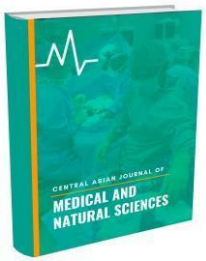

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Ginsenoside Rg1 and Ginseng Alcoholic Extract's Ability to Prevent Tissue Damage and Diabetes-Related Physiological Abnormalities in Male Rats

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Abstract: Because diabetes upsets the oxidative equilibrium, it increases the risk of hepatic tissue problems. There aren't many efficient therapies available for this common ailment right now. Because ginseng extract and ginsenosides are known to have anti-inflammatory, anti-apoptotic, and antioxidant qualities, this study sought to investigate their potential use as a natural medicine. Eight groups of male rats were used. Distilled water, 200 mg/kg of ginseng alcoholic extract, 25 mg/kg of ginsenosides, and a combination of 200 mg/kg of ginseng alcoholic extract and 25 mg/kg of ginsenosides were given to each of the first four groups. The first group was in good health. Distilled water was provided to the fifth group, 200 mg/kg of ginseng alcoholic extract was given to the sixth, 25 mg/kg of ginsenosides were given to the seventh, and both 200 mg/kg of ginseng alcoholic extract and 25 mg/kg of ginsenosides were given to the eighth. All four of the other groups had diabetes. The animals were put to death after 30 days so that their livers could be removed for histological analysis and blood could be taken for biochemical research. The diabetes group had higher levels of MDA and IL-6, lower levels of GSH, and a higher frequency of liver histological abnormalities as compared to the healthy control group. The groups given only ginseng alcoholic extract, ginsenosides, and a combination of ginseng alcoholic extract and ginsenosides showed improvements in liver histology, decreased levels of MDA and IL-6, and increased levels of GSH when compared to the diabetic group. According to the study's findings, there is a stronger synergistic impact when ginseng alcoholic extract and ginsenosides are used together as opposed to separately.

Keywords: Diabetes Mellitus, Ginsenoside, Inflammatory Cytokines, IL-6 and TNF-α

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1. Introduction

Diabetes mellitus (DM) is a global public health concern[1]. In affluent countries, 12.8% of people have diabetes mellitus, and $110 billion is spent on diabetes-related medical treatment[2]. Insulin synthesis, release, and detection are all carefully controlled by molecular processes. Metabolic abnormalities that contribute to the course of sickness might arise from malfunctions in any pathways involved in these activities[3] .The immune-mediated destruction of pancreatic beta cells is the hallmark of type 1 diabetes mellitus, an autoimmune disease[4]. Because of factors like genetic variability in the major histocompatibility complex (MHC), cytotoxic T-cell antigen, and other immune system-associated genes, autoreactive T cells promote apoptosis, which positively correlates with the onset of symptoms. Adaptive immunity and infection have a major role. It played a major role in the development of TIDM[5].

Diabetes raises serum liver enzymes and causes elevated cholesterol levels and oxidative stress, which damages the liver and aorta[6], [7], [8], [9]. Disorders associated with abnormal lipid metabolism have traditionally been treated using herbal medicine, which uses natural materials as medicinal agents. Traditional medicine has used ginseng to lower cholesterol. According to pharmacological study, this natural chemical can regulate immunological, glucose, and lipid metabolism and has anti-aging, anti-fatigue, and anticancer activities[10]. Due to its various pharmacological and therapeutic advantages on aging, cancer, the cardiovascular system, diabetes, immunological modulation, and inflammation, ginseng is currently of great interest[11].

One well-known medicinal herb used to treat many inflammatory conditions is panax ginseng. The main family of active ingredients in ginseng are ginsenosides, or ginseng saponins. The potential of ginseng extracts, particularly pure ginsenosides, to reduce the expression of pro-inflammatory cytokines (TNF-α, IL-1, and IL-6) and operate as an anti-inflammatory mechanism has demonstrated its anti-inflammatory qualities[12].

2. Materials and Methods

Materials:

The root of the Indian ginseng plant, Withania somnifera, was sourced from China. Subsequently, a 60% methanol solution was employed to extract the root extract. Ginsenosides were procured from the American firm Sigma.

Animals:

Male Sprague Dawley rats weighing 200 ± 10 grams and four months old were used in this experiment. The rats were kept in specially designed cages in a lab setting with a temperature of 22 ± 2°C and equal amounts of light and dark. The enclosures underwent disinfection and sanitization. The animals were given unlimited access to food and water during their two-week acclimatization period.

