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Original Article

Morphometric analysis of fatty liver with the treatment of ginseng root extract.

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Abstract

Background: Metabolic syndrome is the challenge of this era; diseases are initiated with the abnormal functioning liver. This study aims to evaluate the effects of HFD on liver anatomy and to study the morphological changes with its histological findings

Methodology: 50 male albino rats were acclimatized in the animal house of DUHS, divided into 5 groups (GP). GP 1 was on a standard diet for 6 weeks, GP 2 was on HFD for 6 weeks, GP 3, GP 4, and GP 5 were on HFD for 6 weeks. They were treated with ginseng root extract in doses of 100mg/ml, 200mg/ml and 400 mg/ml for 6 weeks. After 12 weeks, GP3, GP4, and GP 5 were dissected, and the liver was isolated for histology and micrometry. Blood was drawn and sent for LFT to correlate morphological changes with enzymes.

Results: Rats on a high-fat diet developed fatty liver with altered LFT. The treated group showed a reversal of changes in morphology and LFT in a dose-dependent manner.

Conclusion: The morphometric analysis revealed that fatty accumulation in liver cells deranges the liver function leading to metabolic syndrome. Ginseng root extract helps in depleting fatty accumulation in the liver.

Keywords

Liver Micrometry, Liver Weight, Liver Size, Hepatic Steatosis, Ginsenoside.

Introduction

This is the era of metabolic syndrome (diabetes mellitus, hypertension, obesity, and fatty liver disease) caused by high blood pressure (hypertension), high blood sugar levels (hyperglycemia), and high levels of lipids (hyperlipidemia)[1]. All these factors are interrelated. There are two leading causes one is an increased intake of fatty substances or a glucose-rich diet, and the other cause is a lack of physical activity[2]. A high-fat diet (HFD) now becomes a part of a sedentary lifestyle. Dietary fat accumulates in adipose tissues, the liver (NAFLD), and on receptors causing insulin resistance (IR). In obesity, fats convert into glucose, increased blood glucose level (hyperglycemia), and hyperlipidemia is the primary cause of myocardial infarction (MI) and stroke. Hyperglycemia is the root cause of diabetes, nephropathy, neuropathy, and retinopathy[3].

The liver is the metabolic factory, it absorb glucose, lipids, and proteins from the small intestine pass toward the liver through the portal vein to form ATP or energy coins through metabolic cycles. In fatty liver disease (hepatic steatosis) liver is not functioning correctly, leading to metabolic disorders. In a research article, it is suggested to write Metabolic associated fatty liver diseases (MAFLD)[4]. NAFLD is correlated with lipolysis of adipose tissues and ectopic fat distribution, causing insulin resistance[5]. Glucose cannot enter cells without insulin. In the absence of glucose, cells need energy, so it signals for lipolysis, an increased amount of lipids in the blood causing an increased uptake of fatty acids by the liver and more synthesis of TG[6].

Korean red Ginseng (KRG) is an herb found in Asia, Africa, China, Korea, and Egypt. It acts as an antihyperglycemic, antihyperlipidemic, and anti-obesity[7]. It works through adenosine monophosphate kinase (AMP kinase) to reduce glucose absorption from the intestine, decreasing plasma glucose levels[8]. It enhances glucose uptake in muscle cells by increasing mitochondrial biogenesis[9]. It also depleted the fat deposition by stimulating the oxidation of fats in adipocytes. It stimulates insulin secretion by beta cells of the pancreas. Genetically, it down-regulates the enzymes required for gluconeogenesis (converting non-carbohydrates into glucose)[10]. This study aims to evaluate the effects of HFD on liver anatomy, study the morphological changes with its histological findings, and to conduct micrometry to determine the size of hepatocytes and the number of fat droplets in the obese group and also in treated groups.

Methodology

In this animal model based experimental study, 50 male albino rats were chosen from the animal house of DUHS. They were divided into 5 groups (GP), GP 1 was on a standard diet for 6 weeks, GP 2 was on HFD for 6 weeks, GP 3, GP 4, and GP 5 were on HFD for 6 weeks. The sample size was calculated using the open epi program, which obtained 6 per group (10 animals were taken in each group for safe side). They were treated with ginseng root extract in doses of 100mg/ml, 200mg/ml and 400mg/ml for 6 weeks. After 12 weeks, GP3, GP4, and GP 5 were dissected, and the liver was isolated for histology and micrometry. Blood was drawn and sent for LFT to correlate morphological changes with enzymes.

HFD was prepared in the animal house by adding animal (cow) visceral fats, butter, and cheese in a standard diet containing carbohydrates (wheat flour) and protein (lentils) in the kitchen of the animal house, DUHS[11]. Ginseng root extract was prepared in the DUHS lab under expert guidance. Ginseng root was obtained from the herbal store, soaked in ethanol for 15 days, then grinded. The filtrate was evaporated and then condensed into a jar. This extract was used in treated groups with the help of a nasogastric tube (NG)[12].

