https://doi.org/10.29309/TPMJ/2024.31.02.7644

The Professional Medical Journal
www.theprofesional.com
2024, Volume, 31 Issue, 02

ORIGINAL ARTICLE

Effect of glycyrrhizin and canagliflozin on treatment of non alcoholic fatty liver disease in a rat model.

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Article Citation: Azam N, Malik M, Zaheer A, Miraj R, Chiragh S, Sharif S. Effect of glycyrrhizin and canagliflozin on treatment of non alcoholic fatty liver disease in a rat model. Professional Med J 2024; 31(02):195-200. https://doi.org/10.29309/TPMJ/2024.31.02.7644

ABSTRACT… Objective: To observe the effect of canagliflozin and glycyrrhizin in prevention of non-alcoholic fatty liver disease. **Study Design:** Randomized Control Trial. **Setting:** Animal House, Post Graduate Medical Institute, Lahore. **Period:** March 2018 to August 2019. **Material & Methods:** It was an experimental study in which twenty-four adult healthy male Sprague- Dawley rats were categorized in four groups. All groups except normal control (A) were fed high fat high cholesterol diet throughout the study period. After 8 weeks group Disease Control (B) was administered distilled water, Glycyrrhizin treatment group (C) was given glycyrrhizin 60 mg/kg, Canagliflozin treatment group (D) was given canagliflozin 10 mg/kg. At the end of study, animals were sacrificed and liver tissue was prepared for histopathological analysis. Data was analyzed by SPSS 25 using Kruskal Wallis ANOVA followed by Mann Whitney U test. P value < 0.05 was considered significant. **Results:** Both showed significantly low grade of hepatic steatosis when compared to disease control. Canagliflozin treated group had significant decrease in hepatic steatosis, hepatic inflammation and ballooning than glycyrrhizin treated group. **Conclusion:** Glycyrrhizin halted the progression of fatty liver disease. The prevention of disease by canagliflozin infers their beneficial effect for treatment of non-alcoholic fatty liver disease.

Key words: Canagliflozin, Glycyrrhizin, Non-Alcoholic Fatty Liver Disease, Sodium Glucose Co-Transporter-2.

INTRODUCTION

Non-alcoholic fatty liver disease is defined as pathological deposition of fat in hepatic parenchyma with no or minimal alcohol intake. The disease spectrum consists of varied range of histological manifestations ranging from simple fatty liver to non-alcoholic fatty steatohepatitis.[1] The main factors associated with the disease include increased urbanization, type II diabetes, metabolic syndrome, obesity, and dyslipidemias.[2,3] The impact of NAFLD on healthcare has increased in recent years. This has commenced the implication of various interventions used in mitigation of non-alcoholic fatty liver disease including lifestyle modification, weight reduction and eradication of risk factors.[4] However, the definitive scientific approach is hither to be established ensuring the development of future practice guidelines in the treatment of NASH.

Canagliflozin is an FDA permitted oral antidiabetic drug which increases glucose urinary excretion by decreasing threshold of glucose in urine at proximal tubule of kidney. The increased caloric deficit due to increased glycosuria is advantageous in monitoring NAFLD without inducing hypoglycemia.[5,7]

Glycyrrhizin is a local herbal remedy derived from glycyrrhiza glabra also called as licorice root. It has well known medicinal effect on dyslipidemias, hyperglycemia and weight reduction. In Asian countries glycyrrhizin is use, as an herbal medicine, to treat viral and drug induced hepatotoxicity leading to chronic liver disease.[8] Glycyrrhizin has a wide range of pharmacological and organic properties due to which its conventional use as antiarthritic, antiallergic, antiviral antihepatotoxic, anti-inflammatory agents is recognized.[9]

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Article received on: 20/06/2023
Accepted for publication: 04/11/2023

The main objective of our study was to observe the effect of both glycyrrhizin & canagliflozin in mitigating progression of non-alcoholic fatty liver disease. The inclusive outcome of the study might subside the development of metabolic syndrome and related parameters.