Experimental Design:

In this study, forty adult male albino rats of similar weight and size were divided into eight groups. Groups 1-4 were in good health, however groups 5-8 developed type 1 diabetes after receiving 150 mg/kg of alloxan subcutaneously. The following is how the groupings were defined:

1. Group 1 (Natural Control): For thirty days, they were fed a regular diet and given distilled water.
2. Group 2 (Natural Control and Ginseng Alcoholic Extract): For thirty days, they were given a regular diet and 200 mg/kg of ginseng alcoholic extract.
3. Group 3 (Natural Control and Ginsenosides): For thirty days, they were given a regular diet and 25 mg/kg of ginsenosides.
4. Group 4 (Natural Control and Ginseng Alcoholic Extract + Ginsenosides): For thirty days, this group had a regular diet in addition to 200 mg/kg of Ginseng Alcoholic Extract and 25 mg/kg of Ginsenosides.
5. Group 5 (Diabetic): For thirty days, this cohort received distilled water and a regular diet.
6. Group 6 (Diabetic Control and Ginseng Alcoholic Extract): For thirty days, this group was given a regular diet along with 200 mg/kg of Ginseng Alcoholic Extract.
7. Group 7 (Diabetic Control and Ginsenosides): For thirty days, this group was given a regular diet and 25 mg/kg of Ginsenosides.
8. Group 8, which included both the infected control group and the group that received Ginseng Alcoholic Extract in addition to Ginsenosides, was given a normal diet and treated for thirty days with 200 mg/kg of Ginseng Alcoholic Extract and 25 mg/kg of Ginsenosides.

Blood serum collection:

After the 30-day trial period ended, the animals were fasted for 12 hours and then given intramuscular injections of 5 mg/kg of ketamine and 35 mg/kg of xylazine to sedate them. Cardiac blood was used to extract blood serum, which was then kept at -20°C for the study's biochemical tests. The rats were then dissected, and the liver was removed for pathological analysis.

Biochemical tests in blood serum:

Malondialdehyde (MDA) was tested using the Guidet and Shah methodology, glutathione (GSH) was quantified using the Al-Zamely et al.[13] protocol, and interleukin-6 (IL-6) was measured using ELISA in compliance with the BT Lab kit instructions.

Histological preparations:

After the animals were dissected, the liver was removed and cleaned with physiological saline. Hematoxylin and eosin staining was used to prepare the materials for microscopic histological sections. After the processing of the microscope slides was finished, they were examined under a light microscope, and pictures were taken.

Statistical analysis:

Duncan's multiple range test was used to statistically analyze the data at a significance level ($P < 0.05$).

3. Results and Discussion

MDA and GSH Concentration: The information shown in Figure A and Table 1 shows that the diabetes-induced group had significantly higher levels of malondialdehyde (MDA) and significantly lower levels of glutathione (GSH) than the normal control group. In comparison to the normal control group, the concentration of glutathione (GSH) significantly increased while the concentration of malondialdehyde (MDA) significantly decreased in the normal groups and those treated with Ginseng Alcoholic Extract alone, Ginsenosides alone, and the combination of Ginseng Alcoholic Extract and Ginsenosides. Malondialdehyde (MDA) levels were significantly lower and glutathione (GSH) levels were significantly higher in the diabetic groups treated only with Ginseng Alcoholic Extract, Ginsenosides, and the combination of Ginseng Alcoholic Extract and Ginsenosides. The group that received both ginsenosides and ginseng alcoholic extract showed the biggest improvement in the previously indicated parameters.

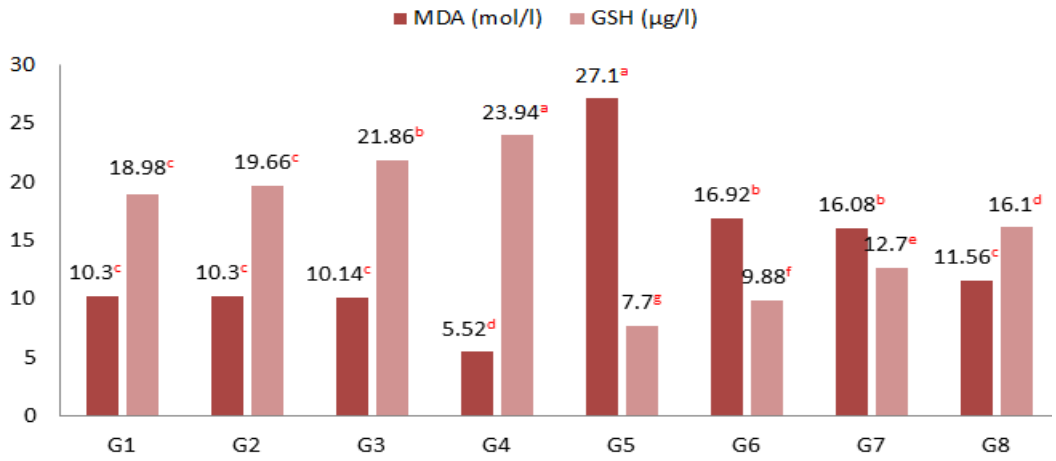


Figure 1. Concentration of MDA and GSH in blood serum.

Our findings indicated that diabetes results in increased MDA levels and reduced GSH levels in the diabetic cohort relative to the normal control group. This results from

heightened blood glucose levels, which augment reactive oxygen species in endothelial cells through advanced glycation end products and their receptors, consequently provoking vascular endothelial inflammation and thrombosis by upregulating the expression of several pertinent genes. Oxidative stress is linked to the onset and advancement of diabetic vascular problems, including retinopathy, nephropathy, and cardiovascular disease[14].