Gross morphological variables are liver size, weight, color, contour, and consistency. Microscopic variables are the number of hepatocytes per reticule and fat droplets per reticule. Biomarkers (LFT) were conducted for confirmation of metabolic alteration.

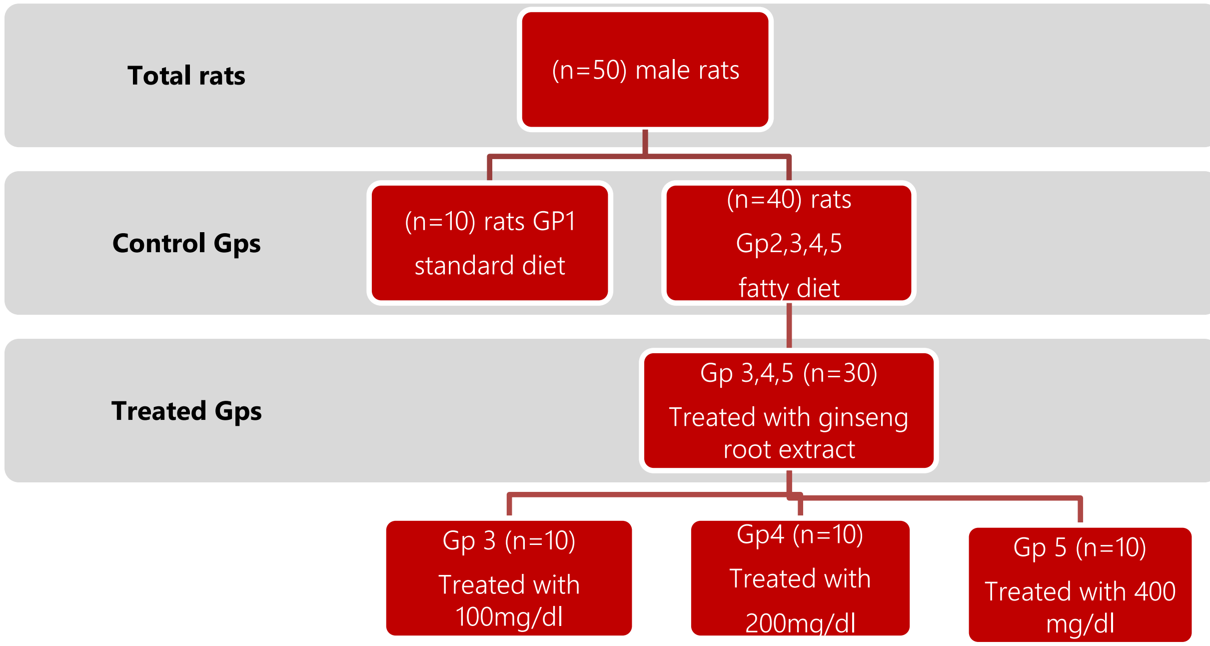

Figure 1: Schematic distribution for each group.

The data was analyzed using SPSS version 20.0 and data were statistically evaluated using the one-way analysis of variance (ANOVA) test to evaluate the significance between various groups. Post hoc Dunnett t-test is applied to compare the groups and P-value of <0.05 is considered statistically significant with a 5% margin of error (α).95% confidence interval (CI).

Results

Liver Morphology

- **Liver Weight**

The liver of rats on a standard diet was found to be dark in color compared to fatty liver, which is dull and large. Weight was increased from a mean of 4.7 gm. to a mean of 9.3 gm. in fatty liver. It was decreased by treating with ginseng root extract (at the dose of 100, 200, and 400 mg/ml) to 7.2, 7.6, and 5.3 gm. in a dose-dependent manner.

- **Liver Size**

The size of the liver also showed some alteration, the mean liver size of rats on a standard diet GP1 was 19.2 mm, and the mean liver size of rats on a fat diet, i.e., obese GP2, was increased to 51.5 mm, in ginseng treated groups GP3, GP4, and GP5 liver size was decreasing to 35.4, 26.2 and 16.7 mm (nearly normal).

- **Fatty Deposition In Liver**

The fatty droplets accumulated in cells increase the cell size. The reticule is used to measure the number of cells per unit area, and the numbers of cells are inversely proportional to their size. In GP1 (control), hepatocytes per reticule were found to be 78; in GP2 (obese), it was found to be 38 (as cells enlarged, decreasing the number); the treated group with ginseng root extract GP3, GP4, and GP5 had 47,53 and 67 cells per reticule (as fat depleted the number of cells increasing per reticule). With the help of high magnification, the hepatocytes containing fat cells were counted per reticule. In GP1 on a standard diet, the mean number of hepatocytes is insignificant, while in GP2 (obese), there is a significant number of hepatocytes containing fat droplets. With the treatment of ginseng root extract in different concentrations, the number of cells containing fatty droplets also decreases as the concentration increases from 200 mg/ml to 400 mg/ml.

