The biochemical role of curcumin nano-suspension in the metabolism of experimentally induced diabetic rats

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A B S T R A C T

This study was designed to determine the biochemical effect of curcumin nano-suspension on alleviating metabolic disorders in experimentally induced diabetic rats. An experimental diabetic rat model was induced by 60mg/kg body weight of streptozoticin (STZ) as a single dose. Curcumin nano-suspension was administrated orally at a dose of 300mg/kg body weight daily for 56 days. Metabolic profile was examined biochemically. Administration of curcumin nano-suspension decreases CK-MB, LDH and AST serum values in STZ- diabetic rats treated with curcumin nano-suspension (203±5.04 and 604±12.85; 1910±7.97 and 4739±120.17, and 125± 12.13 and 174 ± 16.10) respectively, when compared to untreated diabetic rats, it decreases triacylglycerol in serum (124±2.01 and 144±11.4mg/dl) compared to the diabetic rats also, with not so much increase the body weight (242±4.94 and214±18.04gm). On the other hand, there were no changes in concentrations of glucose (346.50±11.04 and 329.50±42.86mg/dl); insulin (0.92±0.66 and1.10±0.18µl/ml) and the total cholesterol level (105±5.04and 112±17.18mg/dl) in serum compared to diabetic rats. In conclusion, oral ingestion of curcumin nano-suspension reverses side effects result from metabolic disorders in streptozotocin induced diabetic rats via its anti-inflammatory and hypolipidemic role.

Keywords: Diabetes, streptozotocin, curcumin nano-suspension.

1. INTRODUCTION

Diabetes mellitus is characterized by chronic hyperglycemia with disturbances in carbohydrate, fat and protein metabolism resulting from defects in insulin secretion and/or insulin action (Nammi et al., 2003).

Yu and Huang, (2010) stated that functional food is defined as foods that provide health benefits depending on its basic nutrition and polyphenols such as curcumin are among these functional food ingredients as a result of its anti-oxidant, anti-inflammatory and anti-cancer properties, furthermore, Chinese medicine used curcumin as an ameolating agent for diabetes (Shishodia et al., 2005).

As a result of poor oral bioavailability of curcumin which include poor aqueous solubility, intestinal metabolism, hepatic metabolism and rapid systemic clearance, it has not yet been approved as a therapeutic agent, although it is extremely safe even at very high doses in both of animal and human studies (Aggarwal and Harikumar, 2009). Thus, nanoparticle technology helps as a prominent solution to the poor bioavailability of some therapeutic agents as curcumin (Ebtihal et al., 2014).

In 2006, Murugan and Pari showed that curcumin significantly reduced blood glucose, serum and liver cholesterol, triglycerides, free fatty acids, hydroxyl methylglutaryl- coenzyme A (HMG- CoA) reductase activity, very low density lipoprotein (VLDL) and low density lipoprotein (LDL) cholesterol levels. While Murugan and Pari, (2006) suggested that curcumin caused a significant increase in

the plasma insulin levels and serum high-density lipoprotein (HDL). Cholesterol was also reversed toward normalization. In addition, there was reduction in oxidative stress and lipid peroxidation in STZ-induced diabetic rats.

The aim of the present study is to determine the biochemical and the anti-inflammatory roles of curcumin nano-suspension in the metabolic disorders and side effects results from induction of streptozotocinin rats.

2. MATERIAL AND METHODS

2.1. Streptozotocin (STZ)

STZ was obtained from (MP Biomedicals, LLC), STZ is used as a single dose of STZ 60 mg/kg body weight dissolved in 0.1M citrate buffer (pH 4.5) in a volume of 1ml/kg body weight freshly prepared and injected within 5 minutes of preparation intrapretonially (Ganda et al.,1976).

2.2. Curcumin nano-suspension

A 1M curcumin with low solubility in water was mixed with 4M sodium bicarbonate buffer, then grinded using mechanical ball mill (350 rounds /sec) for 8 hrs. The colour of curcumin changed from yellow to red as a result of the curcumin sodium salt formation.

Curcumin nanoparticles were then dispersed into 50 ml of distilled water making aqueous solution which was filled in a reactor that was immersed in a water bath adjusted at 11ºC. Afterwards, this reactor was placed in an ultrasound apparatus (VCX-750 commercial sonicator) and sonication was applied in continuous mode at 100 Watt in a glass reaction vessel with thin and indented bottom for uniform and more efficient energy transmission (Hassan et al., 2014).