The function of ginseng alcoholic extract in reducing MDA and increasing GSH levels relative to the diabetic group is ascribed to the antioxidant and lipid-lowering characteristics of ginseng root. Ginseng comprises saponins, sugars, volatile chemicals, organic acids and their esters, proteins, enzymes, sterols and their glycosides, peptides, nitrogenous compounds, lignin, flavonoids, vitamins, inorganic elements, and other constituents[15].

Ginsenosides' role in lowering MDA and raising GSH levels in comparison to the diabetic group is explained by their importance in regulating the synthesis of endothelium antioxidant enzymes. In order to reduce oxidative stress and lessen the degenerative changes in tissues associated with diabetic cardiomyopathy, they alter the expression of genes that encode GSH-Px and SOD1[16]. By increasing antioxidant levels and reducing the production of free radicals and reactive oxygen species (ROS), ginsenoside reduces oxidative stress, prevents hepatic cell death, and reduces intracellular lipid accumulation[17].

IL-6 Concentration: The findings in Figure B and Table 1 indicated a considerable elevation in IL-6 concentration in the diabetes-induced group relative to the normal control group. The concentration of IL-6 considerably decreased in the normal groups and those treated with Ginseng Alcoholic Extract alone, Ginsenosides alone, and the combination of Ginseng Alcoholic Extract and Ginsenosides, compared to the normal control group. In the diabetic groups treated with Ginseng Alcoholic Extract alone, Ginsenosides alone, and the combination of Ginseng Alcoholic Extract and Ginsenosides, the levels of IL-6 considerably decreased compared to the diabetic group. The group administered Ginseng Alcoholic Extract combined with Ginsenosides had the most significant reduction in IL-6 content, followed by Ginsenosides alone, and finally Ginseng Alcoholic Extract.

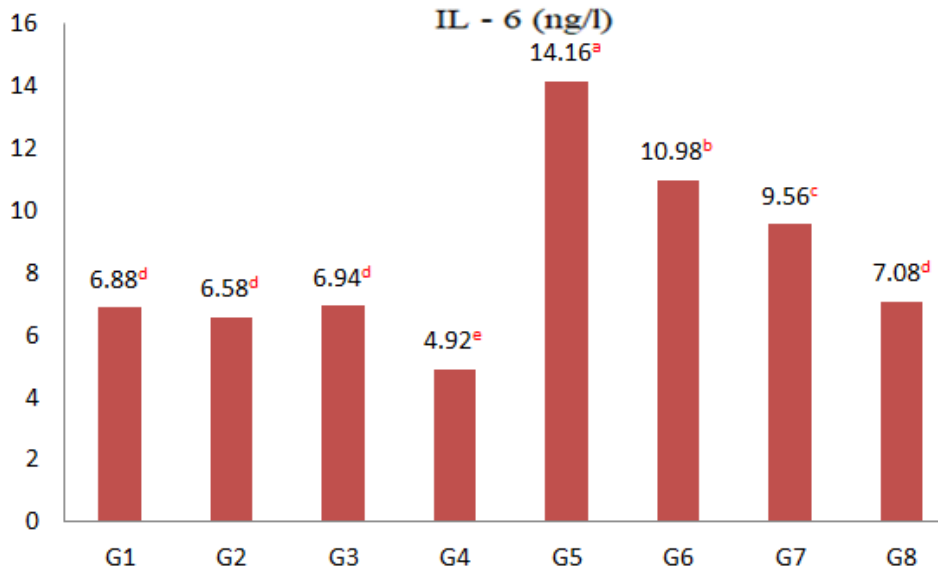


Figure 2. IL-6 concentration in blood serum.

Table 1. Impact of Ginseng Alcoholic Extract and Ginsenosides on the blood concentrations of MDA, GSH, and IL-6 in male albino rat experimental groups

Parameters / Groups	MDA (mol/l)	GSH (µg/l)	IL – 6 (ng/l)
Control	10.30±1.20c	18.98±0.78c	6.88±0.70d
Ginseng Alcoholic Extract	10.30±1.20c	19.66±0.68c	6.58±0.48d
Ginsenosides	10.14±1.35c	21.86±0.56b	6.94±0.34d
Ginseng Alcoholic Extract + Ginsenosides	5.52±0.67d	23.94±0.75a	4.92±0.38e
Diabetic	27.10±1.60a	7.70±0.88g	14.16±1.00a
Diabetic + Ginseng Alcoholic Extract	16.92±1.03b	9.88±0.56f	10.98±0.81b
Diabetic + Ginsenosides	16.08±0.98b	12.70±0.57e	9.56± 0.62c
Diabetic + Ginseng Alcoholic Extract+ Ginsenosides	11.56±0.48c	16.10±0.83d	7.08±0.45d

- **The values denote the arithmetic mean ± the standard deviation.**
- **Numerals succeeded by distinct letters in a vertical arrangement denote a statistically significant difference at a probability threshold of (P > 0.05).**

Figure 2 and Table 1 demonstrate a substantial elevation in IL-6 levels in the induced diabetes group relative to the normal control group. Increased IL-6 levels are a significant predictor of diabetes, as it is an anti-inflammatory cytokine that facilitates B cell development, stimulates T cells, and modulates the acute phase response[18]. Low-grade inflammation arises from the buildup of lipids in adipocytes. Continuous activation of inflammation leads to the recruitment of inflammatory cells, especially phagocytes, into adipose tissue, which in turn stimulates the generation and secretion of inflammatory cytokines, such as interleukin-6[19].

