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ORIGINAL RESEARCH

Effects of chronic oral sodium benzoate on glucose homeostasis using adult mice as experimental models

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Abstract

Introduction: Sodium benzoate (NaB) is a widely used food preservative and antimicrobial substance. Sodium benzoate is a major preservative in soft drinks. Large scale consumption of soft drinks is a risk factor for T_2DM. Studies have shown that sodium benzoate decreases leptin levels which can lead to diabetes mellitus. Since studies exploring the effects of chronic oral sodium benzoate on models of glucose homeostasis are lacking, we intend to evaluate and compare the effects of chronic administration of sodium benzoate on glucose homeostasis in experimental models of adult mice.

Materials and methods: The study aims to evaluate the effects of chronic oral sodium benzoate at a dose of 62 mg/Kg/day on glucose homeostasis using adult mice as experimental models through a Prospective interventional animal experiment study and to evaluate the histological changes produced in the pancreas.

Results: Within-group analysis was performed from baseline to 6 months in the animals of test groups to detect any significant changes in the blood sugar values. Significant changes were noted after120 minutes (baseline versus after 6 months), the p-value was 0.045 which was statistically significant. There was no significant difference in other time frames. Among the 10 slides prepared from the pancreas of experimental mice of the test group, two slides showed a reduction in the Islet cell nest number. No changes were noted in the control group animal tissues.

Conclusion: Sodium benzoate taken orally for 6 months at a dose of 62 mg/kg/day produced a significant change in glucose homeostasis. Insulin resistance was observed at 120 minutes as per IPGTT test. It also produced histological changes like reduction in islet cell number which may be due to oxidative stress. Long term, studies may be necessary to confirm these unfavorable effects and depending on the results of these studies there might be a necessity for the regulation of the use of sodium benzoate.

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Introduction

Sodium benzoate (NaB) is a widely used food preservative and antimicrobial substance in salad dressings, pickles, vinegar, carbonated drinks, jams, fruit juices, sauces and also present in medications and shampoo. Chemically it is the sodium salt of benzoic acid. The chemical formula of sodium benzoate is $C_7H_5NaO_2$[1].Sodium benzoate and Potassium benzoate are commonly used food preservatives that are listed among the "generally regarded as safe" (GRAS) compounds by the United States Food and Drug Administration and can be present in foods at a concentration up to 0.1%[2,3]. Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) expert committee on food additives recommends an acceptable daily intake of sodium benzoate as 5 mg/kg body weight[4].

After ingestion of sodium benzoate rapid absorption occurs both in humans and animals. Studies showed that maximum plasma concentrations are reached within one to two hours[5].In the liver, the benzoate reaching through the diet is conjugated with glycine and will produce Hippurate. So, the deficiency of glycine is a limiting factor for the metabolism of sodium benzoate. This process mainly occurs in the mitochondrial matrix of the liver and kidneys. So, the intake of preservatives increases both benzoate and Hippurate levels in the body[6].

Animal studies have demonstrated that sodium benzoate given as oral gavage at a dose of 200mg/kg/day for 4 weeks showed anxiety and motor impairment in rats. Short term (4 weeks) consumption of sodium benzoate can impair memory performance and increase oxidative stress in mice[7]. Sodium benzoate is a major preservative in soft drinks. Large scale consumption of soft drinks is a risk factor for T_2DM. Studies have shown that sodium benzoate decreases leptin levels which can lead to diabetes mellitus. In a study done on acute exposure to GRAS levels of sodium benzoate, the results show that sodium benzoate does not affect insulin and glucose homeostasis, but further studies will be necessary to explore the metabolic impact of chronic benzoate exposure[8]. A study conducted in patients with renal insufficiency showed that the metabolite of sodium benzoate, Hippurate can accumulate and impair basal and insulin-stimulated glucose uptake into cells in culture[9]. This study concluded that this could explain the altered glucose homeostasis observed in these patients[10]. Another study showed a contradictory result where high dose intravenous sodium benzoate produced Hyperglycemia[11].Since studies exploring the effects of chronic oral sodium benzoate on models of glucose homeostasis are lacking, we intend to evaluate and compare the effects of chronic administration of sodium benzoate on glucose homeostasis in experimental models of adult mice.