2.3. Experimental animals

A total number of 54 white male albino rats weighing between (230±20g) were purchased from The Animal House Unit (University of Benha, Faculty of Veterinary Medicine, Animal Breeding and Research Center). The animals were kept under hygienic conditions for at least two weeks before the initiation of the experiment. The animals were kept under a 12h light-dark cycle and ambient temperature was maintained at 25◦C. Animals were allowed free access to water and were fed on uniformly basal diet.

2.4. Induction of diabetes

Rats were subjected to hyperglycemia by intrapretonially injection of freshly prepared STZ (dissolved in 0.1M citrate buffer, pH 4.5) as a single dose (60 mg/kg body weight) in a volume of 1ml/kg body weight. The blood samples were collected from retro-orbital venous plexus of eyes by using fine capillary glass tubes and used directly for blood glucose determination after three days of (STZ) injection. Rats with blood glucose level ranged from 280–350 mg/dl were considered diabetic and included in the study (Camet al., 2003).

2.5. Experimental design

The animals were divided into 3 equal groups (18 rats for each). Group one served as a control normal group and was injected with the same volume of citrate buffer used to dissolving of STZ. The remaining 36 rats were subjected to hyperglycemia by STZ injection. The hyperglycemic rats were divided into 2 groups as follows:

Group two: (STZ-Diabetic) was given saline by an oral gavage and served as control positive.

Group three: (Diabetic + curcumin nano - suspension) was supplemented orally (with an oral gavages) at a dose of 300 mg/ kg body weight/day in a volume of 1ml / rat (Nishiyama et al., 2005).

After 56 days of treatments and in the basal fasting state, experimental rats of each group were weighed separately.

Blood samples were collected from retro-orbital venous plexus of eyes in clean, dry screw capped tubes. Samples were allowed to coagulate at room temperature for 30 minutes and centrifuged at 3000 RPM for 15 minutes. The clean clear serum was aspirated by pasture pipettes and received

in dry sterile sample tubes, processed directly for glucose determination then kept in deep freeing at -20°C until used for the other biochemical analysis.

2.6. Biochemical Analysis

Serum glucose, insulin, total cholesterol and triacylglycerol were determined according to the methods used by Trinder, (1969); Babaet al., (1979); Allain et al., (1974) and Schettler and Nussel, (1975) respectively. Creatine kinase-MB (CK-MB), Lactate dehydrogenase (LDH) and Aspartate amino transferase (AST) levels in serum determined according to the methods described by Urdal and landaas, (1979); Kornberg (1955) and Reitman and Frankel, (1957).

2.7. Statistical analysis

All data were presented as the mean ± Standard Error (SE). The data were evaluated by a one-way ANOVA.

3. RESULTS

Data in table (1) and figure (1) revealed that, STZ-diabetic group showed significant decrease in the body weight and serum insulin level accompanied with significant increase in serum values of glucose and triacylglycerol levels. There was a non-significant change in serum total cholesterol level compared to the control normal rats. Regarding to curcumin nano-suspension treated –diabetic rats, the obtained results showed a significant decrease in serum triacylglycerol level. However, there was non-significant effect the body weight recovery and serum values of fasting glucose, insulin and total cholesterol compared to STZ-induced diabetic rats.

Data in table (2) and figure (2) revealed that, there was marked increase in CK-MB, LDH and AST serum values of STZ-induced diabetic rats when compared to the control normal rats. On the other hand, our findings revealed significant decrease in CK-MB, LDH and AST serum values in STZ- diabetic rats treated with curcumin nano-suspension when compared to untreated diabetic rats.

Table (1): effect of curcumin nano-suspensionon body weight and serum level of (glucose; insulin; total cholesterol and triacylglycerol) in Streptozotocin-induced diabetic rats:

Parameter	Group (1)	Group(2)	Group(3)
Body weight (gm)	402 ±14.98[a]	214±18.04[c]	242±4.94[bc]
glucose (mg/dl)	117.50±4.40[b]	329.50±42.86[a]	346.50±11.04[a]
insulin (µl/ml)	3.75±0.11[a]	1.10±0.18[c]	0.92±0.66[c]
Totalcholesterol (mg/dl)	98.50±4.15	112±17.18	105±5.04
triacylglycerol (mg/dl)	115±1.35[b]	144±11.4[a]	124±2.01[b]

Groups1, 2 and 3: Control group, Diabetic group and STZ-curcuminnano-suspension treated group. Data are represented as (Means ±S.E).Values with different letters within the same row significantly differed at (p< 0.05).

