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EFFECT OF REPAGLINIDE AND CURCUMIN COMBINATION ON OXIDATIVE STRESS AND BIOCHEMICAL PARAMETERS IN STZ INDUCED DIABETIC RATS

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ABSTRACT
Aim: Repaglinide is an antihyperglycemic agent used for the treatment of diabetes mellitus. It has a good safety and efficacy profile in diabetic patients with complications in renal impairment. Curcumin is a diaryl heptanoid and is the principal curcuminoid of the turmeric. Both repaglinide and curcumin have antioxidant properties. The main aim of the present study was to examine the protective effect of repaglinide in the presence of curcumin in streptozotocin (STZ) induced diabetic rats. **Methods and Results:** For this study all the rats were divided into four groups (n=6). Diabetes was induced into all the rats by STZ 55 mg/kg, intra peritoneally. Group I represents diabetic rats as control and received normal saline orally, group II rats administered repaglinide orally for 28 days, group III rats administered curcumin orally for 28 days and group IV rats administered with curcumin and followed by repaglinide for 28 days orally. Blood samples were collected on 1st day, 7th day, 14th day, 21st day and 28th day and used for further analysis. STZ administration destroys the β-cells and glucose in the blood of diabetic rats was increased and there by increases the SGOT, SGPT, total cholesterol, reactive oxygen species (ROS) and lipid peroxidation and decreases the total proteins, and serum insulin levels. By administration of repaglinide, curcumin and repaglinide+curcumin combination reverses the results significantly. **Conclusion:** Combination of curcumin with repaglinide showed significant changes in various serum biochemical parameters and antioxidant status than repaglinide and curcumin alone groups.

KEYWORDS: Antioxidant, Biochemical parameters, Curcumin, Lipid peroxidation.

INTRODUCTION
Diabetes is one of the oldest diseases[1] and is recognized as the most common metabolic and endocrine disorder.[2] By various pathways reactive oxygen species (ROS) are generated disproportionately in diabetes. Usually ROS damages different body organs by peroxidation of membrane lipids, oxidation of proteins, DNA and other intracellular macro molecules. Structural damage to liver and pancreas as well as complications of diabetes like nephropathy may result from oxidative stress.[3]

Curcumin is a diaryl heptanoid and is the principal curcuminoid of the turmeric.[4] It is a well-known antioxidant[4] and exhibit more pharmacological activities like anti-cancer,[5] antidiabetic,[6] antibacterial,[7] anti-inflammatory,[8] and anti-microbial,[9] etc.

Repaglinide is a first short acting new class of insulin secretagogue used in the treatment of diabetes. As it is both rapidly absorbed and eliminated, it is suitable for administration preprandially giving good overall glycemic control without the risk of hypoglycemia.[10] This drug acts on the sulfonylurea receptor SUR1 of the pancreatic B-cell but at a different site to sulfonylureas.[3] It has been reported that repaglinide treatment decreases the lipid hydroperoxide concentration and increases the activity of super oxide dismutase.[11] Based on the above facts the oxidative stress parameters like total antioxidant status and malondialdehyde (MDA) plays very important role in the diabetes. By considering these facts we have studied the effect of total antioxidant status, lipid peroxidase (MDA) along with total proteins, triglycerides, total cholesterol, serum insulin and liver function parameters like serum glutamate oxaloacetate transaminase (SGOT), and serum glutamate pyruvate transaminase (SGPT).

MATERIALS AND METHODS
MATERIALS
Chemicals
Repaglinide was obtained as gift sample from Novartis, Hyderabad, India. Curcumin was obtained as a gift sample from Nisarg Biosciences, Hyderabad, India. Ascorbic acid, 1, 1- diphenyl-2-picryl hydrazyl (DPPH), 1,1,3,3 – tetraethoxy propane (TEP), Streptozotocin and

thiobarbituric acid was purchased from Sigma-Aldrich, Bangalore, India. HPLC grade methanol was purchased from Merck, Mumbai, India. Merck analytical kits were used to estimate the serum biochemical parameters. Water used for analytical purpose was double distilled, filtered by using direct-Quv Millipore. All other chemicals used for study were of analytical grade.

