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Research Article
Protective Effect of Maternal Prebiotics Against Neonatal Jaundice Induced by Phenylhydrazine in Rats

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Abstract

Background and Objective: The nutritional environment *in utero* renders defensive mechanism in the neonates. Hence, the present study was framed to elucidate the protective effect of maternal prebiotics administration in neonatal jaundice. **Materials and Methods:** Rats pups of Wistar strain were fed prebiotic fiber diet, oligofructose (40%, (w/w), *ad libitum*) throughout pregnancy and lactation was induced with jaundice by intraperitoneal injection of phenylhydrazine hydrochloride (50 mg kg^{-1} b.wt., dissolved in saline). At the end of the experimental period, the pups were killed, blood samples and liver tissues were isolated and tested for various oxidative markers, anti-oxidant enzymes, serum markers such as; bilirubin and SGOT, SGPT and liver function marker enzymes. **Results:** The offspring's of maternal prebiotics administration renders protective effect in liver enzyme markers such as; ALP, SGOT and SGPT which was demonstrated by the unaltered levels of the enzymes compared to jaundice group. In addition, cellular metabolizing enzymes such as; SDH, GDH, GGT were also restored with reduced oxidative markers and improved antioxidant enzymes compared to jaundice induced rats. **Conclusion:** Thus, the study demonstrated that the supplementation of maternal diet with the prebiotic fiber oligofructose elicits a long-term beneficial effect in offsprings in terms of hepatoprotection.

Key words: Maternal prebiotics, oligofructose, neonatal jaundice, hepatoprotection, phenylhydrazine hydrochloride

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Jaundice is a complex disease and is characterized by yellowish skin color, caused by the accumulation of bilirubin bile pigments in the mucous membranes and sclera[1]. Many varieties of jaundice like pre-hepatic, hepatic and post-hepatic jaundice have been identified which are either congenital or acquired[2]. Neonatal jaundice is critical which occurs in the newborn child[3,4]. Neonatal jaundice is one of the prevalent diseases in children. It is estimated that 60% of normal and 80% of premature babies are identified with clinically detectable jaundice as soon as delivered which causes readmission of the children to post birth[5]. The jaundice is mild and transient in most of the neonatal cases, occurred due to the immature liver excretory pathway for bilirubin that leads to enhanced bilirubin accumulation[5]. The bilirubin produced endogenously can be toxic, especially in infants[6]. The hyperbilirubinemia in infants is caused by two ways. The major factor is the relative absence of intestinal bacteria in the first week of new borne results in the failure in newborn babies' transformation of bilirubin to stercobilin. Moreover, the increased hyperactivity of the beta-glucuronidase enzyme in the sterile gut and the mild alkaline pH of the proximal intestine are identified to cause hyperbilirubinemia[7,8]. The administration of probiotics can decline the enterohepatic circulation by altering the gut microbial population and decreasing the function of the beta-glucuronidase enzyme[9].

Phototherapy is the widely used method for the treatment of severe hyperbilirubinemia but without proper diagnosis of neonatal hyperbilirubinemia, it may cause an extreme toxication in the development of kernicterus. Apart from their toxicity, bilirubin is an essential component and plays a positive role in physiological activities[10,11]. Moreover, it is used to protect the membranes against oxidative stress *in vitro*[12]. Recent days, functional foods are gaining much interest due to their potential health applications. Apart from providing nutrition, functional foods have an ability to enhance the health factors or decrease the risk of disease[13,14]. It was shown that probiotics supplementation during pregnancy and breastfeeding provide immunity in the infant[12]. Various investigations have been conducted on the utilization and safety of probiotics in newborns[15,16]. Supplementation of probiotic Mami Ai decreases the breast milk jaundice through improving the gut microflora[17]. Thus, the advantages of dietary fiber hold an important part of a healthy meal[18], because it favored the growth of gut beneficial microbes such; as *Xylanibacter*, *Prevotella*, *Bifidobacterium*, the clostridial cluster XIVa and *Faecalibacterium prausnitzii* and suppressed the growth of harmful species such as *Firmicutes* and Enterobacteriaceae[19].

