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Original Article

Intermittent Fasting as a Non-Invasive Strategy to Mitigate Diabetes-Induced Complications in Rats

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ABSTRACT

Introduction: The global burden of diabetes mellitus is steadily increasing. Abnormal eating patterns and excess energy intake contribute to obesity and type 2 diabetes mellitus (T2DM). Although dietary modifications such as intermittent fasting have gained attention, evidence supporting their beneficial role in T2DM is limited. **Objective:** To evaluate the beneficial effects of intermittent fasting on physiological, biochemical, and histological changes in a rat model of type 2 diabetes mellitus. **Methods:** Healthy adult Wistar albino rats (150–200 g) were used. Diabetes was induced by administering alloxan (150 mg/kg). Animals were divided into four groups: Normal Control (Group I), Experimental Control (Group II), and Intermittent Fasting groups (Group III & Group IV). Physiological, biochemical, and histological parameters were assessed to determine the impact of intermittent fasting. **Results:** Intermittent fasting demonstrated a protective effect by reducing diabetes-induced physiological, biochemical, and histological alterations. Rats in the intermittent fasting groups showed improved outcomes compared to the experimental control group. **Conclusion:** Intermittent fasting may serve as a potential non-invasive strategy to reduce diabetes-related complications. Further studies focusing on molecular mechanisms are warranted to provide deeper insights into its protective role.

Keywords: Intermittent fasting; Type 2 diabetes mellitus; Alloxan; Wistar rats; Physiological changes; Biochemical parameters; Histological changes

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INTRODUCTION

Type 2 diabetes is a global public health crisis that threatens the economies of all nations, particularly developing countries[1]. Poor eating habits and sedentary lifestyle has been well associated with type 2 diabetes[2]. The number of cases of diabetes are increasing day by day, among them type 2 diabetes mellitus has being more prevalent[1]. The International Diabetes Federation has estimated that globally there are 415 million people with diabetes in 2015 and is predicted to increase to 642 million by 2040[3]. Diabetes can affect many different organ systems in the body and over time, can lead to serious complications[4]. Lifestyle changes are crucial to managing the disease, and eating habits play a key role.

The concept and has been practiced by our ancestors for centuries as religious fasting. Intermittent fasting is one of the dietary restriction method. Dietary restriction, such as alternate day fasting and calorie restriction decreases the risk of various chronic diseases including diabetes5. Dietary restriction can be expected to be able to improve diseases that are result of uncontrolled life style[5]. It is suggested that this method, by intervals of fasting, gives body proper time to utilize glucose with the available amounts of insulin in the body. There are various dietary restriction methods, one of them being intermittent fasting, which itself has been developed into various models showing its impact in increasing life span[6,7]. Literature is lacking on the comparative effects of different types of intermittent fasting in types 2 diabetes.

MATERIAL AND METHODS

Healthy adult Albino rats of Wistar strain (150-200 grams body weight) were procured from the Central Animal House Kasturba Medical College Mangalore. All the rats were housed separately in ventilated cages. The rats were maintained atmosphere with 12:12 h natural light: dark cycle and water and food were provided ad libitum, and room temperature (25°C ± 2°C) and humidity (60 ± 10%) were maintained.

Ethical approval: Before the commencement of the experimental protocol, the prior approval was taken from the Institutional Animal Ethical (IAEC) at Kasturba Medical College, Mangalore (KMC/MNG/IAEC/03-2018). All the guidelines proposed by the Committee for Control and supervision of experimentation on animals (CPCSEA), Government of India were followed accordingly.

Induction of Diabetes Mellitus: The animals will be weighed and injected via intraperitoneal route with 150mg/kg of alloxan (Sigma) dissolved in normal saline[8]. Diabetes mellitus was confirmed after 72 hours of alloxan injection by testing the fasting blood glucose levels using Accu-Check Active glucometer and glucose test strip[9].

Animal Grouping : Animals were divided into the following groups (n=6; n: number of animals in each Groups)

Normal Control group (Group I) – Rats fed with normal rat chow.

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Experimental control group (Group II) – High fat diet is given to these rats for 15 days and type 2 diabetes is induced by giving intraperitoneal injection of Alloxan (150mg/kg BW) Intermittent Fasting group[10].

Type I (Group III): These group of type II diabetic model rats are exposed to Intermittent fasting daily (Rats are deprived of food from 5PM to 9 AM) for 2weeks.

Type II (Group IV): These groups of type II diabetic model rats are exposed to type II intermittent fasting (Rats are deprived of food from 5PM to 9 AM for 3days per week) for 2weeks.

