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Original Research

An Experimental Study to Evaluate Anti-Oxidant, Anti-Diabetic and Anti-Inflammatory Effect of Thymoquinone in Streptozotocin Induced Diabetic Rat

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Abstract

Background: This is an experimental study to evaluate the anti-oxidant, anti-diabetic and anti-inflammatory effects of thymoquinone in streptozotocin-induced diabetic rat.

Material and Methods: A total number of 30 Wistar rats were included in the study. Each group contained n=6 rats. Rats in Group 1 were maintained on normal pellet diet (NPD) and water ad libitum while rats in the remaining groups (2 to 6) were given high-fat diet (HFD). After 4 weeks of high fat diet all rats were fasted from 7 am to 3 pm (118). Rats on normal pellet diet were given citrate buffer and rats on high HFD were given a single intraperitoneal injection of Streptozotocin in the dose of 40 mg/kg BW.[11] STZ at a dose of 40 mg/kg was prepared in cold citrate buffer (pH 4.5, 0.1 M) and given intraperitoneally.[12] Rats were stabilized after one week of STZ injection.

Results: Our analysis for between-group differences in mean weights found statistically significant associations for the group A vs. B ($p<0.001$), A vs. C ($p<0.001$), B vs. D ($p=0.04$) and B vs. E ($p<0.001$). The highest positive mean difference was noted for group B vs. E (68 ± 15.61 g) followed by group B vs. D (48 ± 15.61 g) and group C vs. E (42 ± 15.61 g).

Conclusion: Our study involving diabetic rat model to understand the effect of TQ's anti-diabetic, anti-inflammatory and anti-oxidant properties as compared to metformin showed that the combination of TQ + metformin had superior properties as compared to either of these drugs administered alone. The increase in mean weight was significantly higher in TQ group as compared to the combination group (E) ($p<0.001$). And among monotherapies, metformin showed a similar effect on weight gain as TQ ($p=0.47$). Reduction in RBS levels were significantly lower in combination group (E) as compared to TQ alone ($p<0.001$). And among monotherapies, metformin showed similar effect on lowering of RBS levels as TQ ($p=0.48$). Reduction in IL-6 levels was significantly lower in combination group (E) as compared to TQ alone ($p<0.001$). Even among monotherapies, metformin showed a statistically significant lowering of IL-6 levels as compared to TQ ($p<0.001$). Reduction in MDA levels was significantly lower in the combination group (E) as compared to TQ alone ($p=0.03$). And among monotherapies, metformin showed similar effect on lowering of MDA levels as TQ ($p=0.99$).
patients.

Introduction

Diabetes Mellitus (DM) is a chronic metabolic disorder. In diabetes, glucose metabolism gets impaired due to insulin resistance to peripheral acting insulin, or insulin secretion is impaired by beta pancreatic cells. According to the International Diabetes Federation, the global prevalence of diabetes mellitus was 537 million (20 –79-year age) in 2021, with 90.2 million in the South East Asia Region and among them 74.2 million in India. DM proving to be become a global burden as this number is expected to rise to 783 million by 2045.[1] Due to chronic

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hyperglycemia, there is increased oxidative stress by stimulating mitochondrial enzymes, which results in overproduction of reactive oxygen species and its detrimental effect on organs. Due to reactive oxygen species, beta cell dysfunction occurs in diabetes.[2-6] It is also associated with increase in lipid production which causes hyperlipidaemia in addition to vascular complications - microvascular and macrovascular in different organs. Complications like retinopathy, nephropathy, neuropathy, and cardiovascular diseases occur.[7] Type 2 diabetes mellitus (T2DM) is prevalent in more than 90% of population, it mostly affects people older than 45 years but also seen in adolescent, children due to sedentary life style, obesity, eating habits. In T2DM there is a decreased sensitivity to insulin which is why insulin production increases for glucose homeostasis in body known as insulin resistance, but after some time insulin production decreases causing T2DM to set in.[8] A complex pathological condition known as insulin resistance occurs when the cellular responses of insulin in insulin-dependent cells such the liver, muscle, and adipocytes are reduced. That may be caused by pancreatic beta-cell failure, mutations in the insulin and PPAR-γ receptor, up-regulation of protein tyrosine phosphatase 1B (PTP1B), increased cellular oxidative damage, expression of the genes for inflammatory cytokines, and mitochondrial dysregulation.[9] Diabetes encompasses various disorders characterized by elevated blood glucose level (hyperglycaemia)[10]

Our study aims to demonstrate the anti-diabetic, anti-oxidant and anti-inflammatory effect of Thymoquinone in Streptozotocin induced type 2 DM in male Wistar Rats. we have compared to standard drug metformin.