Most of the prebiotics were made from the nondigestible oligosaccharides, among them, fructans are the most used prebiotics[20-24]. Oligofructose (OFS) and inulin potentially modify the gut microbial composition by enhancing the growth of *Bifidobacteria*[25]. The oral supplementation of indigestible inulin-type fructans reduced the tumor size in hepatic and mammary tumor mouse models[26,27]. While, the prebiotic role of dietary fiber in the food imparts resistant against gastric acidity and enzymes like α-glucosidase, maltase, isomaltase and sucrose and also brings changes in the gut microbiota are correlated with prevention or postponement of CVD with hypercholesterolemia, osteoporosis, diabetes, gastrointestinal infections and gut inflammation[28,29]. Hence, in the present study aimed to examine the beneficial effect of maternal prebiotic fiber oligofructose administration against the development of neonatal jaundice.

MATERIALS AND METHODS

All the work related to this study was conducted in affiliated institutes. The analytical part and animal study were conducted in the month of March-December, 2018.

Experimental animals: The experimental procedures and protocol were done after obtaining the proper approval from the institutional animal care and monitoring committee and performed as per the guidelines for the care and use of laboratory animals (CHKX201807). Total 24 numbers of virgin female Wistar rats were caged in a temperature and a humidity-controlled room provided with a 12 h light-2 h dark cycle. After acclimatization, female rats were mated with Wistar males in wire-bottom cages. On the day, when the copulation plug was found, the females were isolated and grouped separately.

Jaundice induction: Females rats were grouped into 12 in each group. The control group received a normal diet and experimental diets containing prebiotic fiber oligofructose (OFS) (40% (w/w), *ad libitum*) continued throughout pregnancy and lactation. Following birth, the litters were also continued with the same diet for 2 weeks.

After 2 weeks, every 12 pups from those two groups were earmarked and segregated for the final experimental group as follows. Group 1: Normal control, Group 2: Jaundice induced, Group 3: Prebiotic control and Group 4: Prebiotic+jaundice. Jaundice group animals were induced with the intraperitoneal injection of phenylhydrazine hydrochloride (75 mg kg^{-1} b.wt., dissolved in saline) per day for 2 consecutive days along with the usual diet containing OFS for another 24 h. The animals were anesthetized with isoflurane and fasted blood sample were collected from the trunk blood following decapitation. The blood sample was centrifuged at 1600 g for 15 min at 4°C and plasma was stored at -20°C until analysis. The liver tissues were isolated and washed followed by the grind in a homogenizer by using the physiological saline. The homogenate was centrifuged at 2500 rpm for 20 min at 4°C and the supernatant was collected and stored in -80°C until analysis.

Biochemical marker enzymes: The liver tissue extract was used for the analysis of protein Carbonyl Content (ab126287) and Lipid Peroxidation (ab233471), superoxide dismutase and catalase assay Kit using Abcam assay kits. All other chemicals were used in analytical grade.

Serum marker enzymes: The liver transaminases include aspartate transaminase (AST or SGOT), alanine transaminase (ALT or SGPT), alkaline phosphatase (ALP), cholinesterase (CHE), total bilirubin, conjugated and unconjugated bilirubin and total protein were measured by using Beckman Coulter AU480 Chemistry Analyzer (AU480) with reference standards.

Cellular marker enzymes: Cellular marker enzymes such as; Sorbitol dehydrogenase (SDH), Ornithine transcarbamylase (OTC), gamma-glutamyl transferase (GGT) and glutamate dehydrogenase (GDH) were analyzed as per earlier studies[30-32].

Statistical analysis: The data are stated as mean±standard error (SE). Statistical significance was evaluated by student t-test using GraphPad Prism Software (GraphPad Software, San Diego, CA). The p-value of <0.05 was considered significant.

RESULTS

Physiological parameters: The present study results showed that the maternal rats administered with prebiotics did not exhibit any visual physiological changes, abortions and no other significant differences in the activities compared to control. Also, there was no significant difference in food intake or urine output between the groups were observed during the course of prebiotic administration. A stool consistency score (modified Bristol Stool score) was used for grading stool, the normal stool was graded as 1, soft and poorly formed stool was graded as 2 and the watery stool was graded as 3. With that score, the fecal consistency was evaluated and it was found that little change in fecal consistency of the prebiotic fed rats, but it was not statistically significant compared to control rats fed with normal rat chow (Table 1).

