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Protective Effects Of Sodium Orthovanadate In Rodent Models Of Nafld: A Preclinical Investigation

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Abstract: *One of the most common metabolic liver diseases is non-alcoholic fatty liver disease (NAFLD). Fatty liver disease, the hepatic expression of a collection of disorders associated with metabolic dysfunction, is commonly observed in patients with diabetes mellitus (DM) and metabolic syndrome (MetS). High-fat meals and environmental toxicity caused by heavy metals like cadmium (Cd) are hallmarks of the contemporary period, which poses major health concerns as a result of improper human activities. Oxidative stress, insulin resistance, inflammation, lipid accumulation, and dietary practices are all linked to the pathogenesis of non-alcoholic fatty liver disease (NAFLD). Chronic liver disease, or NAFLD, is initially associated with fatty liver and hepatic insulin resistance, both of which are strongly impacted by PTP1B. The insulin-mimetic activity of vanadium-based compounds, such as Na3VO4, is drawing interest. In order to increase sugar absorption, sodium orthovanadate (Na3VO4) inhibits PTP1B activity and transports the glucose transporter type 4 (GLUT4) channels to the cell membrane. We speculate that by modifying lipid metabolism, lowering oxidative stress, and decreasing hepatic inflammation, SOV may have therapeutic advantages in NAFLD. In this work, we investigate the impact of SOV in two rodent models of non-alcoholic fatty liver disease (NAFLD): (1) Cadmium-induced liver disease and (2) high-fat diet-induced liver disease. To find out, that SOV can slow the evolution of NAFLD, we measure hepatic lipid accumulation, oxidative stress indicators, inflammatory cytokine profiles, and insulin sensitivity. This work may lead to new pharmacological approaches to treat fatty liver illnesses by revealing the therapeutic potential of SOV.*
Keywords: *Fatty Liver Disease, Cadmium, High Fat Diet, Sodium Orthovanadate, Oxidative Stress, Protein Tyrosine phosphatase.*

1. INTRODUCTION

1.1. Overview of Non-Alcoholic Fatty Liver Disease (NAFLD)

Around the world, non-alcoholic fatty liver disease (NAFLD) is a common cause of chronic liver disease. NAFLD is a group of disorders marked by hepatic steatosis in over 5% of hepatocytes with little to no alcohol consumption when no other causes of secondary hepatic fat accumulation (such as excessive alcohol usage) can be identified. Non-alcoholic steatohepatitis (NASH) is the more severe end of the range, whereas non-alcoholic fatty liver (NAFL) is the more benign condition. Cirrhosis and fibrosis can occur in NAFLD [1–3]. NAFLD shows hepatic steatosis without any signs of inflammation, in contrast to NASH, where it is associated with lobular inflammation and apoptosis that may lead to fibrosis and cirrhosis [1–5].According to a review, the prevalence of NAFLD varies between 6 and 35% worldwide, and it is estimated that about 30% of Americans (90 million people) suffer from the disease [6-8].

1.2. Pathophysiology of NAFLD

As of yet, the processes behind NAFLD remain unclear. Although several theories have been put out, insulin resistance appears to play a key role in the aetiology of type 2 diabetes and non-alcoholic fatty liver disease [9,10]. It has been revealed that NAFLD is linked to the genetic variant of PNPLA3 (patatin-like phospholipase domain containing 3), an enzyme that hydrolyses triacylglycerols in adipocytes and encodes I148M (rs738409 C/G), regardless of the metabolic syndrome [11,12]. Similarly, TM6SF2 (transmembrane 6 superfamily member 2), a genetic variation of the lipid transporter found on the ER (endoplasmic reticulum) and ER-Golgi compartments, encoding E167K (rs58542926 C/T), results in a loss of protein function and promotes triglyceride accumulation in the liver [13]. Steatosis, lipotoxicity, and inflammation are the three "hits" that make up the pathological development of non-alcoholic fatty liver disease [14]. IKKβ (inhibitor of nuclear factor kappaB [NF κB]) is upstream activated in steatosis, which increases signalling of the transcription factor NF-κβ (nuclear factor kappaβ). Pro-inflammatory mediators such as TNF-α (tumor necrosis factor-alpha), IL-6 (interleukin-6), and IL-1β (interleukin-1 β) are produced when NF-κβ is activated. In order to mediate inflammation in NASH [16,17], these cytokines help activate and recruit resident hepatic macrophages, also known as Kupffer cells [15]. Furthermore, it has been documented that TNF-α and IL-6 contribute to hepatic insulin resistance by up-regulating SOCS3 (suppressor of cytokine signalling 3) [18,19]. Lipid toxicity and organelle failure, primarily mitochondrial malfunction and endoplasmic reticulum stress, are two consequences of excess liver fat [20,21]. An imbalance between the generation of reactive oxygen species (ROS) and protective oxidants leads to oxidative stress. A malfunctioning mitochondrion has a higher potential to oxidize FA. In patients with nonalcoholic fatty liver disease, oxidative stress [22,23] is believed to be the third insult that finally causes hepatocyte death. The pathophysiology of nonalcoholic fatty liver disease (NAFLD) seems to be a vicious loop of steatosis, lipotoxicity, and inflammation that results in intricate alterations in the histological and biochemical properties of the liver [24].