Glucose intolerance can be defined as dysglycemia that comprises both prediabetes and diabetes. It includes the condition of impaired fasting glucose and impaired glucose tolerance and diabetes mellitus.

Methodology

Our study aims to evaluate the effects of chronic oral sodium benzoate on glucose homeostasis using adult mice as experimental models.

Primary objective: To evaluate the effects of chronic oral administration of sodium benzoate at a dose of 62 mg/Kg/day on glucose homeostasis using adult mice as experimental models

Secondary objectives: (1). To evaluate the histological changes produced by chronic oral administration of sodium benzoate at the dose of62 mg/kg/day in the pancreas of adult mice.

(2). To evaluate the effects of sodium benzoate on insulin resistance by Intraperitoneal glucose tolerance test (IPGTT) &Intraperitoneal insulin tolerance test (IPITT).

Sample size: Calculated using G* POWER ® version 3.1.9.2, by assuming an α of 0.05, βof 0.05 and power of 95%. Assuming a 10% attrition rate, the corrected sample size was calculated using the formula [sample size /1-(attrition/100)10]. Ten animals in a group are required to detect a statistically significant difference between the groups and within the group.

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Ten animals are needed in the negative control group to detect changes between the groups to avoid bias due to individual variation. So totally of 20 animals were required for this study.

Animals: In this experimental study, 20 three-month-old male Swiss albino mice weighing 20 to 40 g were used, which were obtained from Sree Chitra Thirunal Institute of Medical Sciences & Technology, Biomedical Technology Wing, Satelmond Palace, Poojappura, Trivandrum, Kerala - 695 012. Reg No: 98/GO/R-SL/BiS/99/CPCSEA. The study got approval from the institutional ethics committee, Sree Gokulam Medical College, Venjaramoodu Trivandrum. (SGMC-IAEC No.005/08Am/M4/2019). The animals were kept and experiments were performed at the animal house of Sree Gokulam Medical College, Venjaramoodu, Trivandrum. The stabilization period (Quarantine) was 10 days. The mice were caged in polycarbonate boxes in stipulated environmental conditions. The animals were given pellet chow and filtered water ad libitum. The mice were placed in two groups of 10 each. (Control and test group). Each animal in the cage has got a unique id for its identification.

Study Design- Prospective interventional animal experiment study.

Study Setting-Animal house of Sree Gokulam Medical College & Research Foundation, Venjaramoodu, Trivandrum-695607

Duration Of Study-Study commenced on 20/05/2019 after obtaining approval from the institutional animal ethics committee. The total study duration was12 months.

Experimental Design

In this prospective interventional study, 20 animals were divided into two groups. The Control group (n=10) received a chow diet and filtered water ad libitum daily for six months. The Experimental Group (n=10), received a daily dose of 62 mg/kg for 6 months via drinking water along with a standard laboratory diet.

Table1: Experimental Design

Group	Daily administration	Route	Duration
Control(n=10)	Filtered water + Chow diet	Oral	6 Months
Test, n=10	Filtered water + Chow diet+ Sodium Benzoate 62 mg/kg	Oral	

Dose calculation of sodium benzoate

Acceptable Daily Intake (ADI) in humans =5mg/kg body weight per day, Conversion factor =12.3, Mice Equivalent dose =12.3 x 5=61.2 mg/kg. Sodium benzoate was given at a dose of 62 mg /kg/day dissolved in drinking water. The daily average consumption of drinking water for each mouse was assessed during the initial quarantine period as 7ml. The required amount of sodium benzoate (62 mg/kg) was dissolved in the estimated quantity of drinking water and given daily orally over 6 months.

The average body weight of mice in the test cage is calculated as 39.2 g in the test 1 cage and35.2 g in the test 2 cage. The required amount of Sodium benzoate was calculated for each group and dissolved in the drinking water. The dose calculations are given below.