Material and methods

A total number of 30 Wistar rats were included in the study. Each group contained n=6 rats. Rats in Group 1 were maintained on normal pellet diet (NPD) and water ad libitum while rats in the remaining groups (2 to 6) were given high fat diet (HFD). After 4 weeks of high fat diet all rats were fasted from 7 am to 3pm(118). Rats on normal pellet diet were given citrate buffer and rats on high HFD were given a single intraperitoneal injection of Streptozotocin in the dose of 40 mg/kg BW.[11] STZ at a dose of 40 mg/kg was prepared in cold citrate buffer (pH 4.5, 0.1 M) and given intraperitoneally.[12]Rats were stabilized after one week of STZ injection. After one week, blood samples were taken by tail vein and their fasting blood glucose levels were checked using a glucometer (Dr. Morepen BG03). Rats with fasting blood glucose level >200 mg/dL were considered diabetic rat and used in the study.[13]

Rats were randomly divided into 5 groups, each group containing n=6 rats and assessed for 28 days as follows.

Group A (Normal Control group): Rats were fed with NPD throughout the experiment

Group B (Diabetic Control group): Rats were given water and HFD ad libitum for 28 days.

Group C (Thymoquinone treated group): Diabetic rats were treated with Thymoquinone (50mg/kg/day) p.o by dissolving normal given by oral gavage for 28 days along with HFD**(121)**

Group D (Metformin treated group): Diabetic rats were treated with Metformin (100 mg/kg/day) p.o by dissolving in distilled water with 0.9%w/v sodium chloride and given by oral gavage for 28 days along with HFD **(122,123)**

Group E (Thymoquinone + Metformin treated group): Diabetic rats were treated with Thymoquinone + Metformin (50 mg/kg/day + 100 mg/kg/day) p.o by oral gavage for 28 days along with HFD**(121–123)** (Dissolving method same which mention above)

The collected data was organized and tabulated in Microsoft Excel (Microsoft office 365) and statistical analysis was done using SPSS (Statistical Package for Social Science) version 23.0 statistical analysis software. The values were represented in number and mean SD.

Results

Table 1: Group-wise distribution of control and experimental rats.

Group	Description	No. of Rats
A	Normal Control Group	6 each
B	Diabetic Control Group	
C	Thymoquinone treated group	
D	Metformin treated group	
E	Thymoquinone + Metformin treated group	
Total		30

Out of 30 rats assigned to this study, Streptozotocin was not given to induce diabetes in six rats, which served as normal control (Group A). The remaining 24 rats were given Streptozotocin injection to induce diabetes. Among these 24, six rats were not given any treatment for diabetes and served as diabetic control (Group B). Remaining 18 rats were assigned equally to three different treatment groups (Group C to E). In **Group C**, rats were given

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Thymoquinone (50 mg/kg/day) p.o. In **Group D**, rats were treated with Metformin (100 mg/kg/day) p.o. In **Group E**, rats were given Thymoquinone (50 mg/kg/day) p.o. plus Metformin (100 mg/kg/day) p.o.

Table 2: Inter-group comparison WEIGHT

Group	Initial Reading (day 0)		Final Reading (day 65)		Difference Gain in weight	
	Mean	SD	Mean	SD	Mean	SD
A	145.83	18.269	199.50	17.43	53.67	8.57
B	156.83	17.736	302.00	15.35	145.17	30.25
C	155.00	17.697	274.33	11.64	119.33	23.44
D	158.17	15.536	255.83	20.83	97.67	20.93
E	153.83	19.260	230.83	26.81	77.00	40.99
ANOVA	F=0.44; **p=0.776**		F=25.51; **p<0.001**		F=10.43; **p<0.001**	

p-value less than 0.05 is considered statistically significant

At day-65 (final reading) there was a statistically significant difference in mean weight among Groups A to E ($p<0.001$). The lowest mean weight was recorded for Group A rats (200 ± 17.43 g) and the highest mean weight was recorded for Group B rats (302 ± 15.35 gms). Group C and Group D rats were also found to have increased mean weights as compared to Group E, which had the lowest mean weight among the three treatment groups.