The anti-inflammatory qualities of ginseng glycopeptides in reducing diabetes-induced inflammation are responsible for the ginseng alcoholic extract's effectiveness in reducing IL-6 levels compared to the diabetic group. Ginseng-derived glycoproteins have significant anti-apoptotic properties and lower levels of IL-1β, IL-2, IL-4, and TNF-α[20]. The mechanism by which ginseng exerts its anti-inflammatory effects involves either the activation of glucocorticoid receptors, which are the targets of anti-inflammatory steroid medications, or an antioxidant mechanism characterized by the inhibition of reactive oxygen species (ROS) production and the activation of Nrf-2, or the activation of the anti-inflammatory peroxisome proliferator-activated receptor gamma (PPARγ)[21].

The reduction of IL-6 concentration by ginsenosides, in contrast to the diabetic group, is ascribed to their inhibitory action on inflammatory mediators, including the suppression of IL-1, IL-6, and reactive oxygen species (ROS)[10]. Ginsenosides diminish the size of endometrial implants in the rat endometrium, considerably enhancing the expression levels of IL-6, IL-1β, and TNF-α, which are linked to inflammation, in a dose-dependent manner in the ginsenoside-treated group (Ross et al., 2021). Chronic ginsenoside administration led to a partial attenuation of the elevation of pro-inflammatory cytokines[19].

Histopathological analysis of the liver: The results of the present investigation for the normal control group, administered distilled water, ginseng alcoholic extract alone, ginsenosides alone, and a combination of ginseng alcoholic extract and ginsenosides, exhibited a normal liver cross-section. The central vein (CV), hepatocytes (HC), sinusoids

(S), and Kupffer cells (KC) were observed to be intact, as illustrated in Figure C, Figure 1-D, Figure 1-E, and Table 2, respectively. The diabetic group administered alloxan displayed multiple histological alterations, including inflammatory cell infiltration (ICI), fibrosis (F), central vein rupture (CVR), hepatic artery thickening (HAT), bile duct fibrosis (BDF), and portal vein rupture. Portal vein rupture (PVR), portal area expansion (PAE), hemolysis (H), and degeneration (D) exhibited considerable increases (+++), whereas necrosis (N) and hemorrhage (He) were moderately elevated (++), as illustrated in Figure C, Figures 7, 6, 5-D, Figures 7, 6, 5-E, and Table 2. Conversely, the diabetic cohort administered solely Ginseng Alcoholic Extract exhibited a moderate reduction in inflammatory cell infiltration (ICI), fibrosis (F), portal vein rupture (PVR), portal area expansion (PAE), hemolysis (H), and necrosis (N) (++), while central vein rupture (CVR), hepatic artery thickening (HAT), bile duct fibrosis (BDF), degeneration (D), and hemorrhage (He) were observed to be mild (+), as illustrated in Figure C, Figure 8-D, Figure 8-E, and Table 2. A moderate (++) reduction in inflammatory cell infiltration (ICI), hepatic artery thickening (HAT), and portal area expansion (PAE) was noted in the diabetic group administered just ginsenosides. Fibrosis (F), portal vein rupture (PVR), hemolysis (H), and necrosis (N) were classified as mild (+), while central vein rupture (CVR), bile duct fibrosis (BDF), degeneration (D), and bleeding were additionally noted. Hemorrhage (He) was seldom (Trace), as illustrated in Figure C, Figure 9-D, Figure 9-E, and Table 2. The diabetic group administered Ginseng Alcoholic Extract + Ginsenosides exhibited a minor reduction in Inflammatory Cell Infiltration (ICI) and Portal Area Expansion (PAE), alongside a rare incidence of Fibrosis (F), Hepatic Artery Thickening (HAT), Portal Vein Rupture (PVR), and Necrosis (N). Central Vein Rupture (CVR), Bile Duct Fibrosis (BDF), Hemolysis (H), Degeneration (D), and Hemorrhage (He) reverted to normal (Nor), as illustrated in Figure C, Figure 9-D, Figure 9-E, and Table 2. Figure C, Figure 10-D, Figure 10-E, and Table 2.