The physiological parameters such as food intake, water Intake, body weight was recorded daily. After 2 weeks of experimental procedures the animals were anesthetized using ketamine (5 mg/kg BW) and blood was collected by cardiac puncture for biochemical estimations[11-12]. Pancreas and liver tissue and given for the histological analysis.

Histopathological studies: Excised pancreas and liver samples were washed in ice-cold normal saline, patted dry, and immediately preserved in 10% Neutral buffered formalin (NBF). They were processed in an automatic tissue processor and embedded in paraffin wax. Sections of 5 µm were cut and stained with hematoxylin and eosin, and later, the microscopic slides were photographed using a light microscope.

Statistical analysis: All the data's were expressed as Mean ±SD and the statistical analysis was done by using the paired't' test and ANOVA. Statistical package SPSS version 17.0 was used to do the analysis p< 0.05was considered as statistically significant.

RESULTS

Group I showed a significant increase in the body weight (P< 0.0001) when compared to other groups. A significant decrease (P< 0.0001) in the body weight was observed in the alloxan induced rats. A significant improvement (P< 0.001) in the body weight was observed in the Group III and Group IV when compared to Group II (Table 1).

Group I showed no significant changes in the food intake when compared to other groups. A significant increase (P< 0.0001) in the food intake was observed in the alloxan induced rats. A significant decrease (P< 0.001) in the food intake was observed in the Group III and Group IV when compared to Group II (Table 2).

Group I showed no significant changes in the water intake when compared to other groups. A significant increase (P< 0.0001) in the water intake was observed in the alloxan induced rats. A significant decrease (P< 0.001) in the water intake was observed in the Group III and Group IV when compared to Group II (Table 3)

Group I showed no significant changes in the blood glucose level up to day 30th when compared to other groups. A significant increase (P< 0.0001) in the blood sugar level was observed in the alloxan induced rats . A significant decrease (P< 0.001) in the blood sugar level was observed on in Group III and Group IV when compared to Group II on 30th day. The decrease in the blood glucose was significant (P< 0.001) in Group IV when compared to Group III (Table 4)

GSH level significantly decreased in Group II (P< 0.001) and significantly increased (P< 0.01)in the Group III and Group IV when compared to Group I (Figure 1) . A sigfnicant increase in the GSH level

Table 1. **Effect of different types of intermittent fasting on body weight in Alloxan induced diabetic rats; Values are expressed as Mean ± SD; g / 100 g of BW.**

GROUPS	0 day (g / 100 g of BW)	30th day (g / 100 g of BW)
Gr. I	142.16±6.64	178.33±3.34***
Gr. II	142.83±4.91	113±4.14***
Gr. III	144.66±2.73252	165.33±5.60***†††
Gr. IV	144.16±3.81	161±13.71***†††

***P< 0.0001; 0 day versus 30th day
✞✞✞P< 0.0001; Group III versus Group IV

Table 2. **Effect of different types of intermittent fasting on food intake in Alloxan induced diabetic rats ; Values are expressed mean ± SEM; ml / 100 g of BW. Values expressed as Mean ± SD (n=6).**

GROUPS	0 day (g / 100 g of BW)	30th day (g / 100 g of BW)
Gr. I	8.33±0.98	9.33±1.50
Gr. II	8.33±1.50	27.66±1.50***
Gr. III	9.6±0.81	17±1.09***†
Gr. IV	8.5± 1.64	16.6±5.31***

✞P✞P< 0.05; Group III versus Group IV
***P< 0.0001; 0 day versus 30th day

Table 3. **Effect of different types of intermittent fasting on water intake in Alloxan induced diabetic rats; Values are expressed mean ± SEM; g/100g of BW; Values expressed as Mean ± SD (n=6)**

GROUPS	0 day (ml/ 100 g of BW)	30th day (ml/ 100 g of BW)
Gr. I	15±1.67	17.83±1.722
Gr. II	17±2.06	33.33±2.44***
Gr. III	17.66±2.65	23.33±2.06*†
Gr. IV	17±3.93	26.66±2.33***

*P< 0.05; ***P< 0.0001; 0 day versus 30th day
✞P< 0.05; Group III versus Group IV

Table 4. **Effect of different types of intermittent fasting on blood glucose level (mg/dl) Values expressed as Mean ± SD (n=6)**

Group I			Group II			Group III			Group IV		
0 Day	17th day	30th day	0 Day	17th day	30th day	0 Day	17th day	30th day	0 Day	17th day	30th day
97 ± 1.095445	97 ± 1.095445	97.6 ± 0.81	96 ± 1.26	294.33 ± 7.941***	333.66 ± 10.61***	95 ± 2.44	301 ± 7.71***	162 ± 8.82***†††	95 ± 2.44	305.33 ± 11.4***	248 ± 8.33***†††

