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EFFECTS OF FASTING ON BODY WEIGHT, SERUM GLUCOSE AND CREATININE AND HISTOTEXTURE OF LIVER AND KIDNEY IN SWISS ALBINO MICE

Tithe Saha¹, Khaled Mahmud Sujan¹, Ziaul Haque² and Md. Kamrul Islam^{1*}

¹Department of Physiology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh;

²Department of Anatomy and Histology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

*Corresponding author: Dr. Md. Kamrul Islam; E-mail: k.physiol@bau.edu.bd

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ABSTRACT

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Fasting means remaining without foods and water from a specific period of time which is important for improving health and increasing longevity. The present study was conducted to investigate the effect of fasting on body weight, serum biochemistry and histomorphological changes of liver and kidney in mice. A total of 18 Swiss Albino Mice (*Mus musculus*), 28-35 days old with an average body weight of 26.2 ± 1 gm were randomly divided into three groups. Group A was considered as control (n=6) and fed on standard mice pellet and fresh drinking water. Group B was considered as 14 hours fasting group (n=6), kept fasting for 14 hrs and Group C was considered as 18 hours fasting group (n=6), kept fasting for 18 hrs. At the end of the experiment, blood and tissues were collected for biochemical and histomorphological examination. Data showed that no significant change was found in body weight, serum glucose and creatinine level in fasting groups. Histopathological studies of liver and kidney revealed that fasting could initiate minor change in the normal structures of liver but no architectural change in kidney. Slight depletion of glycogen was found in hepatocytes of liver. From the present study it can be concluded that fasting may be beneficial as it decreases body weight gain and have not any significant alteration in the liver and kidney histotextures.

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INTRODUCTION

Fasting is partial or total abstention from all kinds of foods and water from a specific period of time. There are three most commonly facts of fasting- caloric restrict (CR), alternate-day fasting (ADF), and dietary restriction (DR) (John et al, 2010). Caloric restrict (CR) means reduction or restriction of kilocalorie (kcal) intake by a certain percentage (typically 20 - 40%) of *ad libitum* consumption. CR has been observed in a different group of species, like: dog, rodent, spider, non-human primate, and zebrafish (Spindler, 2009). CR plays a great role to delay the onset of the diseases like autoimmune diseases, atherosclerosis, cardiomyopathies, cancer, diabetes, renal diseases, neurodegenerative diseases, and respiratory diseases (Vaquero et al, 2009). Alternate Day Fasting (ADF) means alternating 24-hours period: during the “feast period,” fasters may consume food *ad libitum*; during the “fast period,” food consumption is restricted completely and water is allowed *ad libitum* during all times. ADF is responsible to extend the lifespan (Spindler, 2009) by preventing the development of cardiovascular disease, kidney disease, cancers, and diabetes (Mattson et al, 2005; Varady et al, 2007). Dietary Restriction (DR) is a reduction of one or more components of dietary intake (typically macronutrients) with minimal to no reduction in total kcal intake. Research suggests that neither carbohydrate restriction nor lipid restriction extend life (Ayala et al, 2007). On the other hand, protein restriction increases maximum of 20% lifespan (Pamplona et al, 2006) and this extension may be due to the reduction of the amino acid, methionine (Caro et al, 2009). The effect of Ramadan fasting on various parameters has been observed in healthy (Dewanti et al, 2006) and unhealthy populations (Khafaji et al, 2012; Kul et al, 2014). In most of the studies, it was found that Ramadan fasting leads to changes in the metabolic status including blood glucose and lipid (Sadiya et al, 2011). Ramadan fasting has some effects on the circulating levels associated with vascular and metabolic disorders (Saleh et al, 2005; Khaled et al, 2009). Ramadan fasting showed to have effect on lipid profile by increasing HDL and decreasing LDL levels (Mansi 2007; Ibrahim et al, 2008; Lamri-Senhadji et al, 2009). It has been found a significant decrease in serum cholesterol and serum triglycerides (Marbut et al, 2005). The concentration of HDL is strongly inversely associated with the risk for atherogenesis and is known to be a protective lipoprotein against coronary heart disease (Gordon et al, 1977). Total protein, AST, ALT level were significantly decreased but no significant change in urea, creatinine by starvation. The histomorphology of starved liver showed that glycogen depletion and vacuolation and in kidney tissue, congestion of the capillary tufts of the glomeruli, mild degenerative changes of tubular epithelium and cellular infiltration within the paranchymatous tissue due to starvation (Yasser et al, 2016). The glucose level decreased significantly by fasting. The findings suggest Ramadan fasting model for energy metabolism and regulation (Nomani et al, 1989).