MATERIAL & METHODS

The study was conducted from March 2018-August 2019 at Post Graduate Medical Institute Lahore after approval from the institutional ethical committee (UHS/Education/126-18/447). It was an experimental study conducted in healthy male Sprague Dawley rats of 4-5 weeks age, confined in the animal house of PGMI in optimum temperature and hygienic conditions. Twenty-four (24) healthy male Sprague Dawley rats were randomly allocated into four groups by lottery method, after one week acclimatization in the confinement of PGMI animal house, having free access to regular rat chow and water. Rats showing any sign of disease were excluded from study.

For the induction of non-alcoholic fatty liver disease, all rats (group B, C, D) were given 15% beef tallow, 1% cholesterol diet and 0.5% sodium de-oxycholate[10] for 8 weeks except the animals in group A, were fed normal rat chow and water. The normal rat chow (Group A) and high fat high cholesterol diet group (Group B) were treated with distilled water 2ml/kg, whereas glycyrrhizin treated group (Group C) was given 60 mg/kg glycyrrhizin obtained from Sigma Aldrich and canagliflozin treated group (Group D) was given 10 mg/kg canagliflozin imported from Aster pharmacy (U.A.E) for next 8 weeks. All the animals were sacrificed after euthanizing at the end of 16 weeks. The liver was dissected from the animals and saved in 10% formalin. After processing, slides were prepared and stained by hematoxylin (Scharlau, Hematoxylin according to Harris) & eosin (Victor lines, Pakistan) and studied under microscope (OLAMPUS: CX21FS) at 10X & 40X magnification. Slides were analyzed according to NASH Clinical Research Network (CRN) Histologic Scoring System.[14]

Statistical Analysis

The data collected was analyzed by using the Statistical Package of Social Sciences (SPSS 25). Data were checked for normality and homogeneity of variance by Levine's test, presented as mean ± standard deviation (SD). Quantitative variables were analyzed by one way ANOVA followed by Tukey's test. Qualitative variables were analyzed by Kruskal Wallis ANOVA and Mann Whitney U test. A p-value of ≤ 0.05 was considered statistically significant.

RESULTS

Groups	Body Weight (g)	Liver Weight (g)
Normal rat chow group (A)	303 ± 37.8	10.90 ± 2.26
High fat high cholesterol group (B)	316 ± 16.9[D]	13.11 ± 1.90
Glycyrrhizin treated group (C)	325 ± 29.2[D]	12.41 ± 1.42
Canagliflozin treated group (D)	259 ± 40.4*	9.67 ± 2.03
ANOVA	0.024*	0.07

Table-I. Data expressed as Mean ± SE (n=6). One Way ANOVA performed between groups. Multiple comparisons done by post hoc analysis with significance level ≤0.05 (*) vs HFD group. Significance is denoted as compared to canagliflozin treatment group ([D]), within the same duration of treatment.

Group I	Group J	Body Weight		Liver Weight	
		Diff	Sig	Diff	Sig
Normal rat chow group (A)	High fat high cholesterol diet group	-13	NS	-2.21	NS
	Glycyrrhizin group	-21.5	NS	-1.51	NS
	Canagliflozin group	44.17	NS	1.23	NS
High fat high cholesterol group (B)	Glycyrrhizin group	-8.5	NS	0.7	NS
	Canagliflozin group	57.17	0.047*	3.45	NS
Glycyrrhizin treated group (C)	Canagliflozin group	65.67	0.016*	2.75	NS

Table-II. Post hoc multiple comparisons for rats' body weight and liver weight at 16 weeks (n=6)

Groups	Hepatic Steatosis				Hepatic Inflammation				Hepatic Ballooning		
Grade brunt et al	0	1	2	3	0	1	2	3	0	1	2
Normal rat chow group (A)	6	-	-	-	6	-	-	-	6	-	-
High fat high cholesterol group (B)	-	-	1	5	-	1	1	4	6	-	-
Glycyrrhizin treated group (C)	1	4	1	-	-	5	1	-	-	5	1
Canagliflozin treated group (D)	4	2	-	-	-	6	-	-	-	3	3
p value	< 0.001***				< 0.001***				< 0.001***		