***P< 0.001; 0 day versus 17th day
✞✞✞P< 0.001; 17th day versus 30th day

Table 5. **Effect of different types of intermittent fasting on lipid profile in Alloxan induced diabetic rats; Values expressed as Mean ± SD (n=6)**

Parameters	Group I	Group II	Group III	Group IV
Serum Cholesterol	79.33±3.48	76.64±5.36	82.3±9.56	83.78±8.60
Triglycerides	45.46±1.45	44.56±11.88	47.97±6.7	40.27±14.60
HDL	46.95±8.29	20.25±5.89***	113.62±17.30*** †††	111.59±25.78***†††
LDL	34.28±0.74	140.63.15±9.07***	144.80±14.9***	148.14±5.082***

***P< 0.0001; Group I versus Group II, Group III, Group IV
✞✞✞ P< 0.0001; Group III versus Group IV

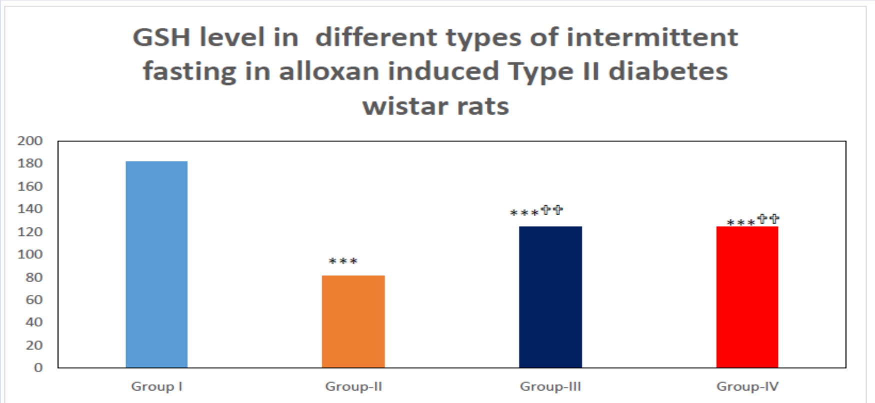

Figure 1. GSH levels

Values expressed as Mean ± SD (n=6),
***P< 0.001; Group I versus Group II, Group III, Group IV
✞P< 0.01; ✞✞✞P< 0.001; Group II versus Group III, Group IV

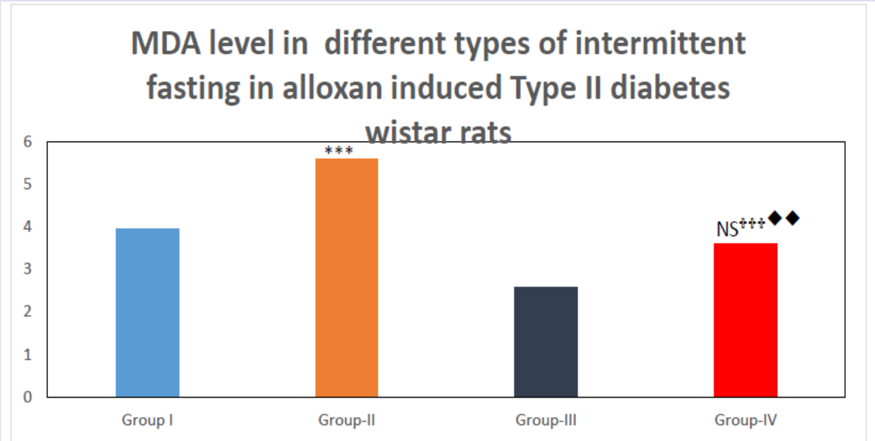

Figure 2: MDA levels

Values expressed as Mean ± SD (n=6),
***P< 0.001; Group I versus Group II, Group III, Group IV
✞✞✞P< 0.001; Group II versus Group III, Group IV
◆◆ P < 0.001 ; Group III versus Group IV

