of ginger extract alone as shown in table 1

Diabetes was recognized as one of the leading causes of morbidity and mortality in the world, while about 2.6 to 7.2 % of the world's population has been diagnosed with DM, it was still expected to increase in future. In spite of the fact that synthetic drugs such as insulin-like substances were the most important therapeutic agents known to medicine, researchers have been making efforts to find insulin-like substances from plant sources for the treatment of diabetes. Previous scientific investigation and clinical studies had confirmed the efficacy of some medicinal plants and herbal preparations in the improvement of normal glucose homeostasis[31,32]

Table (1a): Effect of oral administration of aqueous ginger extract (50, 100, and 200 mg/kg.b.w) on body weight, fluid intake and blood sugar in alloxan induced diabetic and normal rats. Values are expressed as mean (mg/dl ± S.D).

Parameter	Time (wk)	Control (G_1)	Diabetic (G_2)	50 mg/kg (G_3)	100mg/kg (G_4)	200mg/kg) (G_5)	0.5mg/kg drug (G_6)
Body weight (gm)	0	230 ± 8.7	233 ±6.9	235±8.1	232 +7.4	230±6.6	251±7.8
	4	269 ±8.2	250 ± 7.2	258±6.9	262±8.8	266 ±7.1	269 ±6.3
	8	300 ± 9.1	270 ± 7.8	280 ±7.8	288 ±7.4	292 ±8.2	295 ±7.4
Fluid intake ml/day	0	44± 2.5	44 ± 2.4	44±7.2	42 ±2.6	43±1.5	44 ± 2.7
	4	50 ± 1.8	60 ± 2.1	57±1.8	50 ±2.3	53 ±2.9	48± 1.9
	8	54 ± 1.5	80 ± 4.6	54±1.5	53 ±4.4	56 ±4.3	55 ± 1.7
B.sugar mg/dl	0	88 ± 4.5	288 ±7.8	280±8.2	286±9.2	285±8.4	285±8.1
	4	92 ± 3.8	314 ± 9.5	230±7.9	210 ± 8.6	200 ±7.1	166 ±7.1
	8	94± 6.2	379 ±9.9	200±10	184 ±4.6	176 ± 7.7	140 ±7.7

Table (1b): Effect of oral administration effect of curcumin (10, 25, and 50 mg/kg) on body weight, fluid intake and blood sugar in alloxan induced diabetic and normal rats. Values are expressed as mean (mg/dl ± S.D).

Parameter	Time (wk)	Control (G_1)	Alloxan (G_2)	10 mg/kg (G_7)	25mg/kg (G_8)	50mg/kg) (G_9)	0.5mg/kg drug (G_6)
Body weight (gm)	0	230 ± 8.2	233 ±6.4	235±8.1	232 +7.4	230±8.5	253±7.3
	4	271 ±6.2	253 ± 5.8	260±6.2	266±8.8	268 ±8.3	269 ±6.8
	8	306 ± 9.1	274 ± 7.8	283 ±6.3	291 ±7.4	296 ±7.4	295 ±8.6
Fluid intake ml/day	0	44± 2.5	44 ± 2.4	44±7.2	42 ±2.6	43±1.5	44 ± 2.7
	4	50 ± 1.8	61 ± 2.1	58±1.8	52 ±2.3	54 ±2.9	48± 1.9
	8	55 ± 1.5	83 ± 4.7	54±1.5	53 ±4.4	58 ±4.3	55 ± 1.7
B.sugar mg/dl	0	88 ± 4.5	288 ±7.8	280±8.1	288±9.2	285±8.1	277±8.1
	4	92 ± 3.8	312 ± 9.5	230±7.9	212 ± 8.6	190 ±7.1	160 ±7.1
	8	94± 6.2	350 ±9.9	200±10	186 ±4.6	166 ± 7.7	140 ±7.7

Table (1C): Effect of oral administration effect of combination of aqueous ginger extract (50, 10, and 200 mg/kg) and curcumin(10, 25 and 50 mg/kg.b.w) on body weight, fluid intake and blood sugar in alloxan induced diabetic and normal mice. Values are expressed as mean (mg/dl ± S.D).