During the fasting period, serum and urine levels of free carnitine and its renal clearance decreased. Non-significant changes were observed in serum glucose, cholesterol and triglycerides during the fasting period (Jiri et al, 1978). The effects of fasting were studied in various animals (Nieminen et al, 2001; Yasser et al, 2016;) especially human (Mohsen et al, 2015; Unalacak et al, 2011) but the information on that was poorly established in laboratory animals. In connection of that, this present study was conducted to investigate the effects of fasting in mice. The laboratory animals are alternative choice worldwide as an experimental model by means of their genetic, biological and molecular pathways are closely related to humans. With that sense, the present experiment has been undertaken to determine the effect of fasting on the body weight, serum glucose and creatinine and investigate the effect of fasting on histo-morphology of liver and kidney.

MATERIALS AND METHODS

Experimental animals

The mice used for this study were purchased from International Center for Diarrheal Disease Research, Bangladesh (icddr'b), Mohakhali, Dhaka, and reared in a compartmentalized square wooden cages wrapped with wire mesh under controlled conditions of temperature (26-30) °C and relative humidity of 70-80% with natural day light.

Experimental designs

The experiment was conducted in the Department of Physiology, Bangladesh Agricultural University, Mymensingh, during the period of December 2017 to January 2018. A total of 18 Swiss Albino Mice (*Mus musculus*), 28-35 days old with an average body weight of 26.2 ± 1 gm were used. The mice were randomly divided into three groups. Group A was considered as control (n=6) and fed on standard mice pellet and fresh drinking water. Group B was considered as 14 hours fasting group (n=6), kept fasting for 14 hrs and fed on standard mice pellet and water after fasting. Group C was considered as 18 hours fasting group (n=6), kept fasting for 18 hrs and fed on standard mice pellet and water after fasting. For fasting, feed and water were taken out from the cage of mice every evening at 6 pm. In the following morning feed and water were given in the cage of group B at 8 am and group C at 12 pm. But in the control group feed and water were present at all time in the cage. The experiment was conducted for two weeks.

Management practices

The mice cages were kept on a well-ventilated room. In order to prevent spoilage, feeds were kept in air tight poly packed bag. The feed was supplied daily to the mice and fresh drinking water was made available. Mice cages were cleaned regularly with proper hygienic measure and sanitary measures were during the experimental period. Commercial mice pellet was purchased from the local market near Bangladesh Agricultural University, Mymensingh-2202.

Body weight

Initial body weight of each mouse was measured with the help of a digital weight balance. Body weight was taken at first day of experiment and then 7 days' intervals until end of experiments.

Body weight gain was calculated by following formula:

Weight gain (g) = mean final weight (g) - mean initial weight (g)

Percent body weight gain was calculated by following formula:

Percent weight gain (g) = $\frac{\text{mean final weight (g)} - \text{mean initial weight (g)}}{\text{mean initial weight}} \times 100$

Collection of blood and organs

At the end of experimental period, blood samples were collected by sacrificing the mice. The mice were placed in an airtight container with diethyl ether presoaked cotton. They were checked for unconsciousness. The mice were taken out and the blood was collected directly from the heart by a sterile syringe. About 1.5 to 2 ml blood was collected and transferred to another tube without anticoagulant for serum preparation. The liver and kidney were collected and transferred to 10% neutral buffer formalin.

Preparation of serum

The blood containing tubes were placed in upright slanting position at room temperature for 6 hours. They were then incubated overnight in the refrigerator (4° C). The serum samples were separated by centrifugation and collected by using 200 µl pipettes. About 0.4 to 0.5 ml serum was collected from each mouse. Serum samples were stored in capped tube at -20° C for biochemical analysis.

Serum biochemical studies

The serum glucose and creatinine were analyzed in Health Care Center, Bangladesh Agricultural University, Mymensingh-2202.