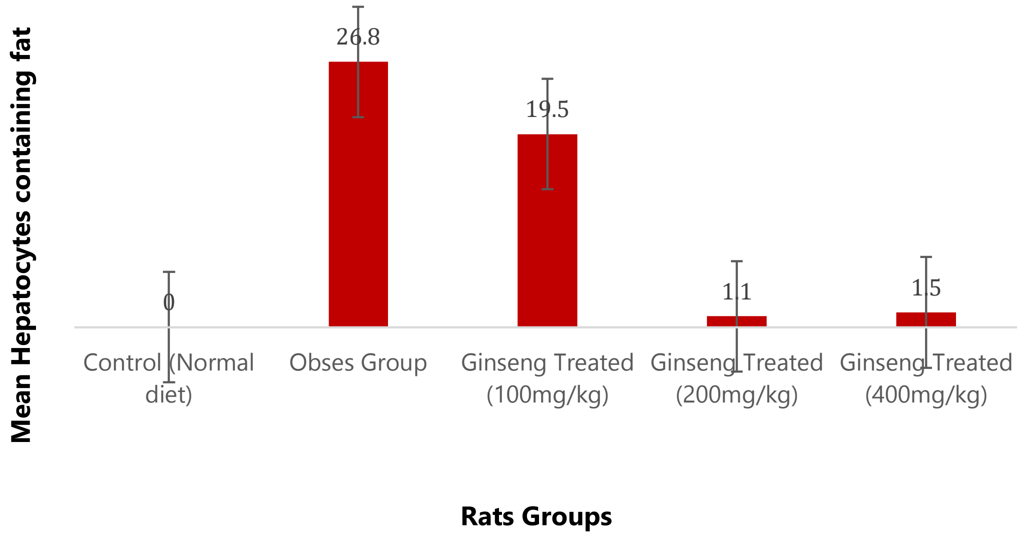

Figure 2: Comparison of mean hepatocytes containing fat droplets in different groups.

The slides were made using H&E stain; fat droplets were depleted and showed as vacuoles on the slide under the microscope. To confirm fatty droplets, Sudan black was used in the frozen section of the liver of rats of different groups (Fatty droplets were visible as black dots on slides).

Group 1(Control)

Figures 3 a and b represent the control group. Figure 3a is stained in H&E, and 3b is stained in Sudan black.

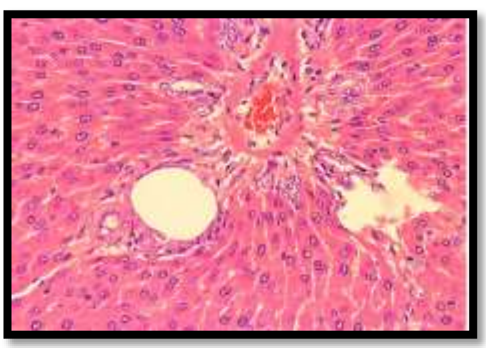

Figure 3a: Photomicrograph of H&E stained 4 μm thick section of Liver showing Portal triad (PT) of rat on control diet (group 1) at ×400.

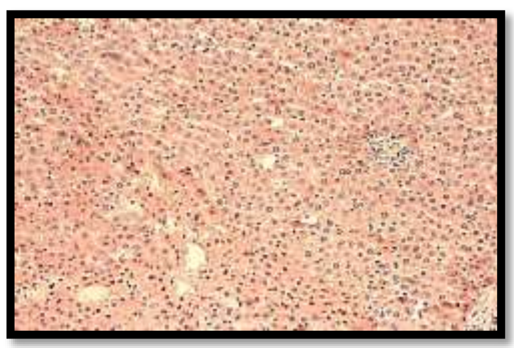

Figure 3b: Photomicrograph of Sudan black stained 5 μm thick frozen liver showing hepatocytes without fat droplet, nucleus, and Central Vein (CV), of rat on control diet (group 1) at ×100.

Group 2 (obese)

Many fat droplets in hepatocytes represent vacuoles in the H&E stain in figure 4a and black droplets in figure 4b.