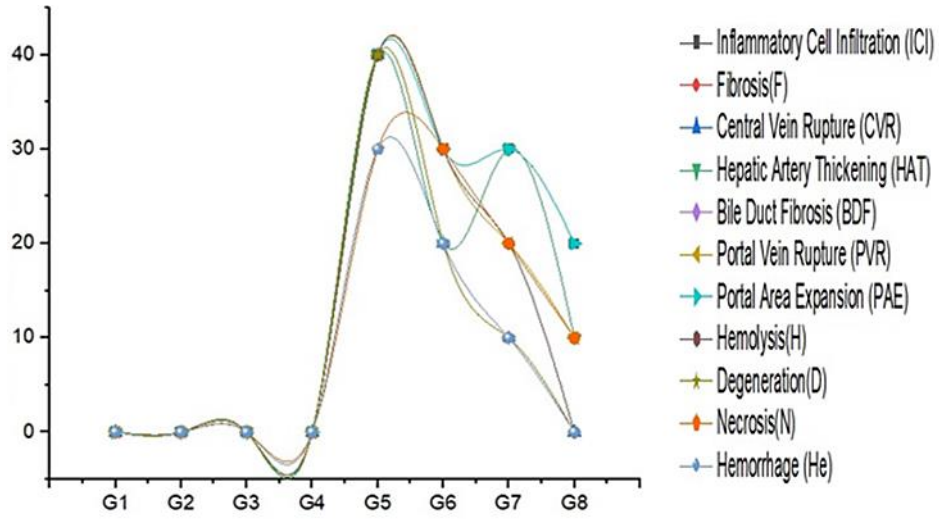
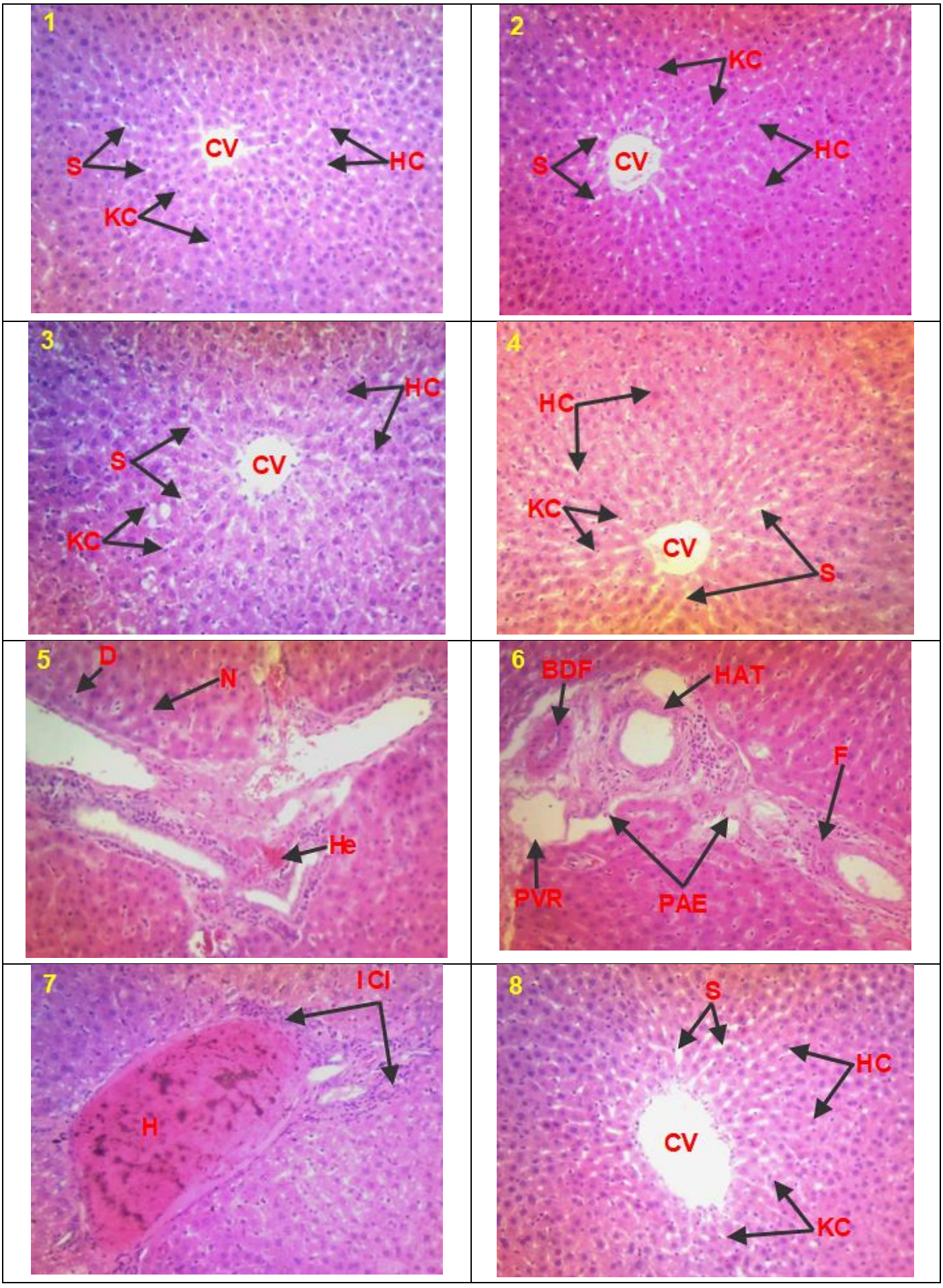