Parameter	Time (wk)	Control (G_1)	Diabetic (G_2)	(G_{10})	(G_{11})	(G_{12})	(G_6)
Body weight (gm)	0	230 ± 8.7	233 ±6.9	235±8.1	232 +7.4	230±6.6	251±7.8
	4	271 ±6.2	250 ± 7.2	260±6.9	268±8.8	272 ±6.3	269 ±6.3
	8	305 ± 8.1	284 ± 7.6	285 ±7.8	290 ±6.5	300 ±5.8	295 ±7.4
Fluid intake ml/day	0	44± 2.5	44 ± 2.4	44±7.2	42 ±2.6	43±1.5	44 ± 2.7
	4	50 ± 1.8	60 ± 2.1	57±1.8	50 ±2.3	53 ±2.9	48± 1.9
	8	54 ± 1.5	80 ± 4.6	54±1.5	53 ±4.4	56 ±4.3	55 ± 1.7
B.sugar mg/dl	0	88 ± 4.5	288 ±7.8	280±8.1	288±9.2	285±8.1	277±8.1
	4	92 ± 3.8	312 ± 9.5	230±7.9	194 ± 8.6	173 ±7.1	160 ±7.1
	8	94± 6.2	350 ±9.9	196±10	176 ±4.6	142 ± 7.7	140 ±7.7

♦Values in parentheses indicated the percentage lowering of blood glucose in comparison with diabetic control. Diabetic control was compared with normal: $P < 0.05$.Each experimental group was compared with its corresponding value before and after treatment: $P < 0.05$.Experimental groups were compared with diabetic control: $P < 0.05$

Herbal therapy had been used in patients with IDDM and NIDDM .The herbal drugs had been prescribed widely because of their effectiveness, fewer side effects and relatively low cost[31] .Several studies revealed the benefits of medical plants like curcumin or ginger which showed hypoglycemic effect and also delay in the development of DM [7,8,9,32,33]. In the present study, alloxan induced diabetic rats showed very highly significantly decrease in final body weight which also accompanied with increasing fluid intake when compared with control group (-ve) after 8 weeks . The obtained results were in agreement with Gupta[30] who reported that, alloxan induced diabetic rats showed signs of loss weight compared with rats non-injected with alloxan. Moreover, Zafar and Naqvi [33] reported that alloxan in a dose of 100 mg/kg induced significant reduction in the body weight of diabetic animals compared with nondiabetic animals.Kota [34] 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. When insulin was deficient and the cells cannot metabolize glucose for energy, the cells compensate by increasing their metabolism of fats and proteins. Thus, the diabetic was usually thin, owing to the loss of fats and proteins from the body structure. Increasing metabolism of fats released large quantities of ketone bodies into the blood, which were intermediate products of fat breakdown, these excreted in the urine. Previous studies have reported that there was a decrease in body weight in diabetic rats and increased in fluid intake when compared with normal rats [35,36], the same study also reported improvement in body weight and feed intake in diabetic rats treated with curcumin compared with diabetic untreated rats. The effect of curcumin treatment may be explained by its ability to inhibit angiogenesis in adipose tissue and decrease differentiation of preadipocytes. In addition, Some researchers suggested that the gradual increase in the body weight was observed in the alloxan diabetic rats treated with curcumin may be due to the retained levels of glucose and insulin levels because of the antioxidant effects of curcumin[37-38]. Moreover, several studies have shown that 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 [39-40].

In addition, Kumar [41] reported that rat injected with Streptozotocin had been shown a marked raise in plasma glucose level and decrease in insulin level. The present results may be explained by alloxan action in β-cells is accompanied by characteristic alterations in blood insulin and glucose concentrations, two days after injection, the hyperglycemia is observed with a Concomitant drop in blood insulin, and finally hyperglycemia develops and blood insulin levels decrease. This might be attributed to alloxan was particularly toxic to the pancreatic, and injection with alloxan leads to the degeneration of the Langerhans islets β-cells[42] . Moreover, alloxan induced destruction of *β*-cells of islets of Langerhans and causing degranulation and reduction of insulin secretion as proposed by[43] . In the current study, we observed that very highly significant alterations in serum glucose concentrations and insulin levels in diabetic groups treated with curcumin, ginger or the both curcumin and ginger mixture as compared with control non-diabetic group (-ve). This result are conforming with the result of [44,45] whose reported significant difference between alloxan diabetic rats treated with curcumin at a dose level of 250 mg/kg for 7 weeks and 100 mg/kg for 8 weeks and non-diabetic control group. Similar results were reported by[22,27] who revealed significant difference between alloxan diabetic rats treated with ginger and normal rat.