1.3. Current Treatment Options for NAFLD

One crucial foundational treatment is lifestyle modification. In the therapy of NAFLD, glucolipid metabolism regulation has remained a priority. Further research is being done on treating NAFLD by focusing on the gut microbiota. Additionally, a number of new metabolic medications, including as PPAR agonists, FXR agonists, and THR-bagonists, are being developed and are currently undergoing clinical trials. There is still more work to be done in the development of novel medications for NAFLD. The complicated heterogeneity of NAFLD, the limitations of therapeutic targets, drug safety, and other factors may be the cause of the suboptimal clinical trial results and the unmet histological endpoint of the majority of therapeutic medications. A thorough grasp of the pathophysiology of NAFLD may aid in the development of medications that, in the near future, can lessen liver fibrosis and inflammation in addition to improving metabolic problems. Naturally, given the medical, financial, and social costs associated with the worldwide NAFLD epidemic, the vulnerable population worldwide, healthcare professionals, and healthcare facilities should raise their knowledge of the condition in order to implement early interventions and improve prevention and control outcomes [25].

1.4. Role of Sodium Orthovanadate in Liver Diseases

There is still a lot of interest in determining whether other medications are feasible, even though the FDA recently approved Resmetirom, the first medication for NAFLD [26]. The surprising therapeutic effectiveness of pharmaceuticals like disulfiram and sorafenib in NAFLD has spurred more study into repurposing well-known treatments for this ailment. [27,28] Previous investigations have identified SOV as a promising treatment for treating diabetes. Studies have indicated that administering SOV and fenugreek together in diabetic animal models can alter blood and tissue lipid profiles and avoid elevated blood sugar levels. Furthermore, SOV has been shown to affect the fatty acid composition in macrophages by raising the amounts of certain fatty acids, acting as a competitive inhibitor of PTP [29-30].This highlights the significance of investigating the efficacy of SOV in treating NAFLD. Although these studies have shown that SOV may affect lipid metabolism, conclusive animal and cellular experiments clarifying the precise

function of SOV in NAFLD are lacking. One study suggested SOV as a possible therapeutic candidate for treating NAFLD using animal models exposed to a high-fat and high-fructose diet [30]. Since PTP1B has been found to be a key factor in the development of NAFLD, SOV, a PTP inhibitor, is expected to help reduce the disease. It is commonly known that SOV affects both P53 and apoptosis. By increasing autophagy, potentially through HIF1α/ATG5 regulation, SOV slows the evolution of NAFLD [31,32].

1.5. Rationale for the Study

An inorganic salt of vanadium, sodium orthovanadate (Na3VO4) is a complex of vanadate and hydrogen peroxide. It is widely used as an inhibitor of protein phosphatases, including protein tyrosine phosphatases (PTPs) and protein serine/threonine phosphatases (PS/TPs), which share structural characteristics with phosphate. The insulin-mimetic activity of vanadium-based compounds, such as Na3VO4, is drawing interest. Na3VO4 inhibits PTP1B activity and facilitates the translocation of glucose transporter type 4 (GLUT4) to the cell membrane, thereby enhancing glucose uptake. Furthermore, the administration of Na3VO4 reduces elevated blood glucose levels in several diabetic rat models. The function of PTP1B as a metabolic modulator has been thoroughly documented in preclinical experiments of diet-induced insulin intolerance and obesity, particularly in mice with globally or tissue-specific deletion of the Ptpn1 gene, [33,34] as well as in human studies. [35]However, PTP1B also plays a significant role in the early stages of NAFLD, a chronic liver disease that is directly linked to fatty liver and hepatic insulin resistance [34,36,37]. Despite this wealth of information, nothing is known about how PTP1B contributes to the development of NAFLD, especially in NASH, where the inflammatory component is essential to the course of the disease.