The required amount of sodium benzoate to be given =62mg/kg/day

1 kg →62mg

1000g→62mg

1g→62/1000mg

39g→62/1000 X 39= 2.418 mg (For test 1 cage)

2.418 mg of sodium benzoate dissolved in 7 ml of drinking water

In 7 ml →2.418 mg

1ml=2.418/7

1/1000 L=2.418/7 mg

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1L=2.418/7 X 1000=345 mg dissolved in 1 L of water
35g→62/1000 X 35=2.17 mg (For test 2 cage)
In 7 ml →2.17 mg
1ml=2.17/7
1/1000 L=2.17/7 mg
1L=2.17/7 X 1000=310 mg dissolved in 1 L of water

Data Collection Procedure

After the initial quarantine and grouping, the health parameters were recorded. Monthly weight recorded in the animal register. The health of the animals was monitored with the help of a veterinary doctor. Blood sugar levels of all animals were monitored every month using a glucometer. Tests for insulin resistance such as Intraperitoneal Glucose Tolerance Test and Intraperitoneal insulin tolerance test was done at the baseline and the end of the study. Animals were sacrificed by CO2 inhalation according to euthanasia protocol approved by the IAEC.

Glucose Analysis: Blood glucose levels were measured at baseline and every month for 6 months by a glucometer. Blood glucose was measured using a one-touch Verio flex blood glucose monitor.

Tests for insulin resistance

1. Intraperitoneal Glucose tolerance test (IPGTT)

After 12 hours fasting, D-(+)- Glucose solution (analytical grade from a local vendor), 2g/kg BW was injected into the mice intraperitoneally and blood glucose levels were measured at 0, 15, 30, 60, and 120 minutes of glucose load using a glucometer. This was done at baseline and the end of 6 months[12]. The procedure steps are given below

20 % Glucose solution preparation[13]

20 g glucose dissolved in 100 ml of distilled water, Volume of IP Glucose injection (microliter) =10 x Body weight (gm) =10 x 35=350 microliter=0.35 ml. This was given intraperitoneally to mice and blood glucose levels were measured using a glucometer at 0, 15, 30, 60 and 120 min after glucose injection.

2. Intraperitoneal insulin tolerance test (IPITT)

Mice would have fasted for 6 hours. Huminsulin regular 40 IU/ml (Lilly company) purchased from the hospital pharmacy, stored at 2-8° C) was given intraperitoneally at a dose of 0.75 U/kg body weight. Blood glucose levels were measured using a glucometer at 0, 15, 30 and 60 min after insulin injection. This was done at baseline and 6 months[12].There was no evidence of an allergic reaction to human insulin in mice. The procedure steps are given below

Weight of each mouse in test cage is estimated
Dose to be given=0.75 IU/kg or 0.00075 IU/gm
The body weight of each mouse is substituted to get the required volume of insulin. If the body weight of a mouse is 35 gm, then the required volume of insulin is calculated as
35 g→0.00075 X 35 =0.026 u insulin
The insulin used for our experiment was Huminsulin Regular 40 IU/ml
1 ml contains 40 units,0.25 ml contains 10 units
0.25 ml insulin +9.7 ml NS contain 10 units
10 ml=10 u ,1ml =1 u
1 ml + 9ml NS = 1 U
10 ml=1u
0.26 ml of 10 ml was taken to get 0.026 U of insulin. This was given intraperitoneally to mice and blood glucose levels were measured using a glucometer at 0, 15, 30 and 60 min after insulin injection.

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Fig 1. Illustrative representation of the GTT experiment(A1) Materials and equipment required for GTT (D- glucose, 1-mL syringes, 25–27-G needles, microsurgery scissors, glucose test strips, glucometer, lab balance).(A2) Material and equipment required for ITT (Insulin, sterile saline, 1-mL syringes, 25–27-G needles, microsurgery scissors, glucose test strips, glucometer, lab balance). (B) Animals are weighed before the beginning of the experimental procedure for the estimation of proper amount of Glucose/Insulin. (C) i.p. injection. (D) Evaluation of blood glucose at different time intervals.