Table-III. Comparison for hepatic steatosis, hepatic inflammation, hepatic ballooning among all groups using Kruskal Wallis test at 16 weeks

(I) Group	(J) Group	P-Value		
		Hepatic Steatosis	Hepatic Inflammation	Hepatic Ballooning
Normal rat chow (A) group	High fat high cholesterol diet group	0.001***	0.002**	0.001***
	Glycyrrhizin group	0.006**	0.001**	0.001***
	Canagliflozin group	0.138	0.001**	0.002**
High fat high cholesterol group (B)	Glycyrrhizin group	0.003**	0.014*	0.005**
	Canagliflozin group	0.002**	0.006*	0.056
Glycyrrhizin treated group (C)	Canagliflozin group	0.075	0.317	0.241

Table-IV. Pairwise comparison for hepatic steatosis, hepatic inflammation, hepatic ballooning among different groups by Mann Whitney U test at 16 weeks

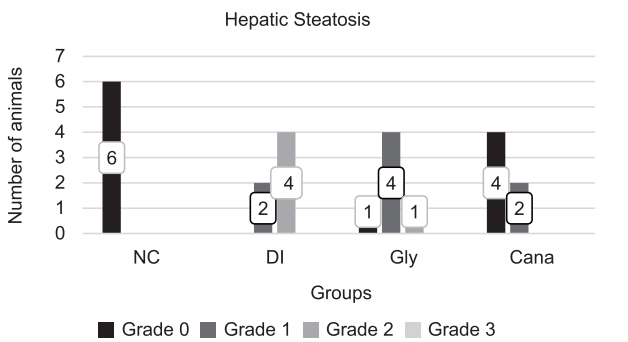

Figure-1. Grades of hepatic steatosis in normal, disease control, glycyrrhizin treated and canagliflozin treated group

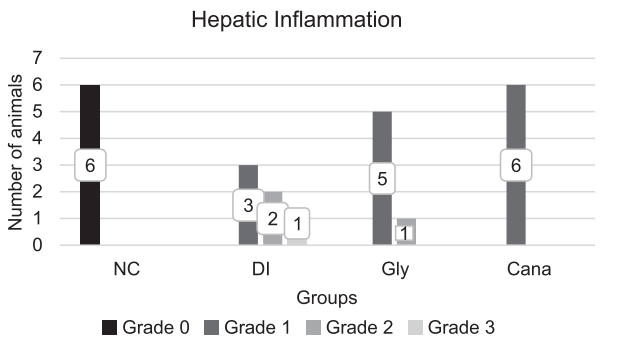

Figure-2. Grades of hepatic inflammation in normal, disease control, glycyrrhizin treated and canagliflozin treated group

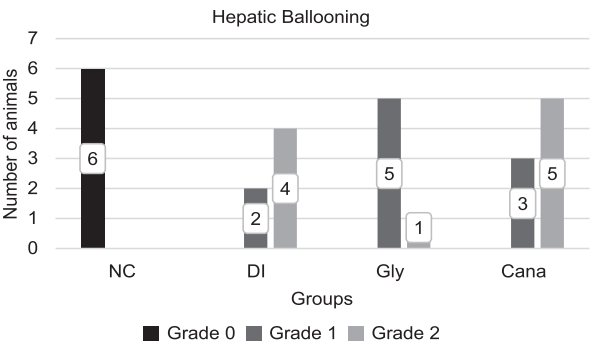

Figure-3. Grades of hepatic steatosis in normal, disease control, glycyrrhizin treated and canagliflozin treated group