Table 1: Changes in various physiological parameters of the control, jaundice induced (J), maternal prebiotics (OFS) and J+O supplemented rats

	Groups			
Parameters	Control	Jaundice induced	OFS	J+O
Body weight (g)	165.0±7.0	160.0±6.0	170.0±5.0	169.0±8.0
Food intake (g)	10.0±2.0	9.0±2.5	11.0±3.0	9.0±3.0
Urine output (mL)	3.1±0.1	2.8±0.2	3.0±0.2	3.2±0.3
Stool consistency (A.U)	1.1±0.1	1.1±0.2	1.2±0.1	1.1±0.1

Antioxidant enzymes: Further on experimentations in the neonatal rats, the levels of serum oxidative stress in the serum samples were found to be elevated with compromised anti-oxidant enzymes in the jaundice group compared to control. The levels of lipid peroxidation ($p<0.01$) (represented in terms of Malondialdehyde levels, the unit of lipid per oxidation) and protein carbonyl ($p<0.01$) was significantly higher (Fig. 1a, b), while the level of SOD and catalase was reduced in jaundice group compared to vehicle-treated saline and OFS controls (Fig. 1c, d). On the other hand, the OFS pre-treated group later induced with jaundice significantly attenuated the levels of oxidative stress markers and improved anti-oxidant enzymes (Fig. 1).

Liver function enzymes: Additionally, the levels of biochemical markers for the liver function such as; bilirubin, total, conjugated and unconjugated forms of bilirubin were also determined in the control and experimental group of rats. The results demonstrated the elevated levels of total bilirubin ($p<0.01$) with an evident increase in both conjugated and unconjugated bilirubin with reduced total protein levels (Fig. 2a-d, respectively). It is speculated that the loss of liver function in jaundice reduced the potential of the liver to metabolize protein in the phenylhydrazine-induced jaundice group that had spiked the marker levels. On the other hand, the levels of altered bilirubin and the protein levels were not significantly affected in the OFS pre-treated group

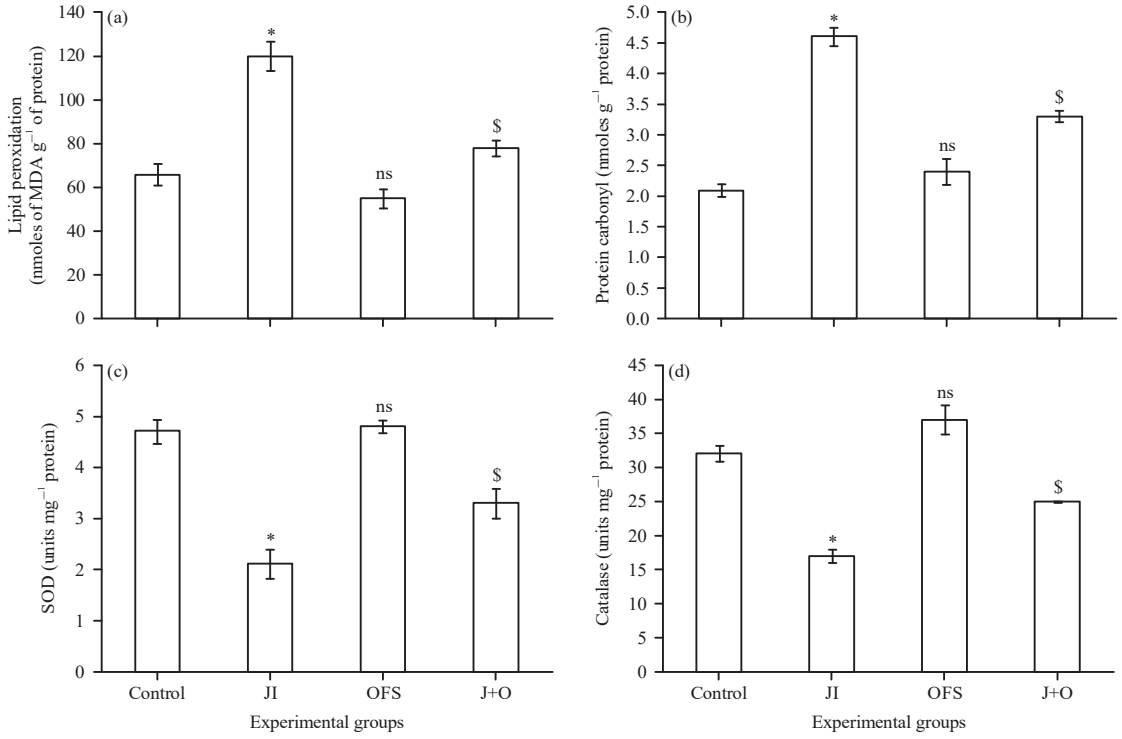

Fig. 1(a-d): Effect of maternal prebiotics on serum oxidation parameters and antioxidant enzymes, (a) Lipid peroxidation, (b) Protein carbonyls, (c) SOD and (d) CAT of control and experimental groups