The hypoglycemic effect of curcumin may be attributed to curcumin supplementation ameliorates muscular insulin resistance by increasing the uptake and oxidative of fatty acids and glucose in skeletal muscles [44].Moreover, Best [46]reported that 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. Furthermore, curcumin protected islets against alloxan induced oxidative stress and corresponding islet damage and dysfunction by scavenging free radicals [47]. These results may in part explain the decrease serum glucose and increase insulin in diabetic rats fed curcumin in our study. Despite the positive results, some studies have shown conflicting results, where[48]) reported similar results of blood glucose in diabetic rats and diabetic rats treated with dietary curcumin, and reported that curcumin did not prevent alloxan-induced hyperglycemia. The underlying mechanisms by which curcumin can lower blood glucose is not fully defined [9].

In the present experiment, the hypoglycemic effect of ginger were in conformity with the results of many studies, the hypoglycemic effect of ginger may be attributed to the bioactive and pharmacological compounds of ginger they may help in suppressing the free radical in diabetes, this will ultimately lead to decreased levels of blood glucose [49]. Chakraborty *et al.* [50] 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. The key enzymes controlling carbohydrate metabolism associated with hyperglycemia in diabetes are α-amylase and α-glucosidase, ginger extract showed the highest α-glucosidase and α- amylase inhibitory activities, the action of ginger extract against these two enzymes found to be correlated with the phenolic contents of gingerol and shogaol in these extracts [51],on the other hand Ginger ethanolic extract has shown insulin tropic action similar to a sulphonylurea drug(chlorpropamide), and enhanced insulin sensitivity at the cellular level. The active compounds of ginger are 6-gingerol, tannins, polyphenolic compounds, normal control group. Diabetic rats treated with ginger flavonoids and triterpenoids of hypoglycemic that aqueous extract or methanolic extract or oil showed maintain cell function related to receptors and membrane transport[52]

Curcumin, one of the major phenolic curcuminoids of turmeric, has been shown to reduce hyperglycaemia and hyperlipidaemia in type 2 diabetic mice as well as STZ-diabetic and alloxan-diabetic rat models[53]. Also, it prevents the oxidation of LDL[54]. Tetrahydrocurcumin (one of the major metabolites of curcumin) exhibited antidiabetic and antioxidant properties in STZ–nicotinamide diabetic rats[53]. Curcumin and tetrahydrocurcumin protected pancreatic b-cells from reactive oxygen species generated in diabetes by scavenging free radicals and reactivating the antioxidant defence system[55]. They significantly increased the tissue GSH level in STZ–nicotinamide diabetic rats, which in turn activated the GSH-dependent antioxidant enzymes (such as GPx and GST) and detoxified the highly reactive intermediates of STZ[53]. However, the antioxidant activity of tetrahydrocurcumin is more potent than that of curcumin. Feeding STZ-diabetic rats with turmeric decreased the TBARS level and increased the activities of antioxidant enzymes such as SOD,CAT, GPx and GST in erythrocytes[56]. The present results are consistent with the aforementioned reports, which may explain the beneficial effects of ginger and turmeric as well as their mixture[57].

Effect of oral administration of Curcumin or ginger alone and their mixture on serum MDA,lipid profile and some enzymatic antioxidant activity in diabetic rats

Table (2) showed the effect of curcumin, ginger or their mixture on serum MDA level and some antioxidant enzymes activity SOD,GPx and CAT in alloxan -diabetic male 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 ,GP_X and CAT enzymes activity as compared with control (-ve) group, with percentage (270%, -40% and -55%, respectively) as percent change from control group. Diabetic groups treated with curcumin, ginger or their mixture showed improvement in MDA level , SOD GPx and CAT enzymes activity, and these 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, but the improvement was in a dose manner .The data also demonstrated that serum MDA level and SOD,GPx, CAT enzymes activity in diabetic group treated with curcumin recorded more significant differences (p < 0.01) when compared with ginger treated group. Treatment with curcumin and ginger mixture showed non-significant difference when compared with control group, which demonstrated highly significant improvement (p < 0.01) as compared with ginger treated group or curcumin treated group.