Histomorphological study

The liver and kidney from each group of mice were collected after completely removal of blood by perfusion with phosphate buffered saline and kept in 10% neutral buffered formalin for 15 days. The well-fixed tissues were processed, sectioned and stained as per standard procedure (Banchroft et al, 1996). in the Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh-2202. The stained slides were observed under Optka Vision Lite 21 and photographs of the characteristic findings were recorded.

Statistical analysis

Data were continuous and normally distributed. One-way analysis of variance (ANOVA) was used to determine the effect of different parameters. The data was placed and stored in Microsoft Excel- 2016 and imported to the software IBM SPSS Statistics 20 for analysis. Descriptive statistics analysis was done to measure the mean, standard deviation and standard error and p value of different parameters. Because of using multiple comparisons, the corrected p value was calculated adjusted at 0.01 and 0.05 considered for level of significance.

RESULTS AND DISCUSSION

Effect of fasting on body weight gain in mice

The average body weight gains and the initial and final body weight of mice of fasting groups and normal healthy control group were presented in table: 1 and figure: 1 and 2 respectively. Here, initial (35.67 ± 1.20 gm) and final (39 ± 1.53 gm) body weight of group A was significantly ($p < 0.05$) increased. In group B, initial (37 ± 0.58 gm) and final (37.67 ± 0.33 gm) body weight was insignificant. Again no significant change was observed in group C in which initial body weight was 37.33 ± 1.86 gm and final body weight was 39.33 ± 1.45 gm. Percent body weight gain of group A was 9.34%, group B was 1.81% and group C was 5.36%. This finding was closely agreeable with the findings of (Ravanshad et al, 2001; Abdulrahman et al, 2006; Haghdoost et al, 2009). In this study, no significant change in body weight in fasting groups but in untreated group significantly increased with the increasing of age. This might be due to lacking of feed intake in fasting groups which was normal in control group. Moreover, Leptin is secreted primarily in fat cells, as well as the stomach, heart and skeletal muscle at the time of fasting which suppressing food intake, thereby inducing weight loss. Fasting can normalize ghrelin levels which known as the hunger hormone that responsible for increasing hunger (Klok et al, 2007).

Table 1. Effects of fasting on body weight gain in mice

Group	Initial body weight (gm)	Final body weight (gm)	Body weight gain after experiment (gm)	% body weight gain after experiment (%)	Level of significance	P-value
Group A (Control)	35.67 ± 1.20	39 ± 1.53	3.33	9.34	*	0.038
Group B (14 hour fasting)	37 ± 0.58	37.67 ± 0.33	0.67	1.81	NS	0.184
Group C (18 hour fasting)	37.33 ± 1.86	39.33 ± 1.45	2	5.36	NS	0.580

** Significant at 1% level ($p < 0.01$); *Significant at 5% level ($p < 0.05$); NS= not significant ($p > 0.05$)

Table 2. Effects of fasting on serum glucose and creatinine in mice

Parameters	Mean \pm SE			Level of significance	P-value
	Group A (Control)	Group B (14 hour fasting)	Group C (18 hour fasting)		
Glucose (mmol/l)	6.40 ± 0.70	6.83 ± 0.18	6.20 ± 0.56	NS	0.681
Creatinine (mg/dl)	1.07 ± 0.07	1.13 ± 0.07	0.93 ± 0.07	NS	0.178