Values are expressed as Mean±SE (n = 12), *: p<0.05 compared to vehicle-treated controls, $: p<0.05 prebiotic treatment in jaundice induction (J+O) compared to jaundice induced (JI), ns: Non-significant compared prebiotics administration (OFS) with vehicle-control rats (C)

demonstrated the maternal administration of OFS. While no significant changes in the level of bilirubin were observed between saline and OFS vehicle control groups (Fig. 2).

Liver marker enzymes: Further to delineate, the role of prebiotics on the liver functions, the enzyme markers with relevance to the hepatic functions were studied. The enzymatic markers such as; SGOT, SGPT, ALP were elevated while CHE was reduced in jaundice induced rats as compare to control (Fig. 3a-d, respectively). The results demonstrated that the elevated ($p<0.01$) liver enzymes in the jaundice group indicate the onset of inflammatory conditions or damage to cells in the liver tissue, while the levels of these markers were not elevated much in the OFS pre-treated group as compared to jaundice-induced group (Fig. 3).

Cellular marker enzymes: In addition to the above markers, liver-specific markers related to cellular metabolism was illuminated in the control, jaundice and prebiotic treated rats. Figure 4a-d showed the level of cellular enzymes such as; SDH, GGT, OCT and GDH, respectively. The listed metabolic enzymes are reported to be significantly ($p<0.01$) elevated in jaundice induced group of animals. The increased level of these enzymes signifies the struggle in the liver cells especially in the mitochondria electron transport system and in the respiratory complexes indicated the direct impact on the chances of the oxidative burst in the liver cells. This disparaging effect was prevented in the OFS pre-treated group promise that the OFS pre-treatment turns out to be a beneficial nutritional supplement in the event of hepatic metabolism.

DISCUSSION

Neonatal jaundice is one of the major diseases among newborn babies throughout the world and aid to prevent the onset is still under trial. Prebiotics play a crucial role in the conservation of healthy intestinal microflora balance by improving the growth and activity of several beneficial gut microbiota[33]. The oligofructose (OFS)