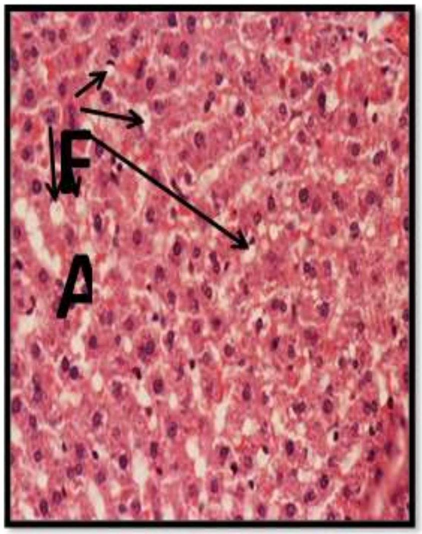

Figure 4a: Photomicrograph of H&E stained 4 µm thick liver section showing fat droplets in rat hepatocytes on a high-fat diet (group 2) at ×400.

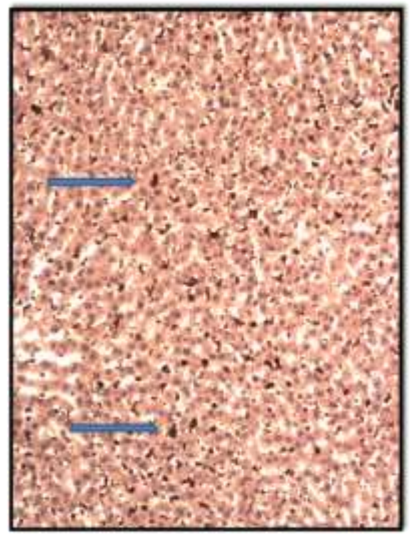

Figure 4b: Photomicrograph of Sudan black stained 5 µm thick frozen section of liver showing multiple fat droplets in hepatocytes of rat on a high-fat diet (group 2) at ×400.

Group 3

Many fat droplets in hepatocytes represent vacuoles in the H&E stain (figure 5a). While black droplets of fats are seen in Sudan black (figure 5b).

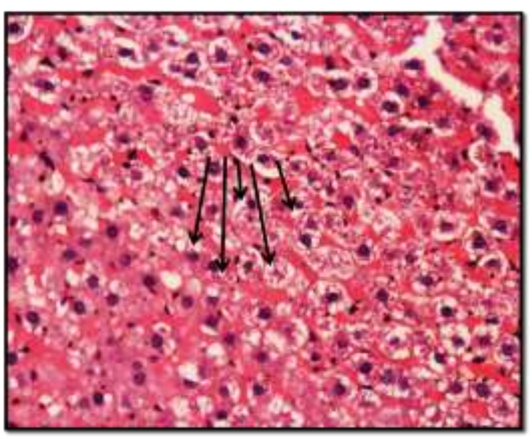

Figure 5a: Photomicrograph of H&E stained 4 µm thick liver section showing Fat droplets (vacuoles) in rat hepatocytes on high-fat diet + Ginseng 100mg/kg (group 3) at ×400.

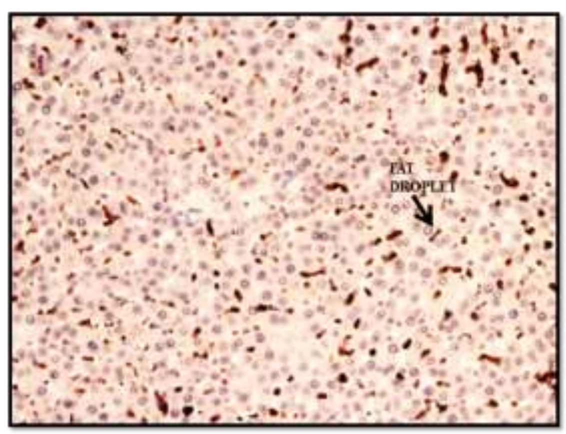

Figure 5b: Photomicrograph of Sudan black stained 5 µm thick frozen liver section is showing Fat droplets (black) of rat on high-fat diet + Ginseng 100mg/kg (group 3) at ×400.

Group 4

Many fat droplets in hepatocytes represent vacuoles in the H&E stain in figure 6a and black droplets in figure 6b.

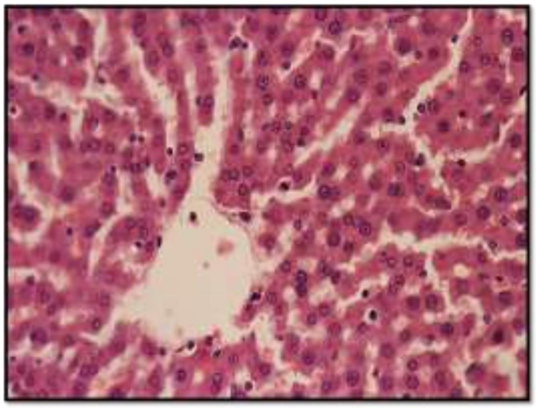

Figure 6a: Photomicrograph of H&E stained 4 µm thick liver section showing fewer Fat droplets (vacuoles) in rat hepatocytes on high-fat diet + Ginseng 200mg/kg (group 4) at ×400.