** Significant at 1% level ($p < 0.01$); *Significant at 5% level ($p < 0.05$); NS= not significant ($p > 0.05$)

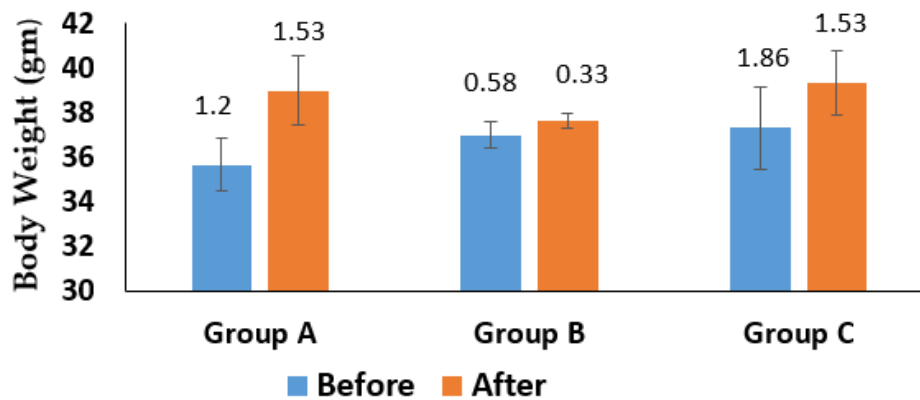


Figure 1. Effect of fasting on body weight

The superscript value above the bars indicates standard error

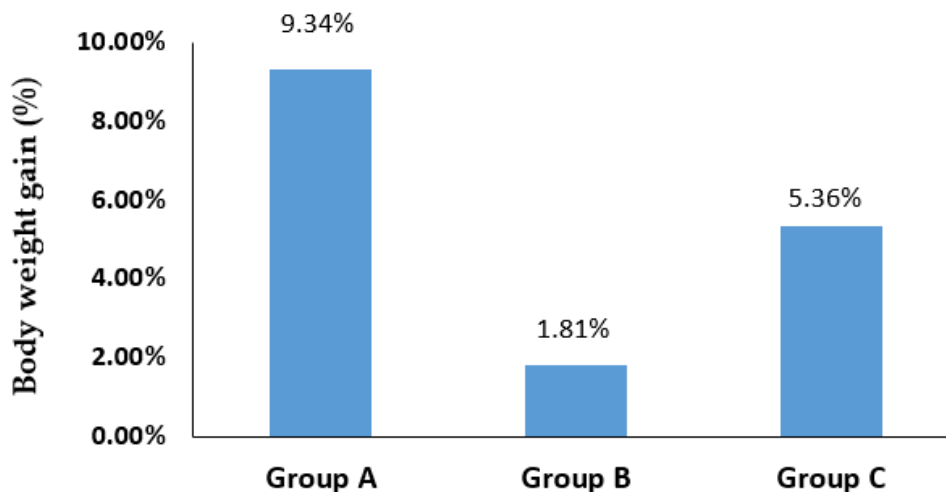


Figure 2. Effect of fasting on body weight gain

The superscript value above the bars indicates standard error

Effect of fasting on serum glucose and creatinine

In the present study, glucose concentration was 6.40 ± 0.70 mmol/l in control group, 6.83 ± 0.18 mmol/l in 14 hrs fasting group and 6.20 ± 0.56 mmol/l in 18 hrs fasting group. There was insignificant change in control and fasting groups (Table 2 and Figure 3). Duration of daily fasting, physical activity and dietary habits are important factors in blood glucose level. So, short duration of fasting might not affect any change on it. This finding supports the report of Abdulrahman et al, (2006) who reported that there was no significant change in blood glucose level in fasting groups. No significant change was found in case of creatinine concentration (1.07 ± 0.07 mg/dl in group A, 1.13 ± 0.07 mg/dl in group B and 0.93 ± 0.07 mg/dl in group C) (Table 2 and Figure 4). So, there was no clear effect of fasting on creatinine. This might be due to fasting duration was short. The finding was closely agreeable with the finding of Yasser et al, (2016) who found no significant change in creatinine level due to starvation. In addition, significantly increase of creatinine level in fasting serum due to dehydration was reported by Maughan et al, (2008); Khaled et al, (2011).

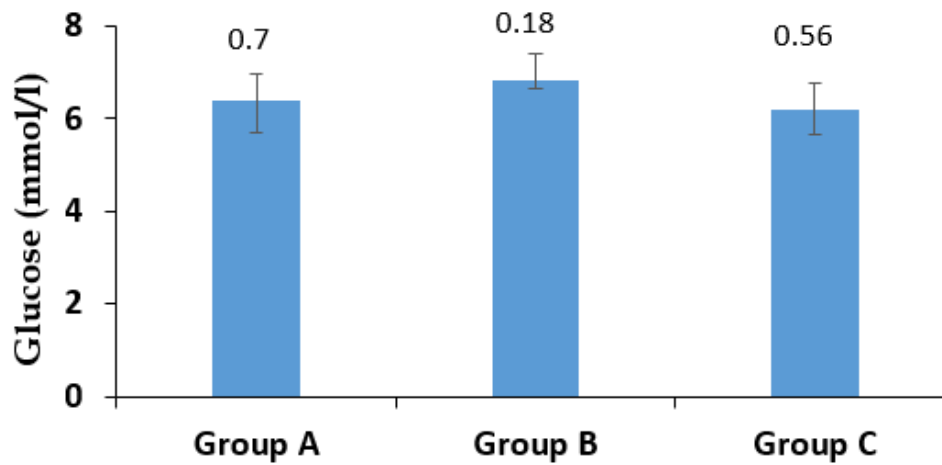


Figure 3. Effect of fasting on serum glucose
The superscript value above the bars indicates standard error