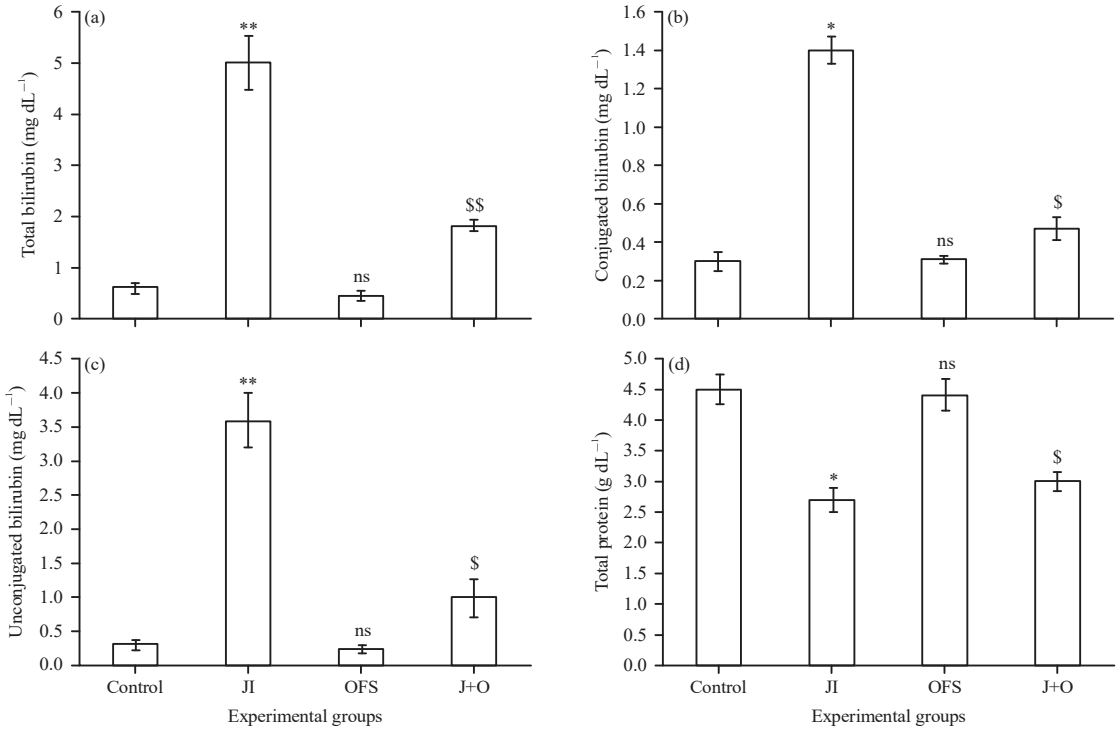

Fig. 2(a-d): Level of liver function parameters of control and experimental groups of rats, (a) Total bilirubin, (b) Conjugated bilirubin, (c) Unconjugated bilirubin and (d) Total protein

Values are expressed as Mean±SE (n = 12), *: p<0.05 compared to vehicle-treated controls, $: p<0.05 prebiotic treatment in jaundice induction (J+O) compared to jaundice induced (JI), $$: p<0.01 prebiotic treatment in jaundice induction (J+O) compared to jaundice induced (JI), ns: Non-significant compared prebiotics administration (OFS) with vehicle-control rats (C)

present in various natural foods contains simple carbohydrates and digestible fiber that nourishes the beneficial bacterial populations resides on the right side of the colon and aids in the absorption of various nutrients[34]. Thus, in the present study, the influence of maternal prebiotics on phenylhydrazine-induced jaundice in rats was illuminated in neonatal rats. Results of the present study showed that OFS administered rats did not elicit any physiological changes while maintaining the overall well-being of neonates support the reports on previous findings of the beneficial effects of prebiotics (short-chain galacto-oligosaccharides/long-chain fructo-oligosaccharides) in attenuating the hyperbilirubinemia in preterm neonates[35]. Moreover, it has been reported that the supplementation of prebiotics (oligosaccharides) in pre-term neonates results in the increased feeding intolerance, enhanced stool frequency and reduced bilirubin level[35]. It has also been reported that the administration of probiotic *Bacillus clausii* for 3 days regularly decreased the requirement as well as the duration of phototherapy in newborn babies[36].

Further on experimentations in the neonatal rats, the levels of serum oxidative stress were found to be elevated with compromised anti-oxidant enzymes in the jaundice group compared to control. While the level of SOD and catalase were tolerated in OFS pre-treated jaundice group compared to vehicle-treated saline and OFS controls evidences the protective role of pre-biotics against the toxin induced liver damage. The observed results are in concordance with the study on the beneficial effect of fructo-oligosaccharides by improving the antioxidative and hepatoprotective effects in D-galactose-treated Balb/cJ mice[37]. On a note to agree on the present findings, studies on supplementation of tocotrienol-rich fraction, a kind of prebiotics from the Palm sources exerted its ability in protecting the liver tissues from phenylhydrazine-induced hyperbilirubinemia through the reduction of oxidative stress and suppression of bilirubin-metabolizing enzymes[38].

Furthermore, the elevated levels of liver function markers such as; total bilirubin with evident unconjugated bilirubin levels speculate that the loss of liver function in jaundice