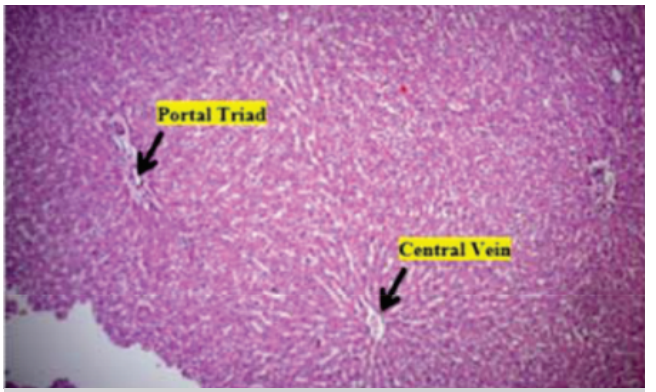

Figure-4. Normal control (Group A) showing normal architecture of liver consisting of one central vein and at least one portal triad (10x; H&E)

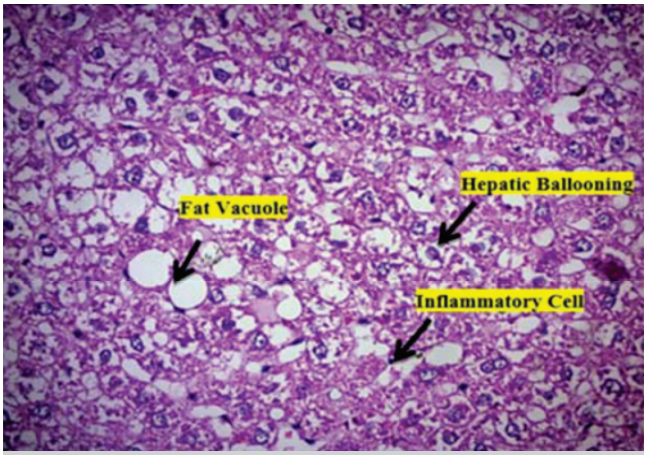

Figure-5. High fat high cholesterol diet (Group B) showing grade three hepatic steatosis, grade three inflammation and grade two hepatic ballooning (40x; H&E)

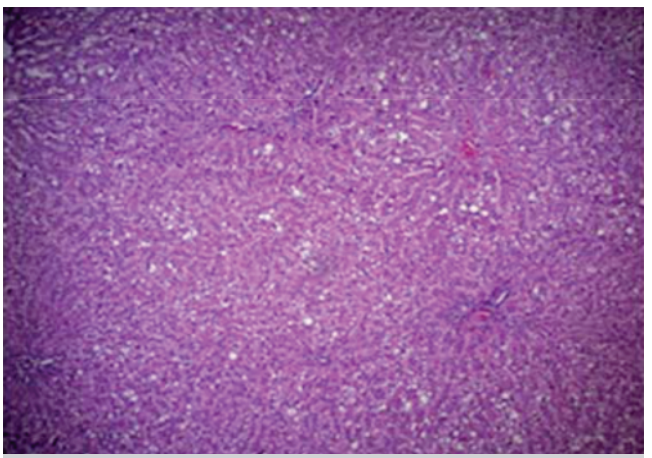

Figure-6. Treatment Group C (HFHC Diet+Glycyrrhizin) showing grade one steatosis (10x; H&E)

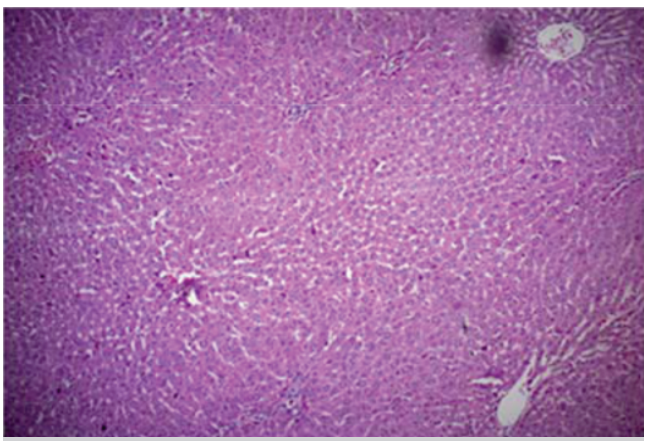

Figure-7. Treatment Group D (HFHC Diet+Canagliflozin) showing grade zero steatosis (10x; H&E)