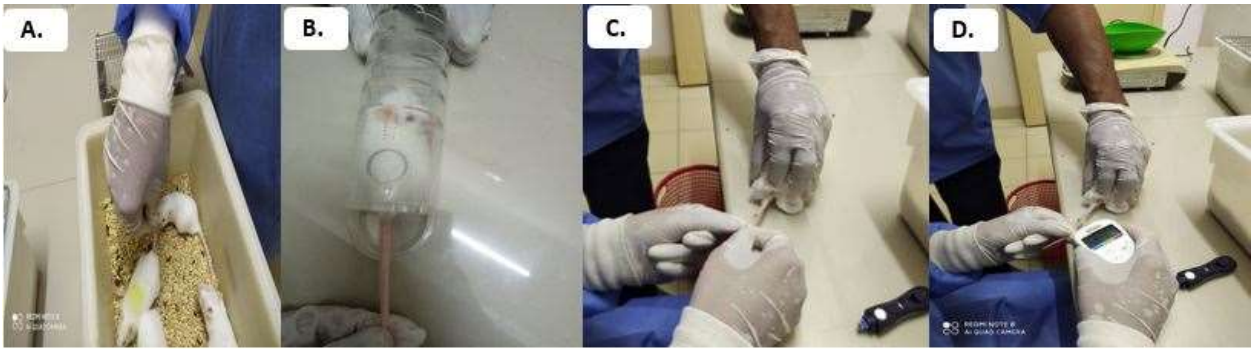

Figure 2.Illustration of blood collection procedure. (A) The blood of the mice to be estimated was identified and taken out from cage. (B) Identifying the lateral tail vein. (C)Pricking the vein with lancet.(D)Annotating glucose with glucometer strip.

Euthanasia

After the completion of experiments, animals were sacrificed using the CO_2 within the euthanasia chamber and tissues (Pancreas) was collected for histopathology studies. The procedure was done as per the recommendations by CPCSEA.

Histological Examination

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Figure 3. Identifying pancreatic tissue for Histopathology

Pancreatic tissue samples were fixed with 4% neutral buffered formalin, dehydrated, and embedded in paraffin. Embedded tissues were 5-micrometre thickness and stained with hematoxylin and eosin (H&E). Histological features like the number & size of islets, insulitis, fibrosis and amyloid deposition were observed using light microscopy. At least 25 different areas of each pancreas slide were observed, and all islets were counted.

Statistical Analysis

The glucometer results were entered in the Microsoft Excel sheet and analyzed with SPSS version1.0.0. 1406.The data were compared within groups and with the negative control using an independent sample **t-test**. For all statistical interpretations, $p<0.05$ was considered as the threshold for statistical significance. Normality was tested using Shapiro- Wilk test.

Results

Our prospective animal experimental study evaluated the effects of chronic oral administration of sodium benzoate at a dose of 62 mg/Kg/day on glucose homeostasis, on insulin resistance by Intraperitoneal glucose tolerance test (IPGTT)&Intraperitoneal insulin tolerance test (IPITT)using adult mice as experimental models. The histological changes produced in the pancreas of adult mice were also evaluated.

a) Evaluation of the long-term effect of Sodium benzoate on glucose homeostasis

Between-group analysis at the baseline-The comparison between blood sugar values of control and test group animals at baseline was done and was found to be non-significant. As shown in table no 2

Table No 2: Comparison of blood sugar values between Control group and test groupat baseline

At baseline	N	Mean	SD	t-value	p-value
Control	10	124.7	12.91	0.731	0.220
Test	10	119.5	18.95		

Independent sample t-test, p>0.05 considered as statistically not significant

Within-group analysis of control groups-The comparison between blood sugar values of control group animals at baseline and after 6 months was done and was found to be non-significant. This has been done to rule out any changes in blood sugar values in the control group animals which can occur other than sodium benzoate administration such as age, stress etc. and is depicted in table no 3.