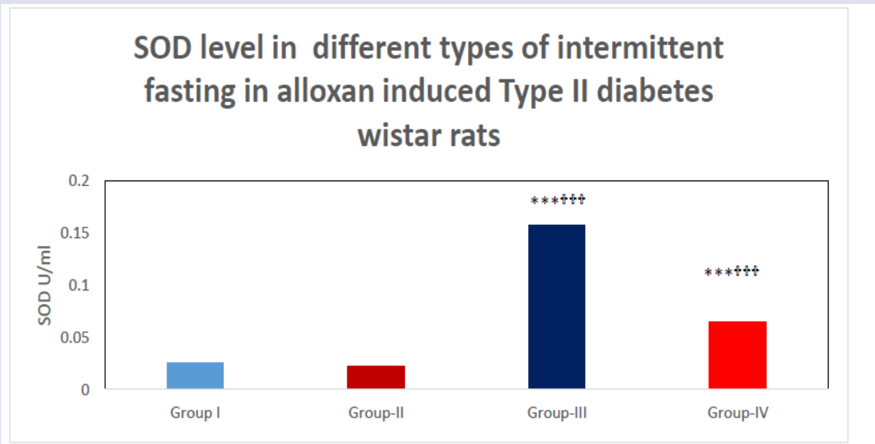

Figure 3: SOD Levels

Values expressed as Mean ± SD (n=6),
***P< 0.001; Group I versus Group III, Group IV
✞✞P< 0.001; Group II versus Group III, Group IV

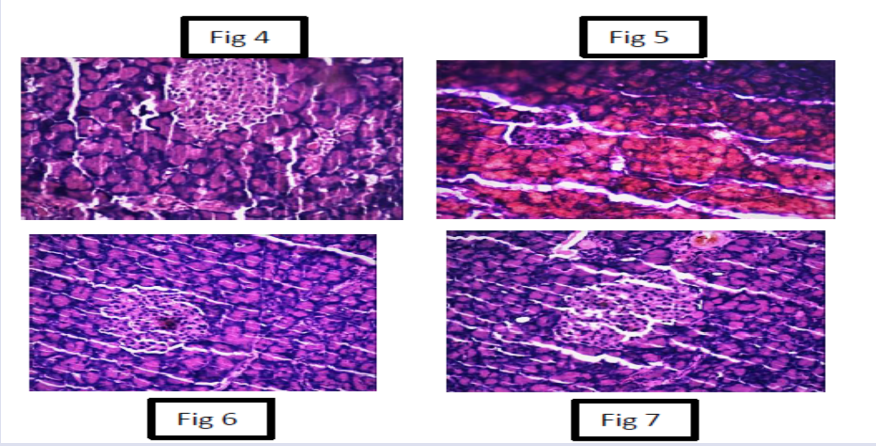

Figure 4,5,6,7. Histopathology of rat pancreas.

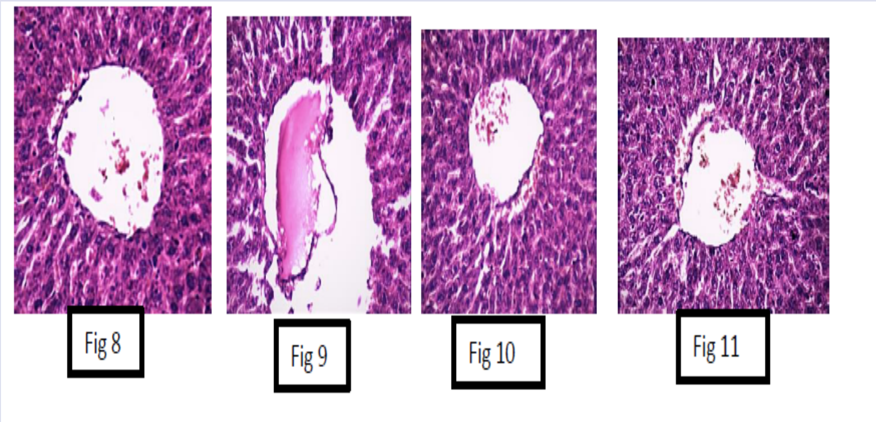

Figure 8,9,10,11. Histopathology of the rat liver cells

was observed in Group III and Group IV (P< 0.001) When compared to group III (Table 5).

MDA level significantly increased in Group II (P< 0.001) when compared to Group I . A sigfnicant decrease in the MDA level was observed in the Group III and Group IV (P< 0.001) When compared to group I. The decreased MDA level was more significant in the Group III (P < 0.001 when compared to Group IV (Figure 2).

SOD level significantly increased in Group III and Group IV (P< 0.001) When compared to group I. The increase observed in the Group III(P< 0.001) was significantly higher when compared to Group II. The decreased MDA level was more significant in the Group III (P < 0.001) when compared to Group IV (Figure 3).