Table 3: Between group differences (Tukey HSD test) for weight

Between group	Initial			Final			Change (gain)		p-value
	Mean diff	SE	p-value	Mean diff	SE	p-value	Mean diff	SE	
A v/s B	-11.00	10.24	0.818	-102.50	11.04	<0.001	-91.50	15.61	<0.001
A v/s C	-9.17	10.24	0.896	-74.83	11.04	<0.001	-65.66	15.61	<0.001
A v/s D	-12.33	10.24	0.749	-56.33	11.04	<0.001	-44.00	15.61	0.06
A v/s E	-8.00	10.24	0.934	-31.33	11.04	0.06	-23.33	15.61	0.58
B v/s C	1.83	10.24	>0.999	27.67	11.04	0.12	25.83	15.61	0.48
B v/s D	-1.33	10.24	>0.999	46.16	11.04	<0.001	47.50	15.61	0.04
B v/s E	3.00	10.24	0.998	71.16	11.04	<0.001	68.16	15.61	<0.001
C v/s D	-3.17	10.24	0.998	18.50	11.04	0.47	21.67	15.61	0.64
C v/s E	1.17	10.24	>0.999	43.50	11.04	<0.001	42.33	15.61	0.08
D v/s E	4.33	10.24	0.993	25.00	11.04	0.19	20.67	15.61	0.68

p-value less than 0.05 is considered statistically significant

Our analysis for between group differences in mean weights found statistically significant association for A vs. B ($p<0.001$), A vs. C ($p<0.001$), B vs. D ($p=0.04$) and B vs. E ($p<0.001$). The highest positive mean difference was noted for Group B vs. E (68 ± 15.61 g) followed by Group B vs. D (48 ± 15.61 g) and Group C vs. E (42 ± 15.61 g).

Table 4: Inter-group comparison of RBS

Group	Mean	SD
A (Normal control)	118.83	6.01
B (Diabetic control)	390.83	38.88
C (TQ treated)	243.00	47.91
D (Metformin treated)	212.67	30.06
E (Metformin + TQ)	166.17	18.67
ANOVA	F=62.85 **p<0.001**	

p-value less than 0.05 is considered statistically significant

At day-65 there was a statistically significant difference in mean RBS levels among Groups A to E ($p<0.001$). The lowest mean RBS level was recorded for Group A rats (119 ± 6.01 mg/dl) and the highest mean RBS level was recorded for Group B rats (391 ± 38.9 mg/dl). Group C and Group D rats were also found to have elevated RBS levels as compared to Group E, which had the lowest RBS levels among the three treatment groups.

Table 5: Between group differences (Tukey HSD test) for RBS

Between group	Mean diff	SE	p-value
A v/s B	-272.000*	18.43	0.00
A v/s C	-124.167*	18.43	0.00
A v/s D	-93.833*	18.43	0.00

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A v/s E	-47.33	18.43	0.11
B v/s C	147.833*	18.43	0.00
B v/s D	178.167*	18.43	0.00
B v/s E	224.667*	18.43	0.00
C v/s D	30.33	18.43	0.48
C v/s E	76.833*	18.43	0.00
D v/s E	46.50	18.43	0.12

Our analysis for between group differences in mean RBS levels found statistically significant association for all the groups under comparison except for Group A vs. E (p=0.11), Group C vs. D (p=0.48) and Group D vs. E (p=0.12). The highest positive mean difference was noted for Group B vs. E (225 ± 18.43 mg/dl) followed by Group B vs. D (178 ± 18.43 mg/dl) and Group B vs. C (148 ± 18.43 mg/dl).

Discussion

Comparison of key study parameters in non-diabetic and diabetic control rats

One of the study objectives was to compare the differences between diabetic and non-diabetic rats in relation to key clinical parameters, including IL-6 and MDA levels. The comparative analysis for key parameters in non-diabetic (normal control) and diabetic control rats suggested statistically significant differences for all the parameters under consideration. The following table depicts the mean value and mean differences for study variables at day-65 for rats in the diabetic and non-diabetic groups.

The mean weight was 103 ± 11.04 (SE) g more in diabetic control group as compared to the normal control group, and the difference was statistically significant (p<0.001). The mean RBS level was 272 ± 18.43 (SE) mg/dL higher in diabetic control group as compared to the normal control group. Thus, the findings suggest that HFD and i.p. injection of STZ were effective in inducing diabetes for the preparation of diabetic rats.

Comparison of weight among therapeutic groups and controls

In our study, diabetic control (B) and both the mono-therapy groups (C, D) showed statistically significant change in mean weight as compared to the normal control (A) group. Despite the considerable difference, no statistically significant association was noted for mean weight between Group A and combination therapy Group E (p=0.06). Additionally, no significant difference in mean weight was noted between rats in standard therapeutic group (D) and novel therapeutic group (C; p=0.47), Group B and Group C (p=0.12), and Group D and Group E (p=0.19). Thus, only metformin and combination therapy showed evident action in lowering mean weight as compared to the diabetic control group.