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Table No3: Comparison of blood sugar values between Control group at baseline and after 6 months

Control Group	N	Mean	SD	t-value	p-value
Baseline	10	124.70	12.91	-0.093	0.927
After 6 months	10	125.30	15.86		

Independent sample t-test, p>0.05 considered as statistically not significant

Within-group analysis of test groups-The comparison of blood sugar values of test group animals at baseline and after 6 months was found to be non-significant and is depicted in table no: 4.

Table No4: Comparison of blood sugar values between Test group at baseline and after 6 months

Test Group	N	Mean	SD	t-value	p-value
Baseline	10	119.50	18.95	0.177	0.870
After 6 months	10	118	19.03		

Independent sample t-test, p>0.05 considered as statistically not significant

Between-group analysis after 6 months-The comparisons of blood sugar values between the control group and test group after 6 months was also done and found to be non-significant. Results are given in table no 5

Table No 5: Comparison of blood sugar values of Control group and test group after 6 months

After 6 months Group	N	Mean	SD	t-value	p-value
Control	10	125.30	15.86	0.932	0.420
Test	10	118	19.03		

Independent sample t-test, p>0.05 considered as statistically not significant

b) Tests for insulin resistance

IPGTT

Between-group analysis of IPGTT at baseline-Comparison of blood sugar values of IPGTT between control and test group animals at baseline were done and showed no statistical difference. Results are depicted in table no 6

Table No6: Comparison of blood sugar values of IPGTT at baseline

Group	N	Mean	SD	t-value	p-value
At 0 minute					
Test	10	89.80	12.01	-0.602	0.254
Control	10	93.40	9.37		
After 15 minutes					
Test	10	203.30	17.12	-0.749	0.679
Control	10	194.6	13.67		
After 30 minutes					
Test	10	128.70	19.28	-0.106	0.681
Control	10	137.70	17.09		
After 60 minutes					
Test	10	106.70	16.28	-0.506	0.450
Control	10	109.90	12.81		

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After 120 minutes					
Test	10	87.10	11.02	-0.665	0.454
Control	10	89.70	12.01		

Independent sample t-test, p>0.05 considered as statistically not significant

Between-group analysis of IPGTT after 6 months- Comparison of blood sugar values of IPGTT between control and test groups after 6 months given in table no 9. The mean score of the test group was 89.80±12.01 and the control group was 87.20±14.10 at 0 minutes. The difference between groups was statistically not significant (p>0.05). The comparison of blood sugar after 15 minutes,30 minutes ,60 minutes and 120 minutes also showed no significant change. The results are shown in table number 7.

Table No7: Between-group analysis of IPGTT after 6 months

Group	N	Mean	SD	t-value	p-value
At 0 minute					
Test	10	89.80	12.01	0.44	0.662
Control	10	87.20	14.10		
After 15 minutes					
Test	10	203.30	17.12	0.728	0.476
Control	10	197.8	16.67		
After 30 minutes					
Test	10	128.70	19.28	0.112	0.912
Control	10	127.70	20.71		
After 60 minutes					
Test	10	106.70	16.28	0.719	0.481
Control	10	100.60	21.31		
After 120 minutes					
Test	10	87.10	11.02	0.418	0.681
Control	10	84.80	13.48		

Independent sample t-test, p>0.05 considered as statistically not significant

Within-group analysis was also performed from baseline to 6 months in the animals of test groups to detect any significant changes in the blood sugar values. Significant changes were noted after120 minutes (baseline versus after 6 months), the p-value was 0.045 which was statistically significant. Given in table no 8.

Table No8: Within Group analysis of IPGTT

Group	N	Mean	SD	t-value	p-value
Test 0Min- Baseline	10	89.80	12.01	-0.603	0.256
Test 0min -After 6months	10	92.70	9.33		
Test 15Min- Baseline	10	203.30	17.12	-0.849	0.779
Test 15min -After 6months	10	209.30	14.36		
Test 30Min- Baseline	10	128.70	19.28	-1.12	0.782
Test 30min -After 6months	10	138.10	18.09		
Test 60Min- Baseline	10	106.70	16.28	-0.607	0.550
Test 60min -After 6months	10	110.80	13.81		
Test 120Min- Baseline	10	87.10	11.02	-0.881	0.045 *
Test 120min - After 6months	10	90.70	6.75		

*** Independent sample t test, p<0.05 considered as statistically significant**

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IPITT

Between-group comparison-the comparison of blood sugar values of IPITT between the control group and test group was done at baseline using an independent sample T-test. But no significant change was noted after 0,15,30and 60minutes of the IPITT test. Depicted in table no 9.