Hepatocellular carcinoma results from fatty liver illnesses, such as alcoholic and non-alcoholic fatty liver disease, which cause progressive changes in hepatic function and alteration as a result of dietary changes, insulin resistance, oxidative injury, and inflammation. By controlling hepatic lipogenesis, insulin resistance, and endoplasmic reticulum stress (ERS), PTPase plays a critical role in the development of both alcoholic and non-alcoholic fatty liver disease. Thus, we speculate that using an antagonist to target protein tyrosine phosphatase in two distinct disease conditions may maintain liver function and change.

2. MATERIALS AND METHODS

2.1 Animal Procurements and Maintenance

We purchased adult Wistar rats (any sex) from a registered breeder that weighed between 150-200 g. Before being moved to the dwelling area, the animals were held in the quarantine area while their health was being observed. For seven days they were acclimated to the central animal house facility's dwelling settings. The animals were kept in polypropylene cages with dust-free rice husk as bedding. They were kept in typical laboratory conditions with a 12-hour light-dark cycle, a regulated temperature of 23 ± 2°C, and a humidity of 40 ± 10%. They were given water ad libitum and fed a standard rodent pellet diet procured from Ashirwad Industries, Mohali. Laboratory animals were cared for in accordance with CPCSEA guidelines. Histopathology of the Liver after sacrificing the animals, the liver was separated, cleaned with ice-cold saline, and preserved for at least 6 hours in 10% neutral buffered formalin (NBF).

2.2 Fatty-liver disease model generation

To induce fatty liver disease: Cadmium (CAD)-induced (n=36) and high-fat diet (HFD)-induced (n=36). Each group was further subdivided into six subgroups (n=6 per subgroup): control, perse control, CAD, sodium orthovanadate (SOV) (5 mg/kg p.o), SOV (10 mg/kg p.o), and standard drug. The CAD group received Cadmium chloride 10mg/l continuously for 6–8 weeks. The HFD group received a high-fat diet (HFD) containing 60% kcal from fat (60% fat, 20% carbohydrates, 20% protein). The HFD was prepared in-house and provided ad libitum. Body weight, food, and water intake were monitored weekly to ensure consistency in consumption and overall health (38,39).

2.3 Measurement of body weight to ensure health

To evaluate the general health status of the animals in the Cadmium (CAD)-induced (n=36) and high-fat diet (HFD)-induced, body weight was tracked during the trial. A digital weighing scale was used once a week to assess body weight. These assessments made sure that any notable shifts in eating habits or variations in weight, which could be signs of metabolic issues or declining health, were found. The effects of Cadmium (CAD) and high-fat diet (HFD) on systemic health and metabolic balance in the model of fatty liver disease were assessed using data analysis (40).

2.4 Assessment of metabolic and liver function parameters in rat serum

At the end Rats' blood samples were taken at the conclusion of the experiment to evaluate a number of haematological and biochemical markers. To reduce pain and discomfort, sterilized glass capillary tubes were used for a retro-orbital plexus puncture, which was carried out under light anesthesia. After being collected, blood samples were placed in the proper tubes; serum extraction was done in simple tubes, and plasma separation was done in tubes treated with anticoagulant (EDTA). After that, the samples were centrifuged for 10 minutes at 3,000 rpm to extract the serum and plasma, which were then kept at -80°C for additional examination. In addition to metabolic markers including cholesterol, uric acid, and triglycerides, the biochemical parameters that were tested included indicators of liver function, such as alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and total bilirubin. Following the manufacturer's instructions, all biochemical assays were carried out using commercially available diagnostic kits. To guarantee precision and repeatability of the data, the assays were carried out using an automated biochemical analyzer (Reckon Diagnostics, Chandigarh; Span Diagnostics Ltd., Surat; and Erba Diagnostics, Baddi) (41,42).