Mean weight for combination therapy group (E) was 231 ± 26.81 g, whereas for standard therapy group (D) and novel therapy group (C) they were 256 ± 20.83 g and 274 ± 11.64 g, respectively. Compared to mean weight in diabetic control group, our finding suggest that the combination therapy may be more effective in reducing mean weight compared to both standard therapies with metformin alone and the novel therapeutic agent TQ. The effects of metformin or TQ alone on lowering weight was almost similar with no statistically significant difference.

A study conducted by **Rani et al.**[14] corroborated our findings, demonstrating comparable results. In their investigation, diabetic rats treated with metformin (150 mg/kg; p<0.001), combined pure glycyrrhizin and Thymoquinone (TQ) (10+10 mg/kg; p<0.001), and a combined glycyrrhizin and TQ nano-formulation (10+10 mg/kg; p<0.01) exhibited significant reversal of diabetes-induced decrease in body weight by day 21, in contrast to diabetic control rats.

Comparison of RBS levels among therapeutic groups and controls

In our study, diabetic control (B) and both the mono-therapy groups (C, D) showed statistically significant change in mean RBS levels as compared to the normal control (A) group. However, no statistically significant difference was noted for mean RBS levels between Group A and combination therapy Group E (p=0.11). Additionally, no significant difference in mean RBS levels was noted between rats in standard therapeutic group (D) and novel therapeutic group (C; p=0.48). However, all the therapeutic groups (C, D, E) showed statistically significant reduction in mean RBS levels as compared to diabetic control group (B). Thus, both monotherapy and combination therapy showed evident action in lowering mean RBS levels as compared to the diabetic control group.

Mean RBS level for combination therapy group (E) was 166 ± 18.67 mg/dl, whereas for standard therapy group (D) and novel therapy group (C) they were 213 ± 30.06 mg/dl and 243 ± 47.91 mg/dl, respectively. Compared to RBS level in diabetic control group, our finding suggest that the combination therapy may be more effective in reducing RBS levels compared to both standard therapies with metformin alone and the novel therapeutic agent TQ. The effects of metformin or TQ alone on lowering RBS levels was almost similar with no statistically significant difference.

In a study **(Alshahrani et al., 2021)**[15] using a rat model, researchers investigated TQ's role in hyperglycaemia-induced insulin resistance within experimental type 2 diabetes. The results demonstrated that TQ treatment significantly decreased elevated levels of glucose, glucose area under the curve, insulin, and DPP-IV in the treated diabetic groups. Administration of TQ at doses of 10

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and 20 mg/kg notably reduced fasting glucose levels compared to the untreated type 2 diabetic control group ($p < 0.001$). Furthermore, TQ treatment led to a significant reduction in high levels of triglycerides and cholesterol (total, LDL, and VLDL), coupled with a noteworthy increase in HDL levels in the treated diabetic groups. Based on these findings, the researchers proposed TQ as a potential alternative natural therapy for managing hyperglycaemia-induced insulin resistance in type 2 diabetes mellitus.

In another rat model study **(Rani et al., 2019)**[14], researchers evaluated the antidiabetic effects of two bioactive compounds, glycyrrhizin and TQ, in comparison to metformin in type 2 diabetic rats. Interestingly, when these compounds were administered as combined nano-medicines at a dose of 10+10 mg/kg, rather than individually, significant reductions in blood glucose and HbA1c levels, as well as notable improvements in body weight and lipid profile, were observed. However, when diabetic rats were treated with glycyrrhizin and TQ separately, no favourable trends were observed in any of the studied parameters. This suggests that the improvements in antidiabetic activity may be attributed to a synergistic effect of the combined nano-formulations. These findings are similar to our study findings where the combined effect of TQ + metformin is observed to be superior than either of these drugs alone.

Conclusion

Our study involving diabetic rat model to understand the effect of TQ's anti-diabetic, anti-inflammatory and anti-oxidant properties as compared to metformin showed that the combination of TQ + metformin had superior properties as compared to either of these drugs administered alone. Increase in mean weight was significantly higher in TQ group as compared to combination group (E) ($p<0.001$). And among monotherapies, metformin showed similar effect on weight gain as TQ ($p=0.47$). Reduction in RBS levels were significantly lower in combination group (E) as compared to TQ alone ($p<0.001$). And among monotherapies, metformin showed similar effect on lowering of RBS levels as TQ ($p=0.48$). Reduction in IL-6 levels was significantly lower in combination group (E) as compared to TQ alone ($p<0.001$). Even among monotherapies, metformin showed statistically significant lowering of IL-6 levels as compared to TQ ($p<0.001$). Reduction in MDA levels was significantly lower in combination group (E) as compared to TQ alone ($p=0.03$). And among monotherapies, metformin showed a similar effect on lowering of MDA levels as TQ ($p=0.99$).

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