Table 2: Serum Malondialdehyde and enzymatic antioxidant activities in control and diabetic rats treated with curcumin or aqueous ginger extract alone or their mixture after 8 weeks.

Experimental group	SOD(U/ml)	GPX(U/ml)	CAT(U/ml)	MDA(µM)
Control	2.1±0.5	3.7±0.8	4.6±1.1	1.8±0.5
Diabetic	0.9 ± 0.3^{Ac}	1.7 ± 0.6^{Ad}	2.1 ± 0.8^{Ab}	6.1 ± 0.9^{AC}
Diabetic+ CUR	1.7 ± 0.6^{Ba}	3.2 ± 0.7^{Bc}	3.9 ± 0.6^{Aa}	2.2 ± 0.8^{Aa}
Diabetic +GIN	1.6 ± 0.4^{Cns}	3.0 ± 0.5^{Cns}	3.6 ± 0.5^{Ca}	2.4 ± 0.7^{Cns}
Diabetic mixture (CUR+GIN)	1.9 ± 0.6^{Da}	3.5 ± 0.7^{Da}	4.3 ± 1.0^{Da}	1.9 ± 0.6^{Da}

Oxidant and antioxidant balance is widely accepted as a fundamental participant in the development and progression of DM and its complications [(58)]. Increasing evidence shows that oxidative stress is elevated under diabetic conditions due to increased production of reactive oxygen species(ROS) and deficient antioxidant defence [(59).] Furthermore, there are many reports indicating that β-cells are particularly susceptible to reactive species because they are low in free radical quenching (antioxidants) enzymes such as CAT, SOD, GPx, and GRx [(60)]. In this study, we noticed an increase in MDA level in alloxan diabetic rats indicating increased free radical production and associated lipid peroxidation. However, treatment of diabetic rats with aqueous ginger extracts significantly reduced the MDA level and the reduction is a dose dependent manner . Accordingly, we presumed that treatment of diabetic rats with aqueous ginger extracts attenuated the production of free radical and peroxidation of lipids thereby preventing oxidative damage of cellular structures. The results obtained from this study also demonstrated that ginger extract has an ability to increase the intracellular activities of SOD,GPx, CAT and GSH enzymes. against a significant decrease in the SOD, CAT, GPx and GSH activities in diabetic rats group(+ve). This is probably indicative of an insufficient antioxidant defence against free radical mediated damage. Treatment of diabetic rats with ginger extracts however produced increased activities of all these antioxidant enzymes. In view of our findings that ginger extracts up-regulate SOD, CAT, GPx activities in diabetic rat, Thus we suggest that *Z. Officinale* synergistically combats oxidative stress by scavenging free radicals and/or augmenting endogenous antioxidant activities thereby improving diabetic condition**.**

Figure (1) showed the effect of curcumin, aqueous ginger or their mixture on serum lipid profile levels in alloxan-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. Diabetic groups treated with curcumin, aqueous 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 the curcumin and ginger 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, aqueous 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**.**