Table No9: Between-group analysis of IPITT result at baseline

Group	N	Mean	SD	t-value	p-value
At 0 minute					
Test	10	129	10.11	-2.09	0.051
Control	10	137.30	7.40		
After 15 minutes					
Test	10	75.90	8.16	-0.835	0.415
Control	10	83.80	28.76		
After 30 minutes					
Test	10	64.10	9.65	-0.791	0.44
Control	10	67.20	7.77		
After 60 minutes					
Test	10	62.50	7.41	-0.254	0.803
Control	10	63.60	11.53		

Independent sample t-test, p>0.05 considered as statistically not significant

Between-group comparison of blood sugar values of IPITT between the control group and test group after 6 months of the experiment using independent sample T-test. But no significant change was noted after 0,15,30and 60minutesof the IPITT test. As shown in table no 10.

Table No 10: Between-group analysis of IPITT result after 6 months

Group	N	Mean	SD	t-value	p-value
At 0 minute					
Test	10	132.30	11.92	-0.667	0.454
Control	10	130.20	10.91		
After 15 minutes					
Test	10	73.50	8.64	-0.991	0.315
Control	10	76.90	9.38		
After 30 minutes					
Test	10	64.50	9.65	-0.243	0.801
Control	10	67.70	7.77		
After 60 minutes					
Test	10	62.50	7.41	0.538	0.758
Control	10	66.70	6.75		

Independent sample t-test, p>0.05 considered as statistically not significant

Within-group analysis -Comparison of blood sugar values between test groups at baseline and 6 months was done to detect any significant changes in the control group and test group. But no significant change was noted. As given in Table 11.

Table No 11: Within-group analysis of IPITT

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Group	N	Mean	SD	t-value	p-value
Test 0 min – Baseline	10	129.00	10.11	-0.667	0.454
Test 0 min -After 6 months	10	132.30	11.926		
Test 15 Min- Baseline	10	75.90	8.185	0.638	0.858
Test 15 Min-After 6 Months	10	73.50	8.644		
Test 30 Min- Baseline	10	64.10	9.655	-.083	0.636
Test 30 min- After 6 months	10	64.50	11.693		
Test 60 Min- Baseline	10	62.50	7.412	.000	0.160
Test 60 Min- After 6 Months	10	62.50	11.825		

Independent sample t-test, p>0.05 considered as statistically not significan

Histopathology result

Histopathology was done to detect the effect of sodium benzoate on pancreatic tissue. Histological features like the number & size of islets, insulitis, fibrosis and amyloid deposition were observed using light microscopy. Among the 10 slides prepared from the pancreas of experimental mice of the test group, two slides showed a reduction in the Islet cell nest number. No changes were noted in the control group animal tissues. The control and test slides are depicted in figure No: 8.