2.5 Assessment of tissue parameters

Using a tissue homogenizer, the samples were homogenized in ice-cold phosphate-buffered saline (PBS, pH 7.4) to create tissue lysates. After centrifuging the homogenates for 10 minutes at 4°C at 10,000g, the supernatants were gathered for further biochemical testing. The Bradford test was used to measure the amount of protein present (43).

2.5.1 Lipid peroxidation assay (TBARS/MDA)

By detecting thiobarbituric acid (TBA) reactive compounds (TBARS), lipid peroxidation was evaluated. In short, 2 mL of TBA (0.375%) and trichloroacetic acid (TCA, 15%) were combined with 100 µL of material in 0.25N HCL. After 15 minutes of heating to 95°C, the mixture was chilled on ice. A spectrophotometer was used to measure the absorbance of the supernatant at 532 nm after the reaction mixture had been centrifuged for 10 minutes at 3,000 g. MDA levels were represented as nmol MDA/mg protein and computed **using an extinction coefficient of 1.56 × 105 M-1 cm-1 (44).**

2.5.2 Glutathione peroxidase (GPx) activity assay

50 mM phosphate buffer (pH 7.4), 1 mM EDTA, 1 mM NaN3, 0.2 mM NADPH, 1 mM reduced glutathione (GSH), and 1 unit of glutathione reductase were all included in the reaction mixture. The addition of 0.25 mM hydrogen peroxide (H2O2) started the process. For three minutes, the drop-in absorbance at 340 nm brought on by NADPH oxidation was seen. The quantity of enzyme that oxidized one µmol of NADPH per minute was considered one unit of GPx activity, and the results were expressed as U/mg protein (45).

2.5.3 Superoxide dismutase (SOD) activity assay

The inhibition of pyrogallol autoxidation served as the basis for evaluating SOD activity. 50 mM Tris-HCL buffer (pH 8.2), 1 mM EDTA, and 0.2 mM pyrogallol were all included in the assay mixture. The sample was added to start the reaction, and for three minutes, the change in absorbance at 420 nm was noted. The amount of enzyme needed to 50% block pyrogallol autoxidation was considered one unit of SOD activity, and the findings were expressed as U/mg protein (46).

2.5.4 Catalase activity assay

The breakdown of H2O2 served as the basis for measuring catalase activity. There were 10 mM H2O2 and 50 mM phosphate buffer (pH 7.0) in the reaction mixture. The sample was added to start the reaction, and

for two minutes, the drop-in absorbance at 240 nm was observed. The quantity of enzyme needed to break down one μmol of H2O2 per minute was considered one unit of catalase activity, and the results were expressed as U/mg protein (47).

2.6 Histo-pathological examinations

For histopathological examination, animals will be sacrificed, and liver tissues were collected, fixed in 10% formalin, and embedded in paraffin. Sections (5 μm thick) were stained with haematoxylin and eosin (H&E) for general morphology. The stained sections were examined under a light microscope, and representative images were captured for comparative analysis (48).

2.7 Statistical analysis

GraphPad Prism® software was used to perform the statistical analysis. Two-way analysis of variance (ANOVA) with a repeated-measures approach was used to examine data on body weight, food consumption, and water intake. To evaluate biochemical parameters, the Student's t-test was used. The findings are displayed as mean ± standard error of the mean (SEM), with $p < 0.05$ designated as the threshold for statistical significance. In cadmium induced fatty liver disease #p<0.05 vs control, @p<0.05 vs Cadmium, *p<0.05 vs Std and Cadmium. Similarly, in high fat diet induced fatty liver disease. # p<0.05 vs control, @p<0.05 vs HFD, *p<0.05 vs Std and HFD was compared.