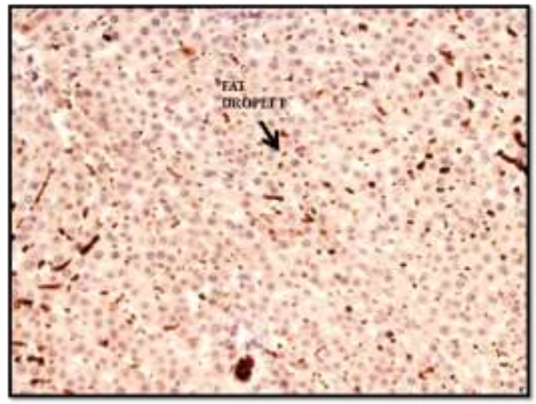

Figure 6b: Photomicrograph of Sudan black stained 5 µm thick frozen section of liver showing Fat droplets (black) in hepatocytes of rat on high-fat diet + Ginseng 200mg/kg (group 4) at ×400.

Group 5

The ginseng depletes fat droplets in hepatocytes (H&E stain) figure 7a and Sudan black stain in figure 7b.

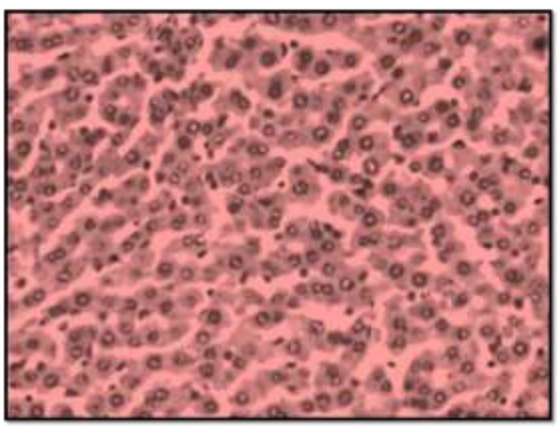

Figure 7a: Photomicrograph of H&E stained 4 µm thick liver section showing congestion but no Fat droplets (vacuoles) in rat hepatocytes on high-fat diet + Ginseng 400mg/kg (group 5) at ×400.

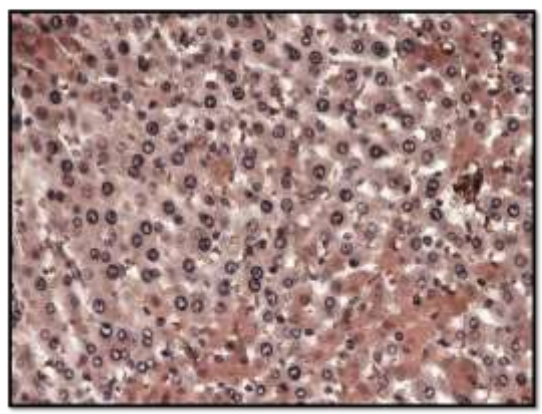

Figure 7b: Photomicrograph of Sudan black stained 5 µm thick frozen liver section showing congestion without Fat droplets (vacuoles) in rat hepatocytes on high-fat diet + Ginseng 400mg/kg (group 5) at ×400.

Liver Function Test (LFT)

HFD altered LFT, and ginseng root extract reversed it to nearly normal levels. Liver functioning is investigated through serum levels of triglyceride (TG), low-density lipoprotein (LDL), serum cholesterol level, and high-density lipoprotein (HDL). Liver biomarkers are serum glutamic-pyruvic transaminase (SGPT), serum glutamic-oxaloacetic transaminase (SGOT), and alkaline phosphatase (ALP). Hepatic injury was measured by measuring liver enzymes and hepatic biomarkers. Liver enzymes markedly increased in the obese group, which was reduced by ginseng. This experiment showed that a fatty diet injured the liver tissues resulting in an increased level of enzymes treated by ginseng root extract administration.