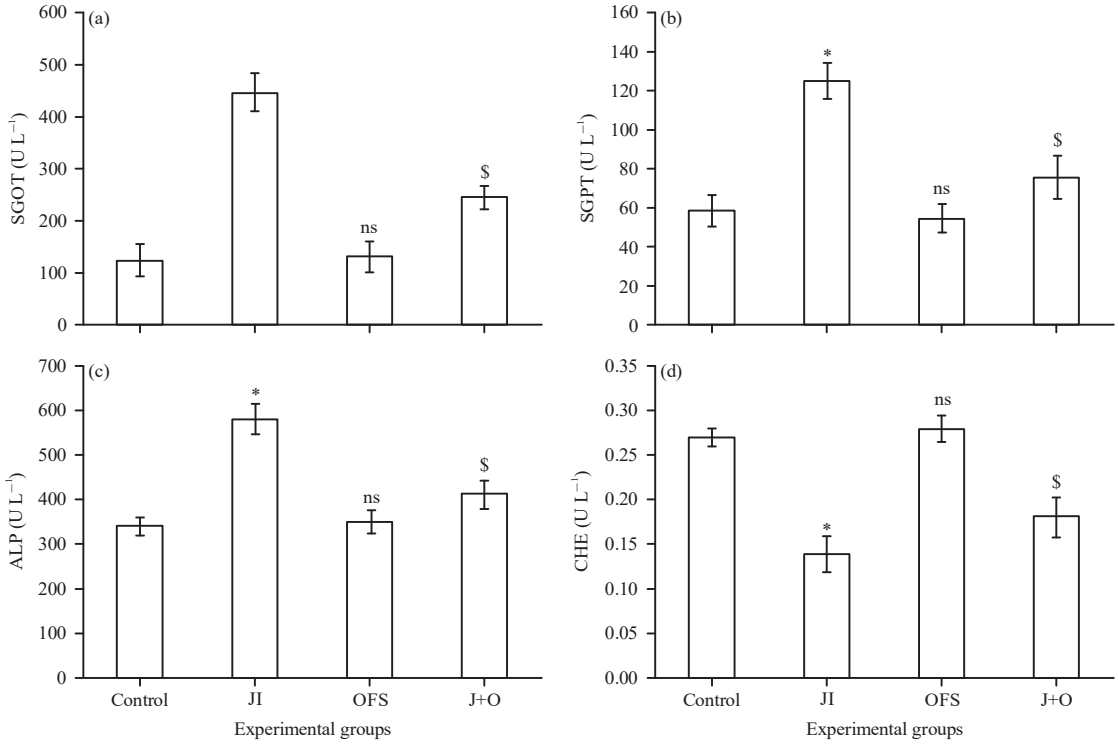

Fig. 3(a-d): Activities of liver marker enzymes of control and experimental groups of rats, (a) Aspartate transaminase (AST or SGOT), (b) Alanine transaminase (ALT or SGPT), (c) Alkaline phosphatase (ALP) and (d) Cholinesterase (CHE)
Values are expressed as Mean±SE (n = 12), *: p<0.05 compared to vehicle-treated controls, $: p<0.05 prebiotic treatment in jaundice induction (J+O) compared to jaundice induced (JI), ns: Non-significant compared prebiotics administration (OFS) with vehicle-control rats (C)

reduced the potential of the liver to metabolize the cellular protein in rats suffering from jaundice and the OFS pretreatment in offspring resist the impact of injury supports the previous findings that the probiotic lowered the incidence of neonatal hyperbilirubinemia[39].

Further to delineate the role of prebiotics on the liver functions, the enzyme markers with relevance to the hepatic cellular functions were studied and the results demonstrated the significantly higher levels of these markers in jaundice-induced group; while the ameliorated levels of these markers were found in OFS pre-treated group proposes the role of prebiotics in normalizing the cellular functions. In consistent with the data, type 2 diabetic patients who consumed the synbiotics consisted of *Lactobacillus sporogenes* (1×10^7 CFU), 0.04 g inulin (HPX) as prebiotic exhibited a reduction in the total bilirubin levels, while no changes in the serum ALP, AST and ALT levels[40]. Conversely, reports on synbiotics containing 7 species of probiotic bacteria and fructo-oligosaccharides supplementation in non-alcoholic fatty liver disease (NAFLD), improved steatosis while impeding its progression[41] with the aminotransferase levels remained static in synbiotic group. It is also demonstrated in human studies that the probiotic treatment reduced the liver enzymes in 22% cases, in spite of reducing the levels of ALT and AST in NAFLD patients[42-44]. Supporting the present study, supplementation of probiotics (*L. plantarum* and *B. coagulans*) with prebiotic inulin decreased the cadmium-induced toxicity in rats by revitalizing the declined ALT, AST, total bilirubin and metal deposition in the liver and kidney tissues[45]. Complementing the present study results, previous reports evidence the beneficial effect of administration of probiotics containing *Bifidobacterium bifidum* and *Lactobacillus plantarum* 8PA3 potentially by decreasing the total bilirubin, ALT, AST, GT and LDH levels in human alcohol-mediated liver injury[46]. Thus the findings of the present study signified that the administration of OFS during pregnancy protected the offspring in utero which needs further proteomics and metabolomics analysis to confirm the downstream signaling.