Figure 3. Graph depicting the proportion of hepatic tissue lesions.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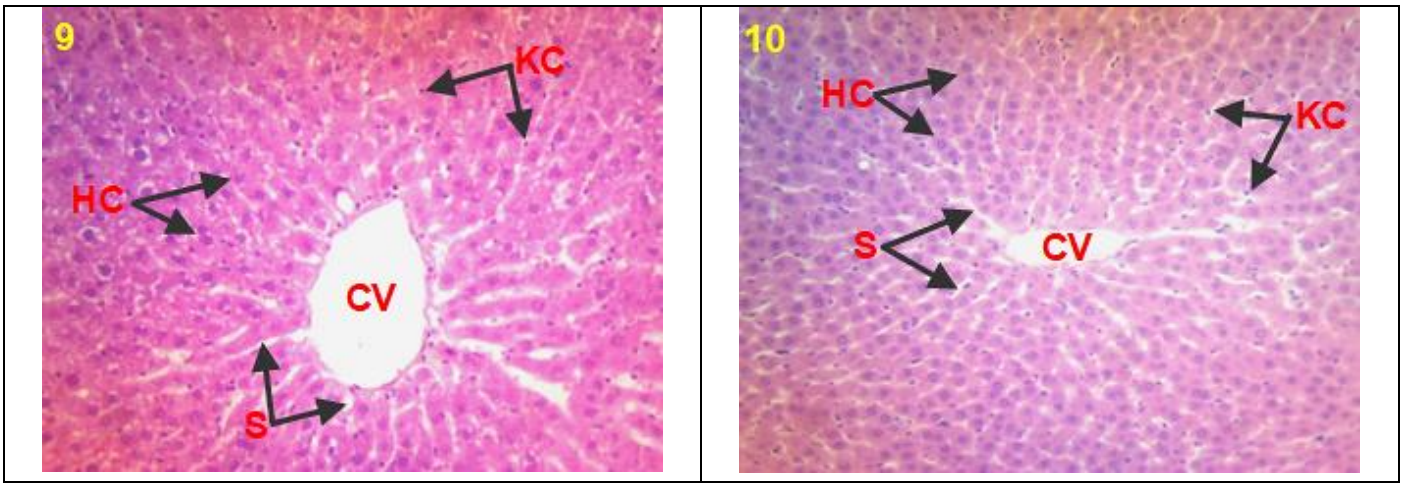
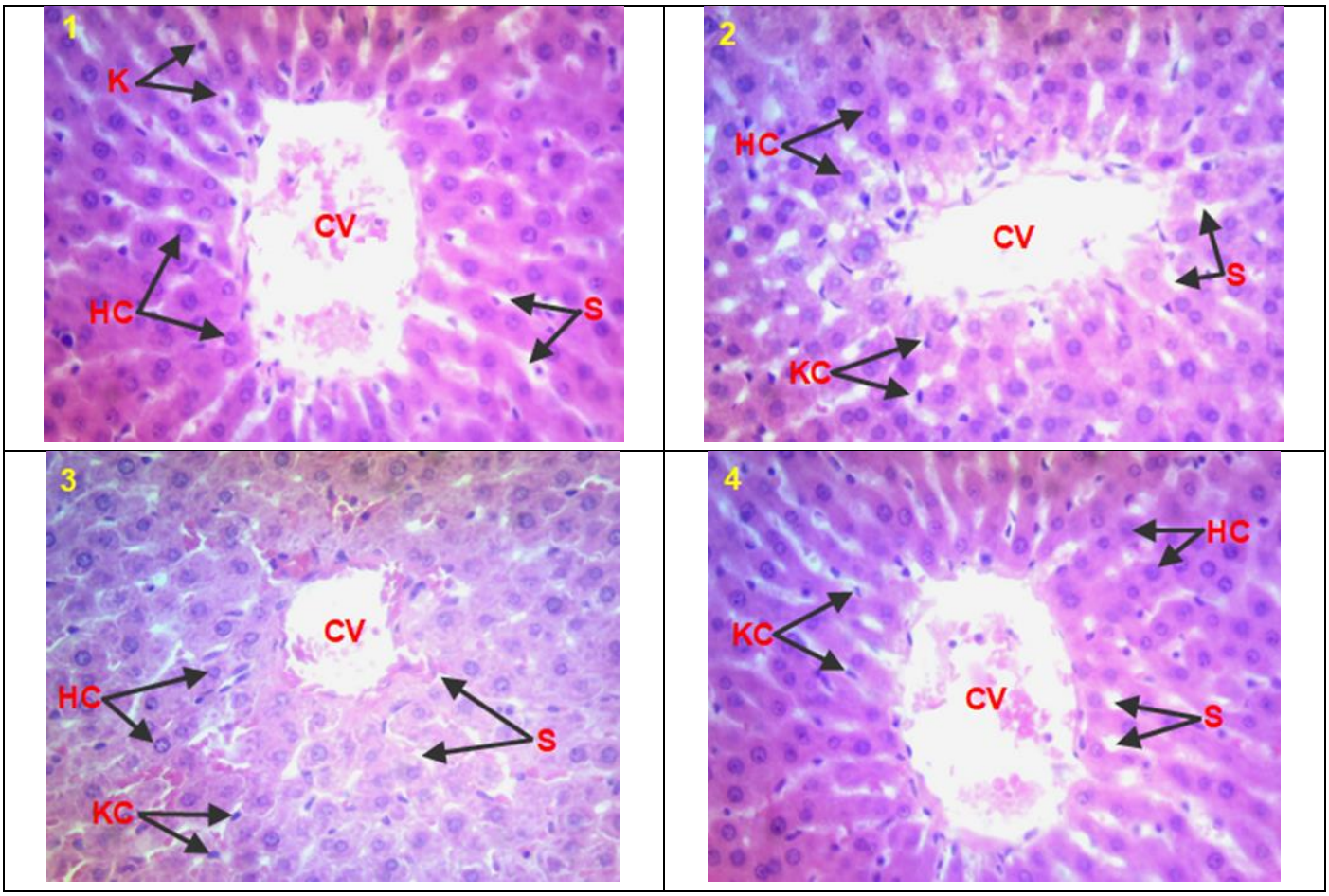