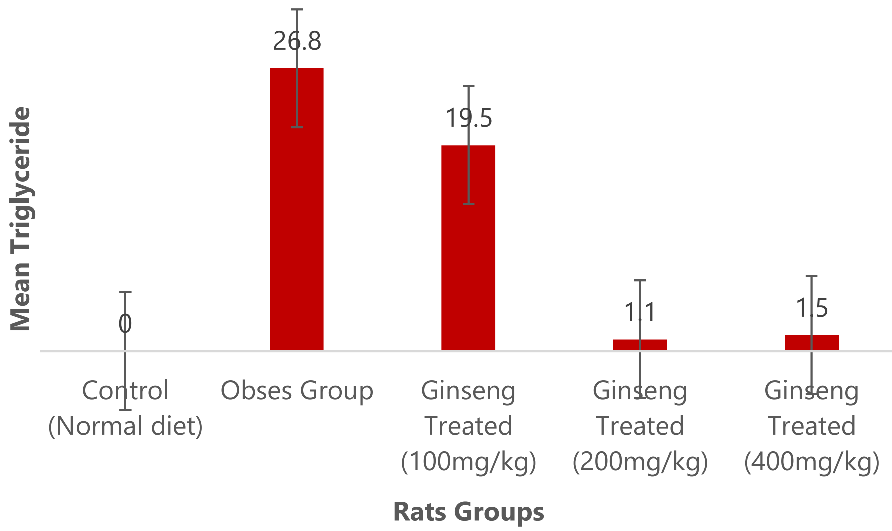

Figure 8: Comparison of mean Triglyceride in different groups.

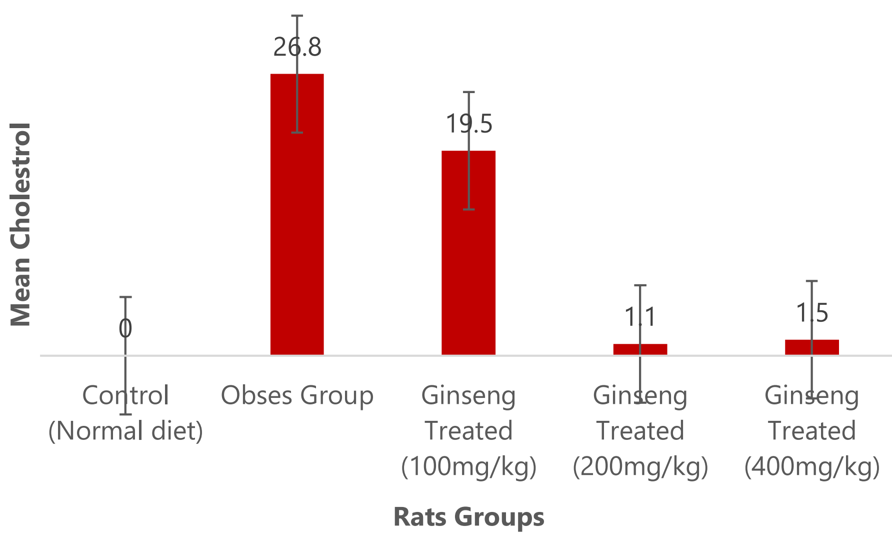

Figure 9: Comparison of mean Cholesterol in different groups.

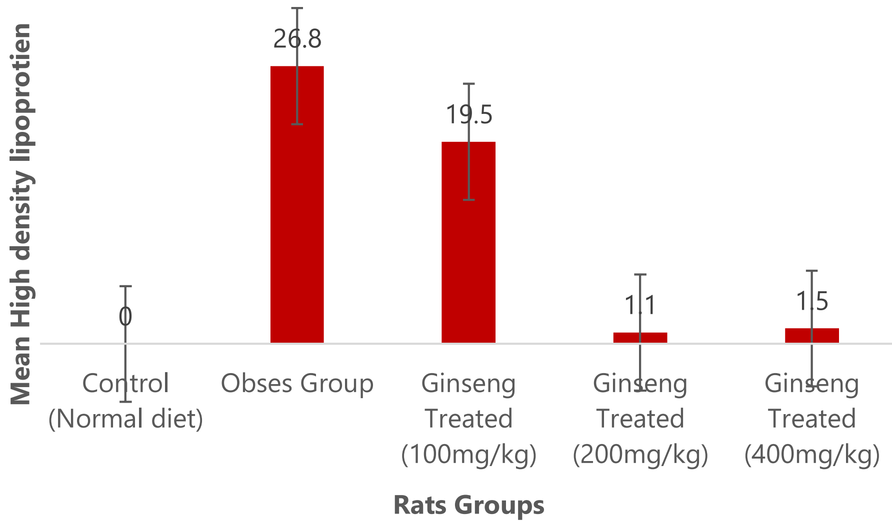

Figure 10: Comparison of mean High-density lipoprotein in different groups.