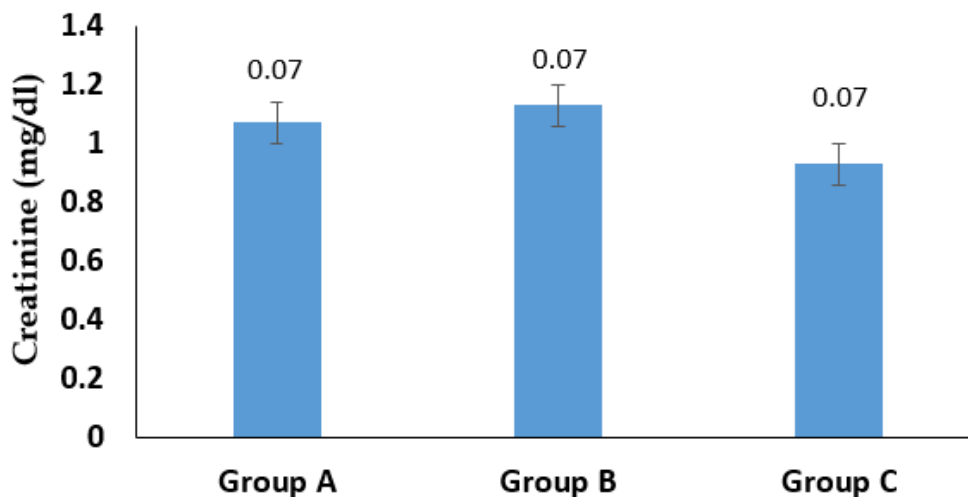


Figure 4. Effect of fasting on serum creatinine
The superscript value above the bars indicates standard error.

Effect of fasting on histomorphological alterations on liver and kidney

In the present study, histomorphology of liver in control group showed normal tissue structures and no detectable changes were found. In fasting groups both 14 hrs and 18 hrs fasting, there was slight depletion of glycogen in the liver hepatocytes. This might be due to gaining energy for body. So, fasting could not cause any significant histomorphological alteration in liver (Figure: 5). It was similar to the work Yasser et al, (2016) who reported that depletion of glycogen occurred in short-period of starvation. Section of kidney of non-treated mice showed normal tissue structures and no remarkable changes were found in cortex and medulla. In fasting groups nuclei were normal, no lesions found in the lining of glomerulus and tubules and no evidence of cellular infiltration were observed of the renal parenchyma.

