

# Evaluation of Metabolic Syndrome Signs in two Different Animal Models

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## ABSTRACT

**Objectives:** In experimental pharmacology fructose induced metabolic syndrome is one among the standardized model used to induce insulin resistance across the globe. Various laboratory guidelines define different methods for the same. Hence the study has been undertaken to standardized and compare between two rat models of metabolic syndrome based on biochemical and histopathological examinations after permission was obtained from the institutional ethical committee. **Methods:** In experimental study wistar albino rats were subjected to evaluate the influence of fructose by two different methods to induce metabolic syndrome. Analysis of data was done with one way ANOVA followed by Dunnet's multiple comparison T test with Post hoc test using "Graphpad prism Instat software". **Results:** Induction of metabolic syndrome was done in current study with using 15 % of fructose in group 1 took 8 weeks with P value 0.0456, considered signify  $t=2.427$  with 7 degrees of freedom, however group 2 which was induced by high diet 60% fructose in less duration of only 2 weeks with two tailed P value is  $< 0.0001$ , considered significant. Changes were higher in Diet induced metabolic syndrome. **Conclusion:** Both models have shown elevated blood glucose level, fructose diet group has shown higher

glucose level in comparison to fructose drinking water group. The elevated glucose level may be because of fructose doesn't stimulate the secretion of insulin from pancreas  $\beta$  cells and also in hypertriglyceridemia there is a reduction in insulin sensitivity this may leads to the formation of hyperglycemia.

**Key words:** Metabolic syndrome, Fructose Diet, Fructose water, Insulin resistance, Insulin sensitivity.

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## INTRODUCTION

Fructose induced metabolic syndrome is one among the standardized model used for experimental research across the globe. There are various methods to induce the fructose induced metabolic syndrome by the means of different diets and water.<sup>1,2</sup> Experimental Metabolic Syndrome models usually characterised by increased serum triglycerides (TG), low serum high density lipoprotein cholesterol (HDL-C), elevated blood pressure, increased fasting plasma glucose, and obesity) in both the models, also has been reported that fructose diet produce increased oxidative stress and nitric oxide dysfunction, affecting the endothelium.<sup>3-5</sup> Hence the study has been undertaken to rule the difference between two rat models of metabolic syndrome based on biochemical and histopathological examinations.

## MATERIAL AND METHODS

24 Wistar Albino rats strains of either sex between 150 to 250 g were obtained from animal house attached to department of Pharmacology, SDM Centre for Research in Ayurveda and Allied Sciences, Udupi. The animals were fed with normal rat diet and water *ad libitum*. They were acclimatized in the laboratory condition for one week prior to the experimentation. The housing provided has the following conditions: controlled lighting of 12:12 h light and dark cycle, temperature of 25°C and relative humidity of approximately 50%. The experimental protocol was approved by the institutional ethical committee under the reference no. IAEC/02/2014-15/HS-07.

## Animal grouping

The selected animals were grouped into three different categories of eight rats each. Group I administered with normal rat diet and water *ad libitum* and served as normal control. Group II administered with 15% fructose in the drinking water along with normal rat diet, considered as fructose water group and group III administered with 55% fructose in diet and served as fructose diet group III.

## Source of Drug

- D Fructose 99 % was obtained from Loba Chemie Pvt Ltd. Mumbai
- Normal rat diet from Pranav agro's 'Amrut' brand rat feed and water given *ad libitum*

## Fructose drinking water model

Fructose drinking water was freshly prepared every day and was prepared based on weight/ volume formula. All the rats in the group II received 15% of fructose in drinking water and prepared by dissolving 15 g of D-Fructose was dissolved in 100 ml of tap water and administered for 8 consecutive weeks to induce metabolic syndrome summarised under Table 2.

## Fructose diet model

Group III has received fructose 55% Fructose in rat food to induce metabolic syndrome in 2 weeks 30 g/Day/Cage by mixing this 45%

**Table 1: Effect of two different fructose induced metabolic syndrome models on Triglycerides, Faecal fat content, Body weight and glucose level**

Group	Blood glucose mg/dl	% change	Triglycerides mg/dl	% change
Group 1-Normal Control	75.66 ± 6.80	-	81 ± 20.038	-
Group 2-Fructose water	135.71 ± 9.40	79.36↑	256.14 ± 5.016	216.22↑
Group 3-Fructose Diet	145.5 ± 6.48	92.3↑	286.75 ± 6.920	254.01↑

Data expressed in Mean ± SEM.

**Table 2: Effect of two different fructose induced metabolic syndrome models on Faecal fat content, Body weight**

Group	Faecal fat content (%)	Body weight changes (g)
Group1-Normal Control	3.05 ± 0.36	6.93 ± 3.669
Group 2-Fructose water	5.77 ± 0.84	84.61 ± 8.48
Group 3-Fructose Diet	6.09 ± 0.96	126.64 ± 8.55

**Table 3: Volume of fructose water Intake per day**

Group	Volume (ml)
Group 1 Normal Control	30.72 ± 1.12
Group 2 Fructose water	34.86 ± 1.50

Data represented in Mean ± SEM, two tailed P value is 0.0456, considered significant,  $t=2.427$  with 7 degrees of freedom.