DISCUSSION

Non-alcoholic fatty liver disease is an emerging global public health threat. It includes a spectrum of simple fatty liver to steatohepatitis (NASH) and hepatic fibrosis and hepatocellular carcinoma. The chief factor in pathogenesis of NAFLD is insulin resistance which results in hepatic de novo lipogenesis and impairs inhibition of lipolysis consequently increasing flux of fatty acids to liver. Insulin resistance also enhances adipose tissue dysfunction that causes secretion of adipokines and inflammatory cytokines causing oxidative stress which extends spectrum of NAFL to NASH.[11,12] Canagliflozin controls obesity in mice by inhibiting insulin resistance in diabetic obese rodents and reduces body weight in non-diabetic subjects. It regulates de novo synthesis of lipid and stimulation of fatty acid oxidation by inhibiting acetyl Co-A carboxylase.[5] It improves lipid profile as well as liver histology in nonalcoholic fatty liver disease.[6,7]

In our experiment, we developed a model for induction of non-alcoholic fatty liver disease in animals by giving high fat high cholesterol diet containing 15% fat, 1% cholesterol and 0.5 % cholate for 16 weeks.[10] High fat and cholesterol combined dietary model ensues better induction of NAFLD as it is similar to the composition of food of convenience containing high saturated fats with high cholesterol.[10]

The variables of the study included body weight, liver weight, liver histopathology comprising of hepatic steatosis, inflammation and ballooning. Quantitative variables were analyzed by one-way ANOVA followed by Tukey's test. Qualitative variables were analyzed by Kruskal Wallis ANOVA and Mann Whitney U test.

Body weight of all the groups increased persistently throughout the study period. At week 16, the post hoc analysis depicted decrease body weight in canagliflozin treated group as compared to glycyrrhizin treated group validating a previous study that net caloric deficit is mainly due to enhanced glycosuria causing a decreased body weight. Another article suggested that it inhibits AMPK based fatty acid oxidation causing decreased lipogenesis.[13]

The histopathological derangements observed in NASH are graded according to CRN.[14] Normal parenchyma of liver with no fatty infiltration was observed in normal control. The disease control group showed marked hepatic steatosis in liver parenchyma as illustrated in various studies.[15] When compared with the normal control group, it had higher grade demonstrating disease progression. The experimental groups showed substantial decrease in hepatic steatosis than disease control; though, the retrieval to normal liver architecture was not attained in all animals. Glycyrrhizin demonstrated imperative reduction of hepatic steatosis grade as mentioned in recent studies.[16,17] Canagliflozin, mitigated steatosis grade in liver of mice markedly[18,19], rats[20,21] and humans.[22] The outcome of the experiment demonstrated that canagliflozin therapy for 8 weeks decreased body weight, liver weight and also exhibited improvement in hepatic steatosis and hepatic inflammation. Since canagliflozin induced weight loss and hypoglycemia, it may have the potential as an anti-obesity drug. However, longer durations of canagliflozin treatment on obesity and the underlying mechanism should be studied in the future

CONCLUSION

1. Glycyrrhizin halted progression to hepatic fatty steatosis, hepatic inflammation and restored hepatic ballooning
2. Canagliflozin caused reversal of disease by restoring hepatic steatosis and hepatic inflammation completely while halting hepatic ballooning.

CONFLICT OF INTEREST / DISCLOSURE

I certify that the submission is *original* work and is not under review at any other publication.

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1	Nada Azam	Lit search, study design, data collection & analysis, write up, proof read & final of manuscript.	
2	Mahwash Malik	Lit. search, data interpretation, write up, prepared finalized.	
3	Akfish Zaheer	Lit. search, data collection & analysis write-up	
4	Rabab Miraj	Lit, search, data collection & analysis write-up.	
5	Sadia Chiragh	Study design, concept, data literature, Proof reading.	
6	Sadia Sharif	Lit. search, data analysis, data interpretaion.