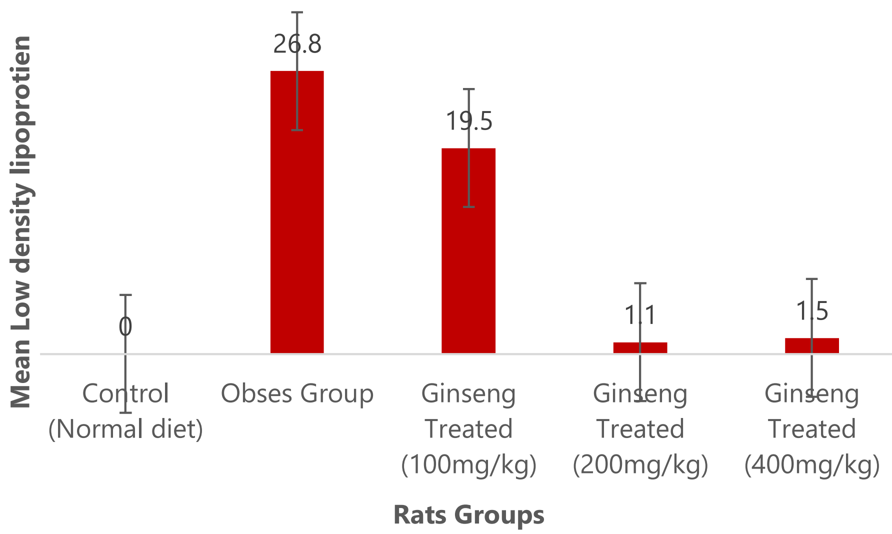

Figure 11: Comparison of mean low-density lipoprotein in different groups.

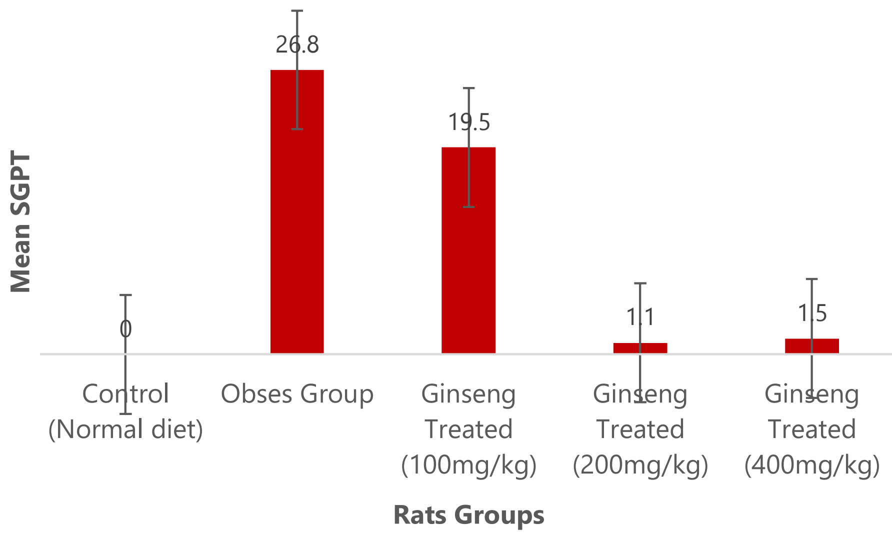

Figure 12: Comparison of mean SGPT in different groups.

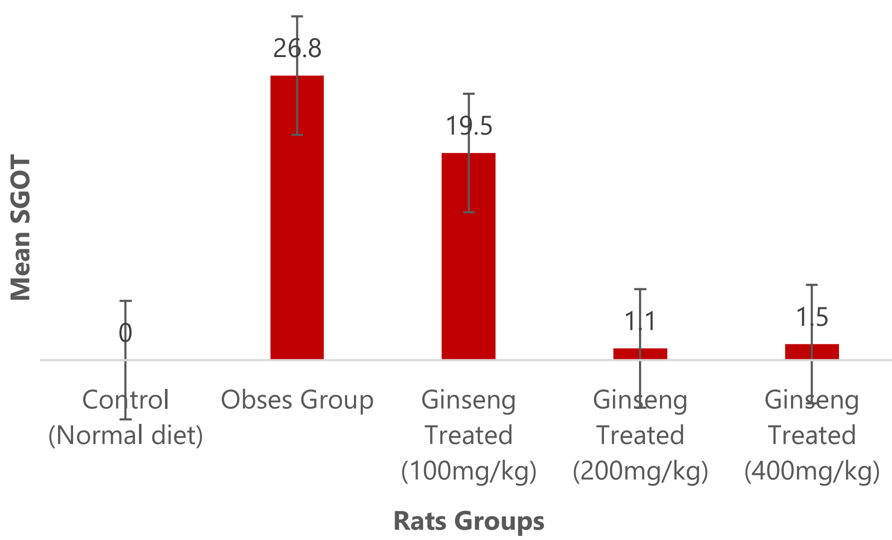

Figure 13: Comparison of mean SGOT in different groups.