**Table 4: Amount of fructose Diet consumption per day**

Group	Weight (g)
Group 1 - Normal Control	2.71 ± 0.04
Group 3 Fructose Diet	5.109 ± 0.28

Data represented in Mean ± SEM, two tailed P value is < 0.001, considered significant,  $t=8.389$  with 7 degrees of freedom.

and normal rat diet and 55% of fructose summarised under Table 3. Physiological parameters such as daily food and water intake were measured every day as per scheduled. The food and fluid intake was measured in by subtracting the measured amount provided to the remaining amount.

**Physical Variables:** Each animal was observed twice daily on weekdays and once daily on weekend, and any sign of ill health, reaction to the treatment and mortality were recorded. Body weight was recorded in the beginning and just before animal sacrifice. The weight of organs like stomach, large intestine and small intestine were recorded after sacrificing at the end of the drug treatment period. Food consumption was measured every day.

**Biochemical analysis:** Blood samples were obtained at the end of experimental period from retro orbital plexus in anesthetized over night fasted rats. The plasma samples were sent for analysis of serum triglyceride and blood glucose.<sup>6,7</sup>

**Histopathological slides:** The tissues are excised out immediately after sacrificing the animals cleaned of extraneous tissues, cut into pieces of appropriate thickness and transferred into 10% formalin solution. The tissues were allowed to remain in it till they are taken for processing. The paraffin blocks were prepared and the 5-10 µm thick sections were prepared, stained with haematoxylin and eosin and mounted in neutral DPX medium. The histological slides were examined under binocular research carl-zeiss's microscope.<sup>8</sup>

## Statistical Analysis

Data expressed as Mean ± SEM and analysis of Data was done with one way ANOVA followed by Dunnet's multiple comparison T test with Post hoc test using "Graphpad prism Instat software".

## RESULTS

The fructose administered through drinking water has shown 79.36 % of increase in the blood glucose level while through diet, it has shown 92.3% increase in comparison to normal control group. And the fructose diet has shown 254% and fructose in drinking water 216.22% in comparison to normal control group (Table 1).

Fecal fat content were increased in fructose diet group while compared to that of fructose administered with drinking water and normal control group (Table 2).

The quantity of fructose water and fructose diet were comparatively increased in comparison to normal control group (Table 3 and 4).

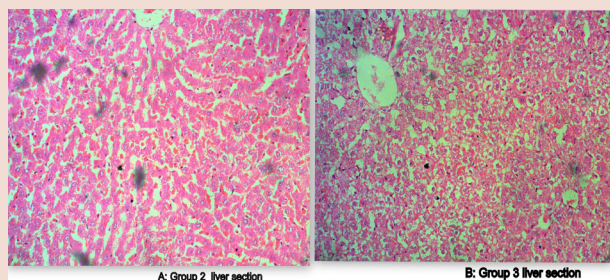
## Histopathological examination

Sections of liver, heart, kidney and pancreas were examined under microscope at different magnifications. The following inference was drawn:

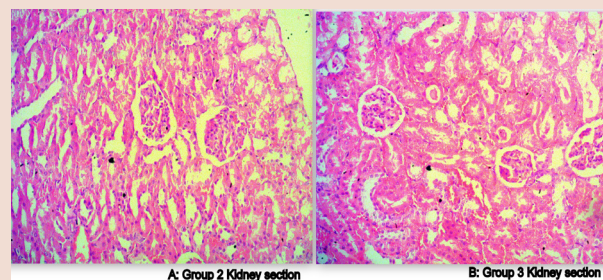
**Liver:** liver sections from fructose administered in drinking water exhibited mild to moderate fatty changes, moderate cell infiltration and hemorrhagic patches of moderate intensity was observed. In few sections multiple lesions were observed.

Liver sections from group 3 comparatively higher degree pathological changes were observed. Moderate to severe cell depletion was observed in almost all the sections, intense focal cell infiltration was observed in sections from four rats. Fatty changes of mild to moderate nature were also observed (Figure 1A-1B).