Animals

Male albino wistar rats (200-280 g) were purchased from Sainath Agencies, Hyderabad, India and used for the study after obtaining permission from the institutional animal ethical committee (CPCSEA Reg. No. IAEC/07/UCPSc/KU/2016). The animals were housed in standard polypropylene cages and maintained under standard laboratory conditions (12 h light/dark cycle, at an ambient temperature of 25±5 °C, 35-60 % relative humidity). The animals were fed with standard rat pellet diet and water *ad libitum*.

METHODS

Induction of diabetes in rats

Male albino wistar rats (200-280 g) were randomly selected and fasted overnight for study and diabetes was induced by intra peritoneal injection of (streptozotocin) STZ 55 mg/kg, in freshly prepared citrate buffer (pH 4.5).[12] After 72 hrs, blood samples were collected from rats by retro orbital puncture and the serum was analyzed for glucose levels by oxidase- peroxidase (GOD) method.[13] The animals with blood glucose level >250 mg/dl were considered as diabetic and were used in the study.

Experimental design

After overnight fasting, rats were randomly divided into four groups (n=6). Group I diabetic rats were used as control, group II administered orally with repaglinide, 0.2 mg/kg[14] daily for 28 days, Group III administered orally with curcumin, 80 mg/kg[12] daily for 28 days, Group IV administered orally with curcumin (80 mg/kg PO) followed by repaglinide (0.2 mg/kg PO) daily for 28 days.

Blood samples were collected through retro orbital plexus and immediately same volume of normal saline was replaced intra peritoneally. The blood was collected at 1st day, 7th day, 14th day, 21st day and 28th day in every group. Serum was separated after centrifugation at 8000 rpm for 15 min and the samples were stored at – 20 °C until analysis.[12]

Estimation of serum biochemical parameters

Triglycerides (TG), total cholesterol (TC), SGOT and SGPT, Serum insulin based on chemiluminescence assay principle are analysed by using semi-autoanalyser.[15,16] Serum total protein content was estimated by the method of Lowry *et al*., 1951.[17] Proteins form chromophoric complex with phenol reagent, which was measured at 610 nm.

General Procedure

The blood samples collected from the animals of the study and serum was separated by centrifugation. Then 0.5µl of serum sample was used to estimate each biochemical parameter by placed at their respective positions in the semi-autoanalyzer [diaSIL-100]. After 30 min of programming the test parameter (s), the corresponding parameter values of the different serum samples were recorded.

Total antioxidant status

The total antioxidant status in serum samples was determined by using DPPH method.[18] A methanolic DPPH solution (0.1 mM, 1 ml) was mixed with serum samples of 1, 7, 14, 21 and 28 days treatment from all the groups. The absorbance was read at 517 nm. Ascorbic acid was used as a reference standard. The standard graph was prepared using different concentrations of ascorbic acid in the range of 1-10 nM/mL in water and the antioxidant status values were expressed in terms of nM of ascorbic acid.

Lipid peroxidation

Lipid peroxides in serum was estimated by the method of Ohkawa *et al.*, 1979.[19] MDA was used as the reference. The standard graph of the MDA was plotted by using different concentrations (1-10 nM/mL) and was prepared by using TEP reagent as the standard and the MDA content in the serum.

Statistical analysis

All the results were expressed as mean±SD. The data were statistically evaluated using one way ANOVA with Bonferroni post-test applied for statistical significance. Values corresponding to $p<0.05$ were considered as significant and $p<0.01$ considered as more significant.

RESULTS AND DISCUSSION

Serum biochemical parameters

The effect of repaglinide, curcumin and repaglinide+curcumin combination was studied in the STZ-induced diabetic rats. The TC and TG levels were 220.14±2.71, 213.23±4.7, 232.86±3.01 and 227.8±3.91, 206.7±4.06, 215.6 ±3. 78 mg/dL respectively, at the 1st day of the treatment. At the 28th day, the levels of TC and TG were found to be 148.9±4.83, 140.7±3.62, 138.2±4.05 and 159.8±2.12, 128.8±1.80, 109.5±1.65 mg/dL, respectively. The lipid profiles of repaglinide, curcumin and repaglinide+curcumin were decreased 32.4%, 34.2% and 40.6% respectively in TC levels, when compared with 1st day treatment. In case of TG levels, 29.8%, 37.7% and 49.2% decrease after 28th day in repaglinide, curcumin and repaglinide+curcumin treated groups, respectively. The results were statistically significant compared to diabetic rats. The results are showed in Table 1. Therefore, the combination of repaglinide+curcumin showed enhanced antilipidemic activity in diabetic conditions. These results are in agreement with earlier study reported by Essam and Ashraf, 2014 in which curcumin showed hypolipidemic

effect on *S.mansoni* infected mice and total serum cholesterol and serum triglycerides were measured and it lowered the TC and TG levels.[20]