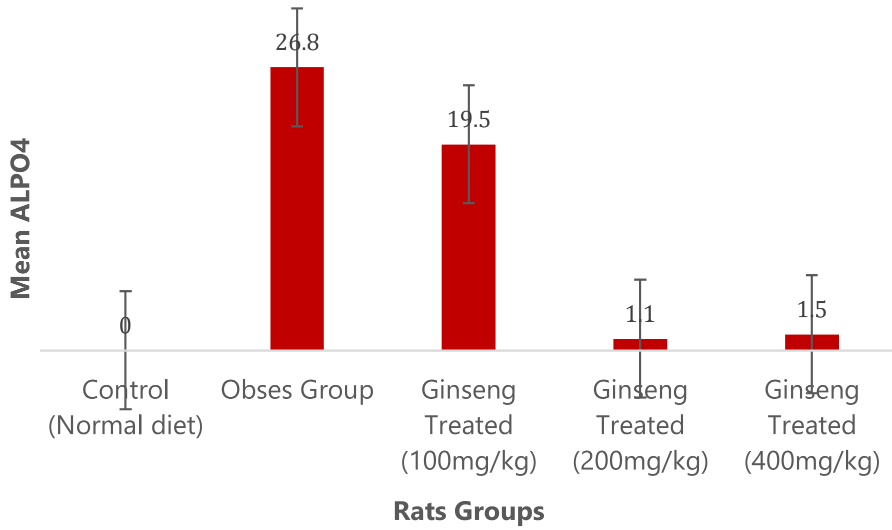

Figure 14: Comparison of mean alkaline phosphates in different groups.

Discussion

Junk food is the root cause of different metabolic diseases; not only is it related to obesity or overweight but also related to mental health disturbances, as shown in research by Bodden et al. in 2021[13]. Deeply fried food contains many calories; fat is deposited in the liver, causing fatty liver and increasing the TG, LDL, and cholesterol while decreasing the HDL, as also proven by the study of Luo in 2021[14]. There are many reasons for fatty liver: alcohol is one of them causing alcoholic fatty liver diseases, as mentioned in the study of purohit[15]. Fatty liver disease without alcohol is known as NAFLD, strongly correlated with metabolic syndrome, named metabolic-associated fatty liver disease MAFLD, also mentioned in a study by Younossi in 2021[16]. According to the survey by van Dijk in 2021, NAFLD is more prevalent in western and Asian countries[17]. It may be caused by a large amount of glucose intake, as the study done by Coelho in 2021[18], contrary to my study, which is focused on NAFLD due to a fatty diet, the same study done by Nasiri in 2021[19].

This study highlights the effect of a high-fat diet on gross morphology as well as the histology of the liver. The high-fat diet was prepared with fats from cow, cheese, and butter, contrary to a high-fat diet prepared by peng in 2021[20]. A high-fat diet changes liver weight, size, and color, also demonstrated by marques in 2021[21]. The deposition of fats (fatty droplets in hepatocytes) alters its morphology. The liver is responsible for metabolisms; its disturbances lead to disturbances in metabolism, leading to MAFLD, as evidenced by Liu in 2021[22]. Rats were used as animal models in this experiment. Rats were used for study in other research, like Makhlouf in 2021 used it instead of humans[23]. Their response is similar to other mammals, giving results in a short time.

An extract of Korean red ginseng was prepared to correct fatty deposition in hepatocytes. This study favors a study done by sung in 2021[24]. Korean red ginseng has parts like stem, leaf, flower, and roots. The most potent part is the root, as mentioned in different studies. Other parts were also used in different studies, like flower, stem, and leaves. In our study, root extracts were used, like the study done by Jiang in 2021[25]. This is a morphometric analysis of hepatocytes, and gross morphological changes were observed like other researchers for measuring histological changes. The reticule was used as it has used by Utela in 2021[26]. Fatty droplets were more clearly visible in slides stained by Sudan Black. Routine staining was also performed for studying hepatocytes, signs of inflammation, or scared as we are focused on fatty droplets, which are more visible on frozen slides using Sudan black staining as it was used by researchers like Alarmouti in 2021[27].

Role of ginseng root extract played a potent role in reversing the fatty accumulation in hepatocytes. Ginseng is used as an antihyperlipidemic in traditional herbal medicine, and it is safe for human consumption. Ginseng can be used as vegetables or salad or as some fruits, as mentioned in the study by Jiang in 2021[25]. This study was focused on fatty liver changes observed by the naked eye and by microscope under high power resolution. This was purely an anatomical study combined with histopathology, highlighting the effects of HFD and Ginseng root extract on the lipid metabolism of the liver.

Conclusion

This study concludes that a fatty diet increases fat deposition in hepatocytes, and it results in increased liver enzymes. The liver function test showed deranged values due to fatty accumulation. The herb ginseng played a key role in reducing fat contents from the liver and positively affected liver enzymes like SGOT, SGPT, and alkaline phosphatase.

Conflicts of Interest

The authors declare no conflicts of interest.

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