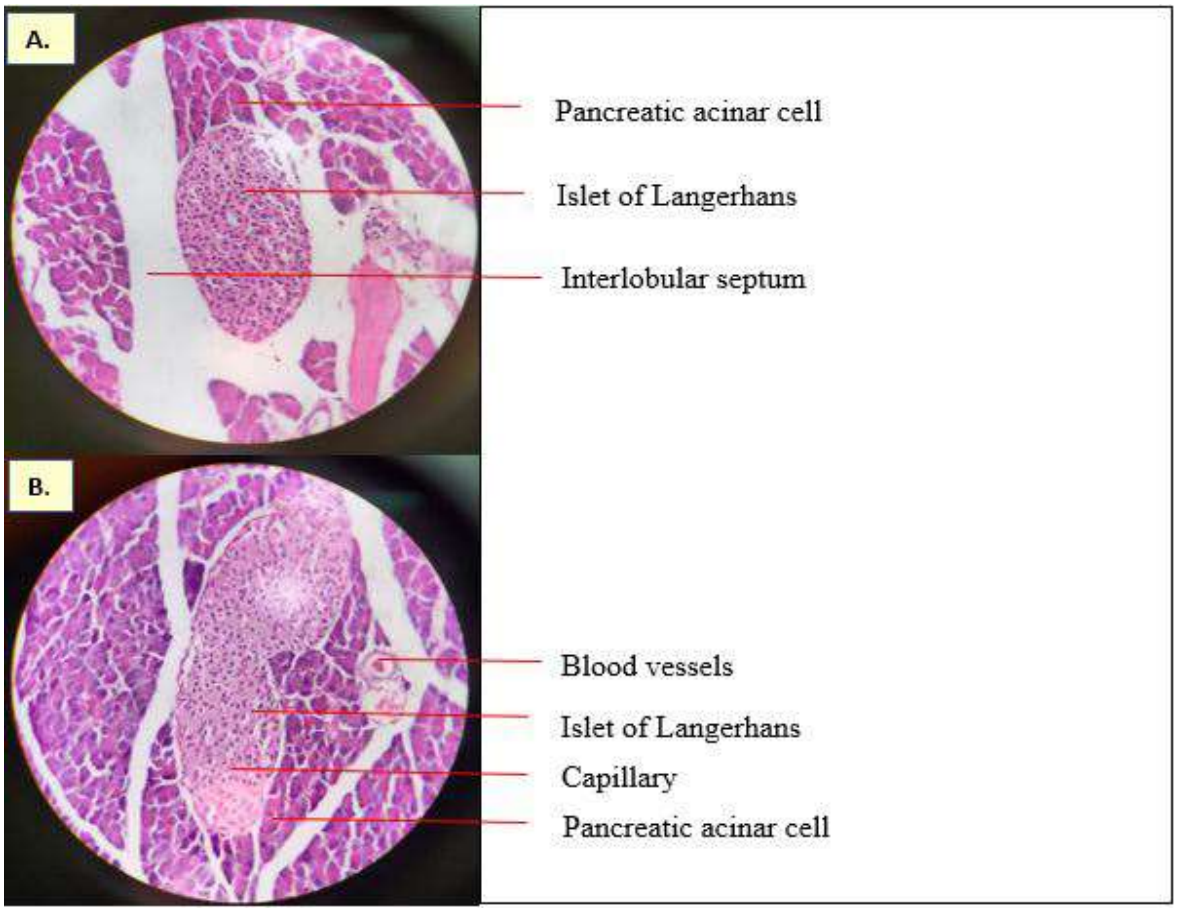

Figure 4. High power view of histopathology of control mice(A) and test mice (B). Normal Features such as Islet of Langerhans, acinar cells, blood vessels are seen. Fibrosis, Amyloidosis etc. which are features of Diabetes are not seen.

Discussion

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In the present study, we evaluated the effect of sodium benzoate administration on glucose homeostasis using adult Swiss albino mice as experimental models for 6 months. The dose used in our study was 62 mg/Kg /Day. In addition to this, we also evaluated the histological changes induced by sodium benzoate in the pancreatic tissue of mice. Insulin resistance was evaluated by two tests IPGTT and IPITT. The between-group analysis of test and control was done at baseline and after 6 months. Within-group (comparison of blood sugar values of test group at baseline and test group after 6 months) analysis was also done. Statistically significant changes were observed in the IPGTT test at 120 minutes. No significant changes were noted in IPGTT at other time intervals. There were no statistically significant changes observed in the results got from IPITT. Short term studies conducted on sheep models showed analogues of benzoic acid can cause increase in plasma glucose concentrations[11].This study concluded that the endocrine pancreas could recognize the benzoic acid chemical structure and which will induce insulin and glucagon secretion in sheep. A clinical study of 14 days duration showed that oral sodium Benzoate administration doesn't produce any significant changes in insulin and glucose homeostasis[8].