Table (2): effect of curcumin nano-suspensionon serum level of creatine kinase-isoenzyme (CK-MB), lactate dehydrogenase (LDH), aspartate aminotransferase (AST) in Streptozotocin-induced diabetic rats:

Parameter	Group (1)	Group(2)	Group(3)
CK-MB(U/L)	129±5.59 [d]	604±12.85[a]	203±5.04 [c]
LDH(U/L)	1118±26.59[d]	4739±120.17[a]	1910±7.97[c]
AST(U/L)	87.50± 1.89[c]	174 ± 16.10[a]	125± 12.13[b]

Groups1, 2 and 3: Control group, Diabetic group and STZ-curcuminnano-suspension treated group. Data are represented as (Means ±S.E).Values with different letters within the same row significantly differed at (p< 0.01).
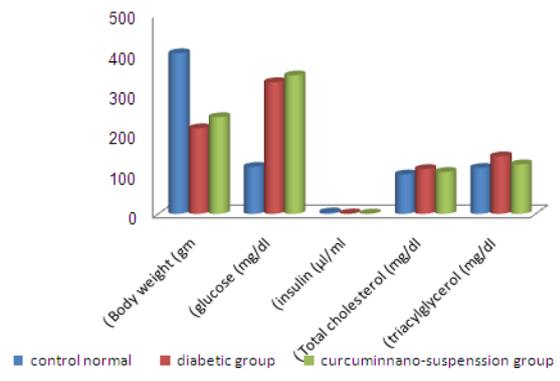
Fig1. Effect of curcumin nano-suspension on body weight and serum level of (glucose; insulin; total cholesterol and triacylglycerol) in Streptozotocin-induced diabetic rats
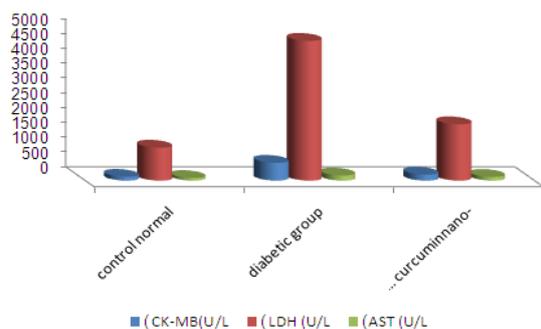

Fig2. Effect of curcumin nano-suspension on serum level of creatine kinase-isoenzyme (CK-MB), lactate dehydrogenase (LDH), aspartate aminotransferase (AST) in Streptozotocin-induced diabetic rats

4. DISCUSSION

The data recorded 3 days after STZ injection showed development of hyperglycemia in the experimental rats; these results came in agreement with Dolan, (1997) who suggested that STZ inhibits the glucose metabolism and insulin secretion of beta cells. STZ-diabetic group was significantly decreased in the body weight, our came in agreement with Juarez-Rojop et al., (2012) who found that sever polyuria and degradation of fat and structural proteins during diabetes result in reduction in body weight of diabetic rats.

STZ-injected rats also developed a significant hyperglycemia and hypo-insulinemia. Our findings agreed with Thomson et al., (2016) who found a reduction in serum insulin level of diabetic rats compared to normal control one. STZ –diabetic rats showed a non-significant

effect in serum total cholesterol level although the triacylglycerol level was significantly increased. These findings are agreed with ElokZubaidah et al., (2014) who found that total serum cholesterol, LDL triglycerides and HDL did not significant changed in diabetic rat model.

In addition, the current study comes in agreement with Thamolwan et al., (2016) who showed that serum levels of AST, LDH and CK-MB marked increased in diabetic rats, and suggested that diabetes mellitus impaired liver, kidney and heart functions.

Regarding to curcumin nano-suspension treated group, data revealed a non-significant recovery in the body weight and non-significant effect on both of serum fasting glucose and insulin levels. This comes in agreement with Majithiya and Balaraman, (2005). Meanwhile there were a significant decrease in serum triglycerides, CK-MB, LDH and AST serum values in STZ- diabetic rats with non-significant effect on the level of total cholesterol in curcumin nano-suspension treated diabetic rats our results agreed with Gutierres et al., (2012) who reported that curcumin -supplemented yoghurt helps in improving physiological and biochemical markers of STZ-diabetic experimental rats. El-Moselhy et al., (2011) stated that curcumin might reduce plasma FFA. Also, Weisberg et al., (2008) showed that oral ingestion of curcumin reverses many of the inflammatory and metabolic disorders associated with obesity and improves glycemic control via reduction macrophage infiltration of white adipose tissue, increased adipose tissue a diponectin production, and decreased hepatic nuclear NF_K- β activity, hepatomegaly and markers of hepatic inflammation in mouse models of T2D.

CONCLUSION

The present study revealed that curcumin nano-suspension is a prominent modified plant has an anti-inflammatory role helps in avoiding side effects results from

diabetes with a limited attenuating effect on the metabolic disorders of streptozotocin induced diabetic rats.

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