Table 1: Lipid profiles of repaglinide, curcumin and repaglinide+curcumin on total cholesterol (mg/dL) and triglycerides (mg/dL) in diabetic rats after oral administration (mean ± SD, n=6).

Group	Total cholesterol (mg/dL)		Triglycerides (mg/dL)	
	1st Day	28th Day	1st Day	28th Day
Control	223.1±4.26	231.8 ±5.91	220.4±2.88	244.9 ±4.70
RP	220.14±2.71	148.9 ±4.83*** (32.4%)	227.8±3.91	159.8±2.12** (29.8%)
CUR	213.23±4.7	140.7±3.62*** (34.2%)	206.7±4.06	128.8±1.80** (37.7%)
RP + CUR	232.86±3.01	138.2±4.05*** (40.6%)	215.6±3.78	109.5±1.65** (49.2%)

Values are Mean±SD; RP: Repaglinide, CUR: Curcumin, Total cholesterol (mg/dL) *** significant at p<0.001 compared to control, **Significant at p<0.01 to control.

The effect of repaglinide, curcumin and repaglinide+curcumin combination in SGOT, SGPT, total proteins and insulin levels in the STZ-induced diabetic rats was showed in the Table 2. The SGOT levels in the treated groups were decreased (20.9%, 26.1% and 29.4%) in repaglinide, curcumin and repaglinide+curcumin treated groups from 1st day to 28th day. This was statistically significant (p<0.01) when compared with diabetic control group. The reduction in SGPT levels in treated groups from 1st day to 28th day was found to be 39.4%, 40.5% and 45.0%. But in case of total serum proteins and serum insulin, the combination shows increased levels than repaglinide and curcumin treated groups.

Table 2: Serum biochemical parameters in diabetic rats after oral administration of repaglinide, curcumin and repaglinide+curcumin (mean ± SD, n=6).

Groups	SGOT (U/L)		SGPT (U/L)		Serum total protein (mg/dl)		Serum insulin (µIU/ml)	
	1st day	28th day	1st day	28th day	1st day	28th day	1st day	28th day
Control	146.3±7.2	152.2±7.4	144.8±5.3	150.9±6.1	3.2±0.4	3.1±0.7	24.3±3.0	20.1±2.6
RP	135.1±4.3	106.8±6.4** (20.9%)	129.5±5.7	78.4±3.2** (39.4%)	3.0±0.5	4.3±0.3** (43.3%)	24.9±1.1	36.7±2.4** (47.4%)
CUR	141.6±6.2	104.6±6.0** (26.1%)	131.2±2.4	78.1±2.7** (40.5%)	3.1±0.3	4.8±0.6** (54.8%)	24.1±0.7	38.3±1.2** (58.9%)
RP + CUR	138.3±4.8	97.6±3.7** (29.4%)	128.5±6.2	70.6±2.2** (45.0%)	3.2±0.7	5.6±0.8** (75%)	22.6±0.6	40.6±1.5** (79.2%)

All values are expressed as mean ± SD, RP: Repaglinide, CUR: Curcumin, Values given in the parenthesis are the percent increase or decrease in respective parameter level; * p < 0.05; ** p < 0.01 when compared with control at the respective time interval.