Insulin resistance was tested by IPGTT and IPITT. In IPGTT test, baseline measurement of blood sugar showed no statistically significant differences between control and test groups (between groups). It was repeated after 6 months. The comparison of blood sugar after 0, 15 minutes,30 minutes,60 minutes and 120 minutes were done for detecting any significant change between groups. No significant changes were noted. Within-group analysis was also performed comparing the sugar values of the test group at baseline and test group after 6 months from baseline to 6 months to detect any significant changes in the blood sugar values. Significant changes were noted at 120 minutes. There were no significant changes in other time intervals (baseline versus after 6 months). The comparison of the results from the IPITT test was done between-group and within-group using an appropriate statistical test. But no significant change was noted after 0,15,30 and 60minutes of the IPITT test. IPGTT is used to assess the body's ability to metabolize glucose[14]. There is a lack of studies that assess the effect of sodium benzoate on insulin resistance which is usually assessed by IPGTT and IPITT. In our study, we evaluated the effect of sodium benzoate on glucose homeostasis and found that the 6-month administration of sodium benzoate produced significant change in glucose homeostasis. Further long-term studies are needed to confirm this.

Histopathology was done to detect the effect of Sodium benzoate on pancreatic tissue. Histological features like the number & size of islets, insulitis, fibrosis and amyloid deposition were observed using light microscopy. Two slides among test groups showed a reduction in the Islet cell nest number. No changes were noted in the control group animal tissues. In a clinical study carried out in human lymphocytes, oxidative stress caused by sodium benzoate has been pointed out for the pancreatic beta-cell damage[15]. Another study conducted in Zebrafish larva showed that sodium benzoate could induce oxidative stress and the study suggested caution in excessive use of this preservative in processed and packeted foods[16]. In our study, two slides among test groups showed degenerative changes.

Clinical studies of short-term duration showed that oxidative stress can be developed as a result of sodium benzoate. This oxidative stress is implicated in the development of pancreatic beta-cell damage and insulin resistance[15]. Antioxidants are the agents that protect our bodies against various harmful oxidants. Superoxide Dismutase enzyme, catalase, glutathione peroxidase, glutathione – s- transferase, glutathione reductase are examples of antioxidant enzymes that present in our body and these enzymes protect our body against oxidative stress. A previous study showed that sodium benzoate administration reduced Glutathione peroxidase, glutathione -S- transferase, Catalase and Superoxide Dismutase enzyme levels[17]. This study also showed that sodium benzoate produces a significant rise in the levels of inflammatory cytokine markers such as TNF-α, IFN-γ, IL-1β and IL-6. This all may contribute towards the

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development of oxidative stress followed by gland damage which could account for glucose homeostasis abnormalities.

Preservatives are added to the food to prevent microbial growth and undesirable chemical changes. But in the present century because of Industrialization, the growing fast-food culture and the lack of stringent regulations, the use of preservatives has increased a lot. But these compounds are toxic and was found to be harmful to humans. Sodium benzoate is one of the most commonly used food preservatives and is found in food items such as vinegar, carbonated drinks, jams, fruit juice, and condiments[15].FDA allows usage of sodium benzoate at 300 mg/1 kg in dairy products such as yoghurt, ice creams and pudding. But an increased dose of sodium benzoate taken for a longer period can lead to health disorders.

Our study concluded that 6-month oral administration of sodium benzoate produced statistically significant changes on glucose homeostasis, on IPGTT done for insulin resistance at 120 minutes. It also produced islet cell atrophy in 2 slides among the test groups. So further long-term studies are required to prove the effect of sodium benzoate as it is one of the major food preservatives used in the present decade.

Conclusion

Sodium benzoate taken orally for 6 months at a dose of 62 mg/kg/day produced a significant change in glucose homeostasis. Insulin resistance was observed at 120 minutes as per IPGTT test. It also produced histological changes like reduction in islet cell number which may be due to oxidative stress. Long term, studies may be necessary to confirm these unfavorable effects and depending on the results of these studies there might be a necessity for the regulation of the use of sodium benzoate.

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