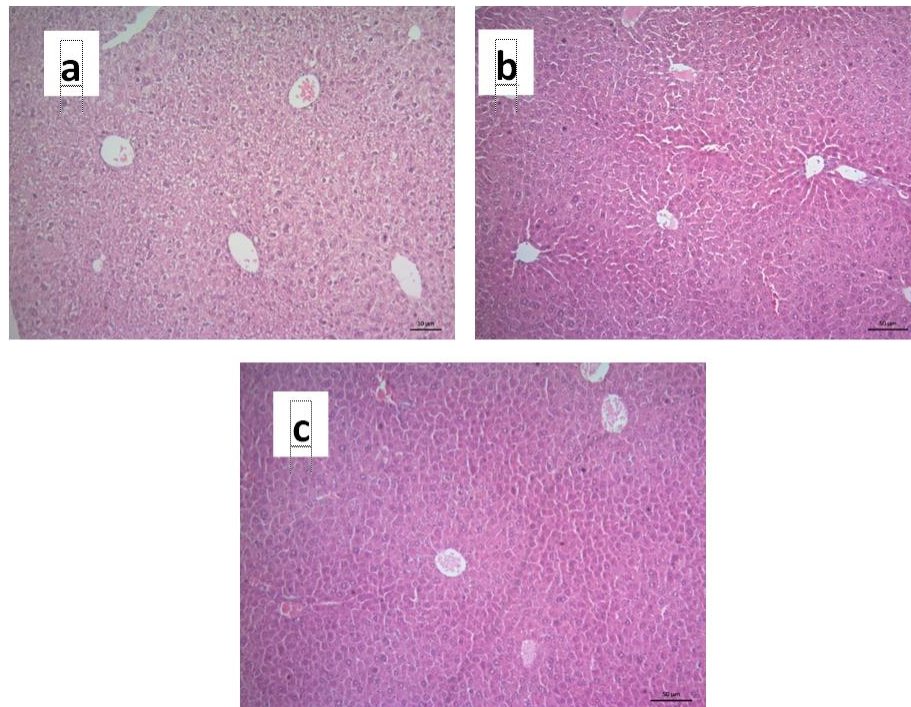


Figure 5. Photomicrographs showing histomorphology of liver (10x), (a) control group; (b) 14 hrs fasting group; (c) 18 hrs fasting group in mice

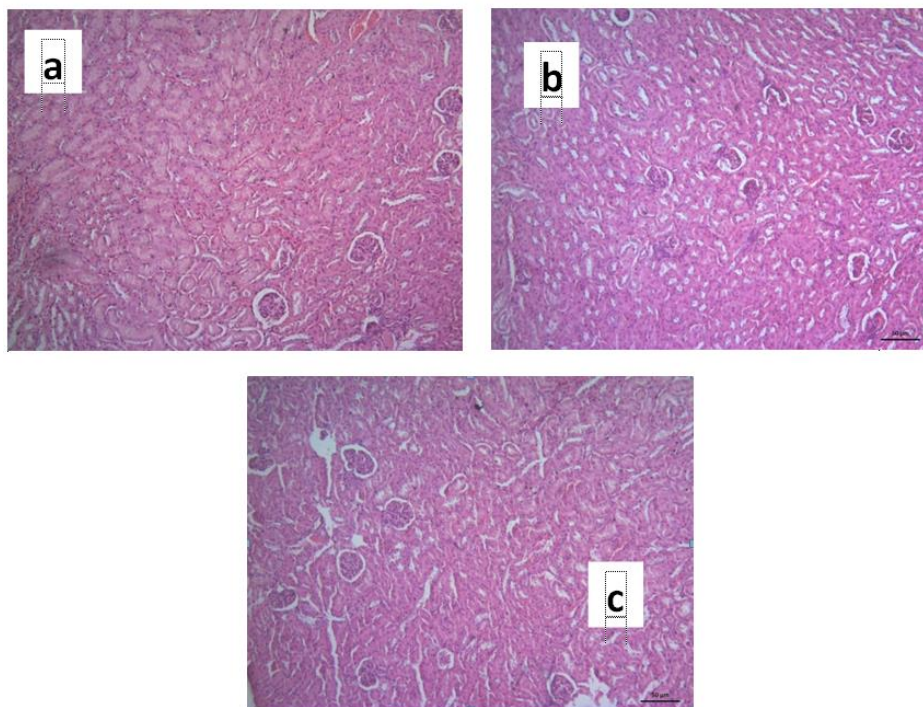


Figure 6. Photomicrographs showing histomorphology of kidney (10x), (a) control group; (b) 14 hrs fasting group; (c) 18 hrs fasting group in mice

Therefore, fasting could not alter any histomorphological changes in kidneys of both fasting groups (group B and group C) (Figure 6). It might be due to short duration of fasting. But this finding was not related with the finding of Yasser et al. (2016) who found that there was congestion of the capillary tufts of the glomeruli and the blood vessels of surrounding renal parenchyma, mild degenerative changes of tubular epithelium and cellular infiltration within the same areas of the paranchymatous tissue due to starvation.

CONCLUSIONS

From the present study it can be anticipated that short duration of fasting may not have harmful effects on organ structures. The results obtained from this study demonstrated that no significant changes were observed in body weight, serum glucose and creatinine level in fasting groups. It can be concluded that fasting may not affect oxidative stress or cellular damage and may have positive effects on serum glucose and creatinine level in healthy subjects. So, fasting is important for the improvement of one's physical health. Finally, the results provided in this study will be the basis for future investigations on health benefits of fasting.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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