Histology results

The histopathology of rat pancreas was shown in (Figure 4-6) . Microscopic investigation of pancreas sections of control rats (Figure 4) showed the normal appearance of islets of Langerhans. However, Group II rats showed pathological changes of both exocrine and endocrine components. The acinar cells were swollen and small vacuoles were observed in almost all acinar cells. On the other hand, Group III and Group IV depicted the evidence of cellular regeneration among the islets of Langerhans (Figure 5 and Figure 6) . Atrophic change of the acinar cells was less severe and the border between exocrine and endocrine portions became more differentiable The regenerative changes was better in group III compared to Group IV.

The histopathology of the liver cells of control groups (Group I; Figure 8) showed eminent hepatocytes with central vein along with portal triad. The damage to the liver cells in the form of damaged central vein, hepatocytes and portal trial could be clearly seen in the (Group II; Figure 9). The damage to the liver cells were reversed in intermittent fasting groups (Group III and Group IV; Figure 10-11). The regenerative changes was better in group III when compared to group IV

DISCUSSION

In the recent years increased modernization has influenced the life style changes impacting on the health of the individual. Obesity has been closely associated with the modern life styles changing patterns[13-14]. The cases of obesity are strongly suspected to increase the risk for degenerative diseases. Oxidative stress has been suggested extensively as a potential mechanism in diabetes promoting the formation lipid peroxidation products and decreasing the antioxidant defense by decreasing the level of antioxidant enzymes like SOD, catalase and glutathione[15], .In diabetes, hyperglycemia has been considered as the major culprit of oxidative stress induced damage inflicted on the various tissues. Oxidative stress and inflammatory mechanisms is well associated with obesity[16]. Impaired glucose and lipid metabolism, such as hyperglycemia, insulin resistance, ectopic fat deposition, and dyslipidemia, are the common pathological features of obesity and are associated with pancreatic and liver abnormalities[17]. Dietary restriction is associated with a decreased risk for obesity related complications .

Degenerative diseases, are frequently caused by life style pattern, one of the reason is uncontrolled dietary patterns. Intermittent fasting is one of the dietary restriction method which has been linked with the various therapeutic approaches[18-19].

The diabetic model induced by Alloxan associated with the high fat diet efficiently simulated the type II diabetic mellitus condition. After alloxan injection, there was severe hyperglycemia which persisted till the last day of experiment. Thus, alloxan might have caused beta cell destruction attributing to severe hyperglycemia . Diabetes mellitus has been characterized with polyphagia, which may be responsible for observed increase in food intake. This is important for the animals to compensate for loss of body weight and fluid. Our study reports shows that , Intermittent fasting in the alloxan induced rats significantly reduced the diabetic symptoms like polyphagia , polydipsia and reduced body weight . This might be attributed to normoglycemic activities of intermittent fasting. Intermittent fasting has significantly improved oxidative stress parameters as observed in this study such as lower concentrations of MDA, increased level of SOD, GSH and the higher concentrations of HDL. Diets high in carbohydrates and polyunsaturated fatty acids tend to decrease apolipoprotein A-1, which is the main component of HDL and functions to decrease oxidized LDL concentrations. Intermittent fasting might have caused decreased intake of calories promoting the production of apolipoprotein A-1 subsequently increasing the HDL concentrations in the blood[20]. The intermittent fasting model has decreased the oxidative stress induced by diabetes. This shows that oxidative stress has been minimized through intermittent fasting. Restriction of calories and control of the amount of calories entering the body are expected to prevent and control oxidative stress in the body subsequently decreasing the oxidative stress. The present reports are in accordance with the study of Ma et al[21] also found that rats subjected to dietary interventions alternating between high fat diets and normal showed good results in the prevention and therapy of the metabolic syndrome. Comparatively, everyday fasting[22] has been showed more significant effect when compared to weekly three days fasting. The significant effect of fasting in this study in diabetic model rats has been well correlated with the significant regenerative changes as confirmed by the histological analysis of pancreas and liver. In conclusion, the dietary approach merits should be considered as a possible approach to prevent or minimize the diabetes and obesity related complications .

CONCLUSION

Dietary restriction can be expected to be able to improve diseases directly related to the improper life style. Literature review shows that intervals of fasting, gives body proper time to utilize glucose with the available amounts of insulin in the body. Intermittent fasting has been developed into various models showing its impact in increasing life span. Literature is lacking on the comparative effects of different types of intermittent fasting in types 2 diabetes. The present study showed the protective effect of different types of intermittent fasting in diabetic model wistar rats by analyzing the physiological, oxidative stress parameters and metabolic profile with histological analysis. Results showed that, intermittent fasting has protective role in minimizing the deleterious effect of diabetes. Based on the types of intermittent fasting in our study , daily intermittent fasting proved to be beneficial. Dietary restrictions could be considered as a possible non-invasive approach in minimizing the diabetes related complications

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