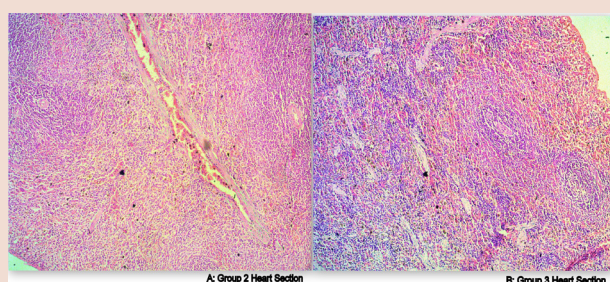
**Pancreas:** Histological examination revealed normal pancreatic cytoarchitecture in all the groups. However, further analysis of the size



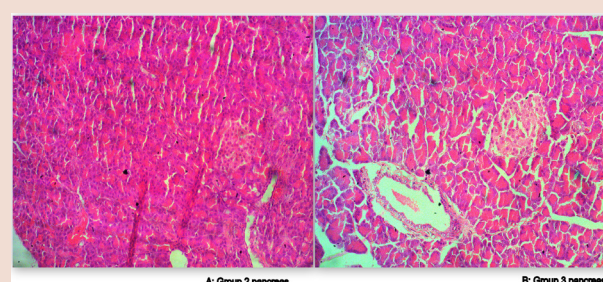
**Figure 1:** A) Group 2 liver section exhibited moderate fatty changes with haemorrhagic patches of moderate intensity.  
B) Group 3 liver section with intense focal cell infiltration was observed along with Fatty changes.



**Figure 2:** A) Group 2 pancreas section exhibited small sized 2; medium sized 13 and large sized 10.  
B) Group 3 pancreas section with 11 small sized, 12 medium sized and one large sized islet.



**Figure 3:** A & B - In both the 2 and 3 group kidney was presented with mild fatty changes.



**Figure 4:** A & B - In both the 2 and 3 group heart was presented with mild myocarditis

and cellularity of islets showed interesting data. The number, size and cellularity were evaluated in 18 fields. It was observed that in group 2 - small sized 2; medium sized 13 and large sized 10. The cellularity was low in one medium in 14 and high in 10 the cellularity was low cellularity in 3 islets; moderate in 15 islets and high in 1 islet. The figures for group 3 sections were 4 small sized, 6 medium sized and 8 large sized islets. The cellularity was low in 5; medium 15 and high in 1 (Figure 2A-2B).

**Kidney:** fructose in drinking water group rats exhibited mild fatty changes in the tubular epithelium but for this the cytoarchitecture was normal. In group 3 mild fatty changes were observed in three rats; six exhibited mild to moderate fatty changes was observed (Figure 3A-3B).

**Heart:** Microscopic examination of heart sections from group 2 revealed almost normal cytoarchitecture, in one rats mild myocarditis features were observed. In group 3 features of Myocarditis were observed (Figure 4A-4B).

## DISCUSSION

The results of present study showed an elevated level of serum TG both models, however the fructose diet has shown comparably higher degree 254% increase in comparison to normal control group. The elevated level of TG in the blood referred as hypertriglyceridemia. It ultimately results in reduction in glucose uptake by insulin sensitive tissues. This further leads to lipolysis and formation of more free fatty acids and glycerol. Further free fatty acids and glycerol enters adipose tissue to form TG.<sup>9</sup> Both models have shown elevated blood glucose level, fructose diet group has shown higher glucose level in comparison to fructose drinking water group. The elevated glucose level may be due to fructose doesn't stimulate the

secretion of insulin from pancreas  $\beta$  cells and also in hypertriglyceridemia there is a reduction in insulin sensitivity this may leads to the formation of hyperglycemia.<sup>10</sup> These can be interpreted in clinical study as independent risk factor for the obesity is lack of exercise which is in term responsible for the Diabetes. Sedentary life style and dietary habits is the determined as the main factor the obesity which further develops with insulin resistance. Among the obesity the more clinical component is visceral obesity and or ectopic fat in comparison to the total obesity, further lead to principal metabolic abnormality known as insulin resistance.<sup>11</sup> In contrast systemic metabolism of glucose, the liver is the primary metabolic clearing house four food that are responsible with development of insulin resistance, Transat, branched amino acid, ethanol and fructose. These four substrates are not insulin regulated as well as they deliver metabolic intermediates to hepatic mitochondria without an appropriate pop off mechanism for excess substrate enhancing lipogenesis and ectopic adipose storage.<sup>12</sup> After the insulin deficient mechanism due to unfolded protein response therefore due to non-availability of specific target based drug mechanism only rationalized based therapy can be useful altering hepatic substrate availability (dietary modification), reducing hepatic substrate flux (high fiber), or increasing mitochondrial efficiency (exercise) can be useful.<sup>13</sup>

## CONCLUSION

Induction of metabolic syndrome was done in current study with using 15 % of fructose in group 2 took 8 weeks P value is 0.0456, considered signify  $t=2.427$  with 7 degrees of freedom, however group 3 which was induced by high diet 60% fructose in less duration of only 2 weeks with two tailed P value is

< 0.001, considered significant. Changes were higher in Diet induced metabolic syndrome.

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## CONFLICT OF INTEREST

The author declare no conflict of interest.

## ABBREVIATION USED

**TG:** Serum triglycerides; **HDL-C:** Low serum high density lipoprotein cholesterol.

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