Figure 4. Figure D(1) Liver slice of the control group exhibiting normalcy. **Figure D(2)** Liver slice from the control group administered alone with Ginseng Alcoholic Extract. **Figure D(3)** Liver slice from the control group administered just Ginsenosides. **Figure D(4)** Liver segment of the control group administered Ginseng Alcoholic Extract and Ginsenosides.

Figures D(5, 6, 7) depict liver slices from the diabetic cohort. **Figure D(8)** Liver slice from the diabetic group administered just Ginseng Alcoholic Extract. **Figure D(9)** Liver slice from the diabetic group administered just Ginsenosides. **Figure D(10)** Liver segment from the diabetic group administered Ginseng Alcoholic Extract + Ginsenosides, H&E 200X.



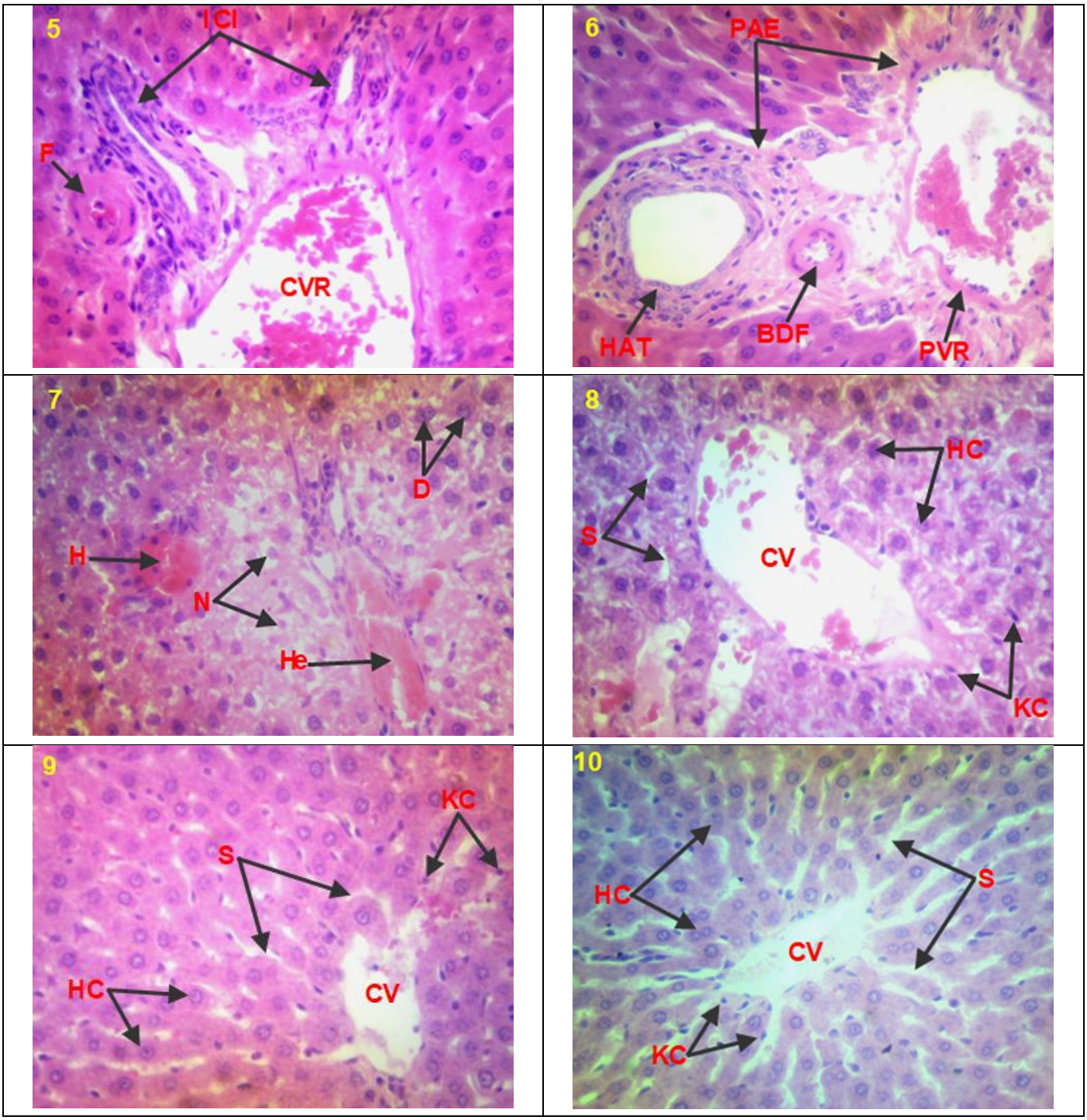


Figure 5. Figure E(1) Liver slice of the control group exhibiting normalcy. **Figure E(2)** Liver slice from the normal group administered just Ginseng Alcoholic Extract. **Figure E(3)** Liver slice from the control group administered just Ginsenosides. **Figure E(4)** Liver slice from the normal group administered Ginseng Alcoholic Extract and Ginsenosides. **Figures E(5, E(6, E(7)** depict liver slices from the diabetic cohort. **Figure E(8)** Liver slice from the diabetic group administered alone with Ginseng Alcoholic Extract. **Figure E(9)** Liver slice of the diabetic cohort administered just Ginsenosides. **Figure E(10)** Liver slice of the diabetic cohort administered Ginseng Alcoholic Extract + Ginsenosides, H&E 400X.

Table 2. Impact of Ginseng Alcoholic Extract and Ginsenosides on the Inhibition of Hepatic Tissue Lesions in Male Albino Rat Experimental Groups.

Groups \ Parameters	Inflammatory Cell Infiltration (ICI)	Fibrosis(F)	Central Vein Rupture (CVR)	Hepatic Artery Thickening (HAT)	Bile Duct Fibrosis (BDF)	Portal Vein Rupture (PVR)	Portal Area Expansion (PAE)	Hemolysis(H)	Degeneration(D)	Necrosis(N)	Hemorrhage (He)
Control	Nor	Nor	Nor	Nor	Nor	Nor	Nor	Nor	Nor	Nor	Nor
Ginseng Alcoholic Extract	Nor	Nor	Nor	Nor	Nor	Nor	Nor	Nor	Nor	Nor	Nor
Ginsenosides	Nor	Nor	Nor	Nor	Nor	Nor	Nor	Nor	Nor	Nor	Nor
Ginseng Alcoholic Extract + Ginsenosides	Nor	Nor	Nor	Nor	Nor	Nor	Nor	Nor	Nor	Nor	Nor
Diabetic	+++	+++	+++	+++	+++	++	+++	+++	+++	++	++
Diabetic + Ginseng Alcoholic Extract	++	++	+	+	+	++	++	++	+	++	+
Diabetic + Ginsenosides	++	+	Trace	++	Trace	+	++	+	Trace	+	Trace
Diabetic + Ginseng Alcoholic Extract+ Ginsenosides	+	Trace	Nor	Trace	Nor	Trace	+	Nor	Nor	Trace	Nor

- **The values denote the arithmetic mean ± the standard deviation.**
- **Numerals succeeded by distinct letters in a vertical arrangement denote a statistically significant difference at a probability threshold of (P > 0.05).**