However, the percentage reduction in SGOT, SGPT levels was maximum in group IV than remaining groups, because curcumin also possess hepatoprotective activity reported by Reham, 2017 and which plays a role in reducing the liver enzymes. The significant (p<0.01) reduction in SGOT and SGPT levels further strengthens the antidiabetogenic effect of curcumin because increased gluconeogenesis and ketogenesis occur in diabetes, which may be due to high levels of SGOT and SGPT.[21]

Further, the increased serum total protein level brought out by curcumin explains the antidiabetogenic effect, as a reduction in protein level takes place in diabetes due to deficiency of insulin, which stimulates uptake of amino acids into muscle and increases protein synthesis. These results are in agreement with earlier study reported by Sandhya *et al.*, 2012, in which combination of curcumin and glimepiride for 28 days pretreatment in STZ induced diabetic rats shown maximum percentage increase in total proteins level than alone pretreated groups.[12] Mosley *et al.*, 2011 reported that curcumin oral administration showed an antihyperglycemic effect and improved the insulin sensitivity.[22]

Antioxidant status

The ascorbic acid was used as standard for the measurement of antioxidant activity. The calibration curve of ascorbic acid was plotted in the linearity range of 1-10 nM/mL. The correlation coefficient was 0.999. The antioxidant activity of treated groups after 28 days was statistically significant (p<0.01), compared with diabetic rats as follows: repaglinide+curcumin treated group > curcumin treated group > repaglinide treated

groups. The combination of repaglinide+curcumin shows synergetic activity than treated groups with repaglinide and curcumin alone. The levels of antioxidant status were showed in Figure 1. Gumieniczek, 2005 reported that repaglinide reduces the oxidative stress in diabetic patients and enhances the antioxidant activity.[3] In another study Mohammad *et al.*, 2014 reported that combination of sodium fluoride, selenium and curcumin extract enhances the antioxidant activity compared to control and reduced the MDA levels.[23]

Lipid peroxidation

The standard graph of MDA was linear in the concentrations range of 1 - 10 nM/ml, with correlation coefficient of 0.999. The lipid peroxide levels were decreased statistically significantly ($p<0.01$) in repaglinide+curcumin treated group when compared with groups treated with curcumin, repaglinide alone and also with control group at the 1, 7, 14, 21, ad 28 days intervals. The co-administration of curcumin exhibited synergetic activity in diabetic rats. The lipid peroxides levels of treated groups were showed in Figure 2. Ghosh *et al.*, 2015 reported that curcumin treated diabetic rats significantly inhibited hepatic lipid peroxidation.[24]

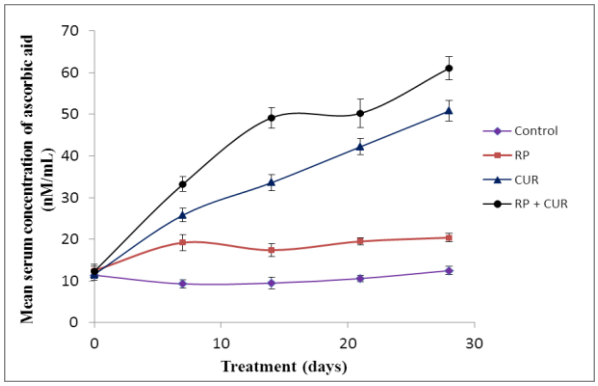

Figure 1: Effect of different groups on total antioxidant levels in diabetic rats (mean±SD, n=6).

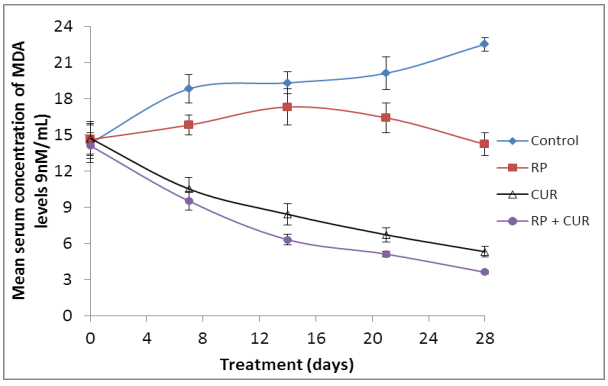

Figure 2: Effect of different groups on lipid peroxide levels in diabetic rats (mean±SD, n=6).

CONCLUSION

Repaglinide+curcumin combination showed significant changes in various serum biochemical parameters. Hence, repaglinide doses may require special attention if used along with the curcumin to avoid complications. This could be important in reducing the dose of repaglinide to achieve desired therapeutic effect with minimal adverse effects.

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