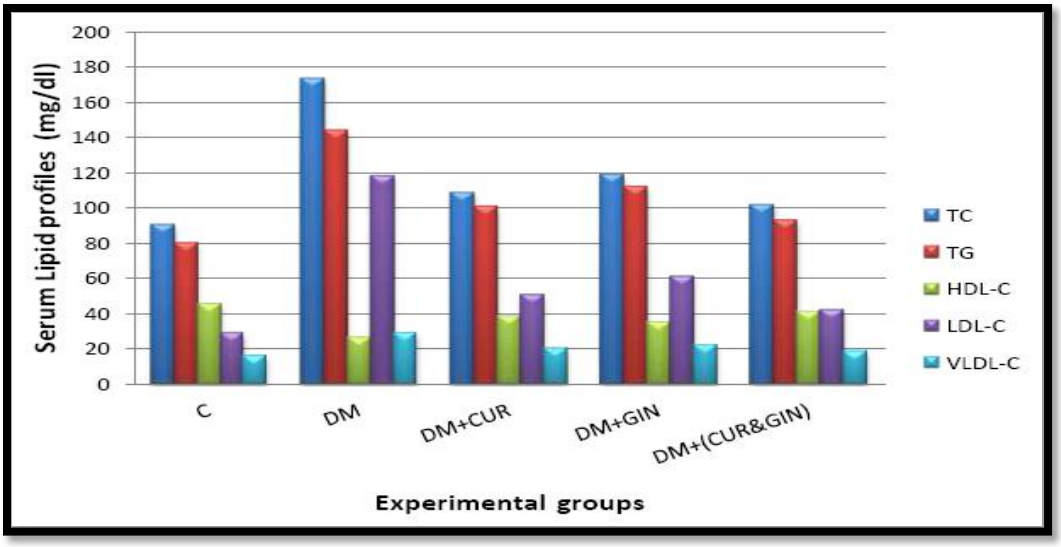

Figure 1. Serum lipid profile levels (mg/dl) in control and alloxan induced diabetic rats (DM) treated with curcumin (CUR), ginger (GIN) or their mixture (CUR & GIN) after 8 weeks.

Effect of oral administration of Curcumin or ginger alone and their mixture on Serum GSH, Vitamin E and C in diabetic rats

Data from table 3 showed that there are a significant reduction of each GSH , Vitamin E and C in diabetic rats compared with non diabetic group, while diabetic rats which received curcumin and aqueous ginger extract showed a significant increase and the improvement was at a dose dependent ,but the most clear improvement was observed in all rats treated with a mixture of curcumin and aqueous ginger extract. Vitamin C may play an important role in physiological reactions such as mixed function oxidation involving incorporation of oxygen into a biochemical substrate. In addition, vitamin C is considered the most important antioxidant in extracellular fluids and its antioxidant function has been shown to efficiently scavenge superoxide, hydrogen peroxide, hydroxyl, peroxyl and singlet oxygen radicals [(61,62)].Vitamin C has been reported to efficiently scavenge free radicals before they can initiate lipid peroxidation, and contribute to stability of cellular and basal membranes [(63)]. Although both vitamins serve as free radical scavengers in biological system, vitamin C is hydrophilic and exerts its effect in the extracellular space, trapping radicals in the aqueous phase [(64)], while vitamin E is a lipid soluble antioxidant within the cells, where the reactive metabolites are actually produced (65). Furthermore, vitamin C interacts with tocopheroxyl radical and generates the reduced tocopherol.

Table 3: Serum non enzymatic antioxidant levels in control and diabetic rats treated with curcumin or aqueous ginger extract alone or their mixture after 8 weeks.

Experimental group	GSH(µM)	Vit E(mg/dl)	Vit C(mg/dl)
Control	3.3±0.92	0.45±0.12	1.34±0.23
Diabetic	1.33 ± 0.56^{Ad}	0.22 ± 0.11^{Ad}	0.66 ± 0.16^{Ad}
Diabetic+ Curcumin	2.88 ± 0.78^{Bc}	0.37 ± 0.16^{Ba}	1.05 ± 0.14^{Ba}
Diabetic +Ginger extract	2.61 ± 0.64^{Cns}	0.32 ± 0.13^{Cns}	0.96 ± 0.11^{Cn}
Diabetic +mixture (CUR+GIN)	3.1 ± 0.59^{Da}	0.41 ± 0.15^{Da}	1.23 ± 0.20^{Da}

Elevated levels of lipid peroxide in DM may be due to the alteration of function of erythrocytes membrane, and a deficiency of the antioxidant activity of vitamins (C and E) has been related to higher concentration of peroxide(66). There may be imbalance between production and scavenging of free radicals produced due to the lack of antioxidant system (67). The mean plasma Vit.C in type 2 diabetes mellitus was 0.48mg/dl which was significantly lower than that of control group (1.18mg/dl), This result was in agreement with other observations (the biochemical evidence of ascorbate deficiency in the presence of diabetes could be due to impaired tubular reapportion or increased oxidation (68).

Conclusion:-

This study demonstrated that curcumin and aqueous ginger mixture possesses significantly reduction in hyperglycemic, hyperlipidemic and antioxidant effect in alloxan induced 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 mixture of both curcumin and ginger could be excellent adjuvant support in the therapy of diabetic mellitus and prevent its complications.

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