- **(Nor) signifies normal, (Trace) denotes rare, (+) represents low, (++) suggests moderate, and (+++) signifies high.**

Our findings in the livers of the diabetic cohort revealed hepatic histological abnormalities, corroborating the results of Tahir et al.[6]. which reported increased levels of triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and very low-density lipoprotein cholesterol (VLDL-C), alongside diminished levels of high-density lipoprotein cholesterol (HDL-C) in diabetic patients relative to the healthy control group. The increased cholesterol level results in hepatic histological abnormalities, such as inflammatory cell infiltration, fibrosis, bile duct sclerosis, and congestion[7]. This is because of the negative effects of diabetes, such as hepatic and cardiovascular dysfunction, which are brought on by high blood levels of harmful lipids like cholesterol and low-density lipoprotein cholesterol (LDL)[2].

Ginseng alcoholic extract's ability to increase antioxidant levels and decrease free radical levels is thought to be responsible for correcting liver histological abnormalities[22]. Saponins, carbohydrates, volatile oils, and amino acids are all found in ginseng. The main saponins in ginseng are called ginsenosides, and they are important regulators of lipid metabolism. By triggering the AMP-activated protein kinase (AMPK) pathway and increasing energy expenditure in skeletal muscle and hepatic tissue, they control the reduction of pancreatic lipase activity and simultaneously restrict lipogenesis[23]. Ginsenosides' protective effect against diabetes mellitus is attributed to their ability to reduce liver tissue issues. This is accomplished by reducing oxidative stress and inflammation through increased expression of SOD and GSH[24]. In addition to its importance in reducing oxidative stress and carbohydrate-induced liver damage,

ginsenosides increase SOD activity while decreasing the amount of fat vacuoles in liver tissue and MDA expression[14].

4. Conclusion

The current study's findings show that diabetes induction led to elevated MDA and IL-6 levels, decreased blood serum GSH concentrations, and the development of hepatic tissue lesions.

According to our research, ginsenosides and ginseng alcoholic extract had a beneficial effect on raising GSH levels while lowering blood MDA and IL-6 concentrations. Additionally, compared to the diabetic cohort, they worsen liver tissue damage. The potency of ginsenosides, the main components of ginseng saponins, and the active components of ginseng are responsible for this activity. The findings showed that ginsenosides and ginseng alcoholic extract work better together than when used alone to reduce the negative consequences of diabetes.

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