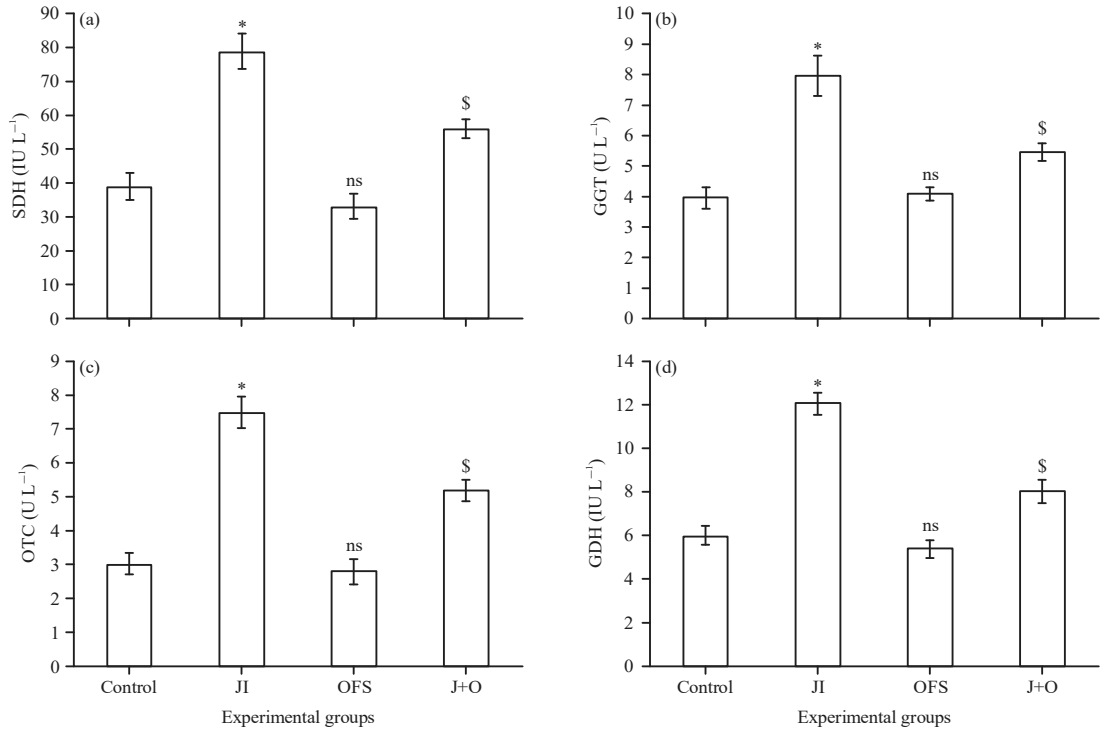

Fig. 4(a-d): Levels of cellular marker enzymes of control and experimental groups of rats, (a) Sorbitol dehydrogenase (SDH), (b) Gamma-glutamyl transferase (GGT), (c) Ornithine transcarbamylase (OTC) and (d) Glutamate dehydrogenase (GDH)
Values are expressed as Mean±SE (n = 12), *: p<0.05 compared to vehicle-treated controls, $: p<0.05 prebiotic treatment in jaundice induction (J+O) compared to jaundice induced (JI), ns: Non-significant compared prebiotics administration (OFS) with vehicle-control rats (C)

CONCLUSION

Results of the present study showed that the oligofructose (OFS) exerted the hepatoprotective effects in neonatal jaundice induced by phenylhydrazine. The maternal prebiotics administration with OFS facilitated the protective effects on liver enzymes ALP, SGOT and SGPT. In addition, maternal prebiotics restored the activities of various cellular metabolizing enzymes and decreased the oxidative stresses (decreased MDA and protein carbonyl contents) and enhanced the antioxidant enzymes such as; SOD and catalase. On the whole, it is concluded that the administration of maternal prebiotics (OFS) is a valuable supplementation that provides hepatoprotection to the offsprings.

SIGNIFICANCE STATEMENT

The study demonstrates that the beneficial effect of supplementing the maternal diet with the prebiotic fiber oligofructose (OFS) could have a long-term beneficial effect in offsprings in terms of hepatoprotection. Thus, the study results will help the researchers to uncover the critical areas of potential benefits of maternal mediated in utero environment that many researchers were not able to explore in the cases of neonatal jaundice.

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