3. RESULTS

3.1 Experimental Design and Treatment Protocol

Non-Alcoholic fatty liver was produced by Cadmium model and High Fat Diet model for eight weeks. The test drug Sodium Orthovanadate (5 and 10 mg/kg) and Standard drug were administered to animals for eight weeks. At the end of the study on 57th day, the animal's blood sample were collected for biochemical studies and then the animals were sacrificed for tissue parameters assessment and histological studies (**Fig. 1**). Throughout this period, SOV was administered at doses of 5 mg/kg and 10 mg/kg body weight in both models. After the eight-week treatment, blood samples and liver tissues were collected for comprehensive analysis. Liver toxicity markers were assessed from serum samples, while antioxidant enzyme activity was evaluated using liver tissue lysates. Additionally, histopathological examination of liver sections was performed to observe morphological changes associated with liver damage and the protective effects of SOV. This study design allowed for a detailed assessment of SOV's therapeutic potential in mitigating Cadmium and diet-induced liver damage at both biochemical and histological levels.

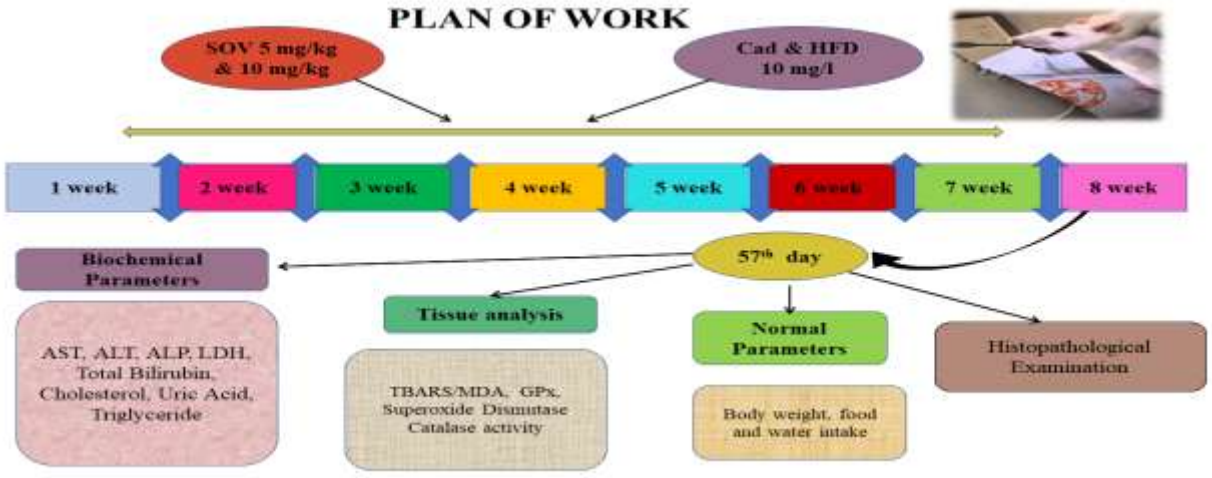

Figure 1: Working model to study the effects of SOV on CAD and HFD-induced fatty liver disease models.

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Rats were subjected to CAD and HFD for 8 weeks to induce fatty liver disease. SOV was administered at the doses of 5 mg/kg and 10 mg/kg body weight in both models, throughout the study period. After 8 weeks, blood and liver tissues were collected for analysis. Liver toxicity parameters were assessed from serum, while antioxidant parameters were measured from liver tissue lysates. Histopathological examination of liver sections was performed to evaluate morphological changes associated with liver damage and the potential protective effects of SOV.

3.2 Effect of SOV on body weight change CAD and HFD -Induced hepatotoxicity model

The CAD-induced fatty liver group exhibited a significant increase in body weight, while SOV treatment (5 mg/kg and 10 mg/kg) led to a dose-dependent reduction in body weight, with SOV (10 mg/kg) showing a more pronounced effect. The standard drug resulted in the most significant weight reduction, surpassing both SOV-treated groups, though SOV (10 mg/kg) demonstrated a weight reduction effect comparable to the standard drug. Similarly, in the HFD-induced fatty liver group high-fat diet consumption significantly increased body weight, whereas SOV treatment dose-dependently reduced weight, with SOV (10 mg/kg) showing a more substantial effect than SOV (5 mg/kg). The standard drug exhibited the greatest weight reduction, with a more pronounced effect than SOV (10 mg/kg), though SOV (10 mg/kg) demonstrated a similar efficacy (**Fig. 2**). Overall, the CAD and HFD group showed a more effective response in reducing oxidative stress biomarkers and a high-fat diet may alter the metabolic response to treatmen.

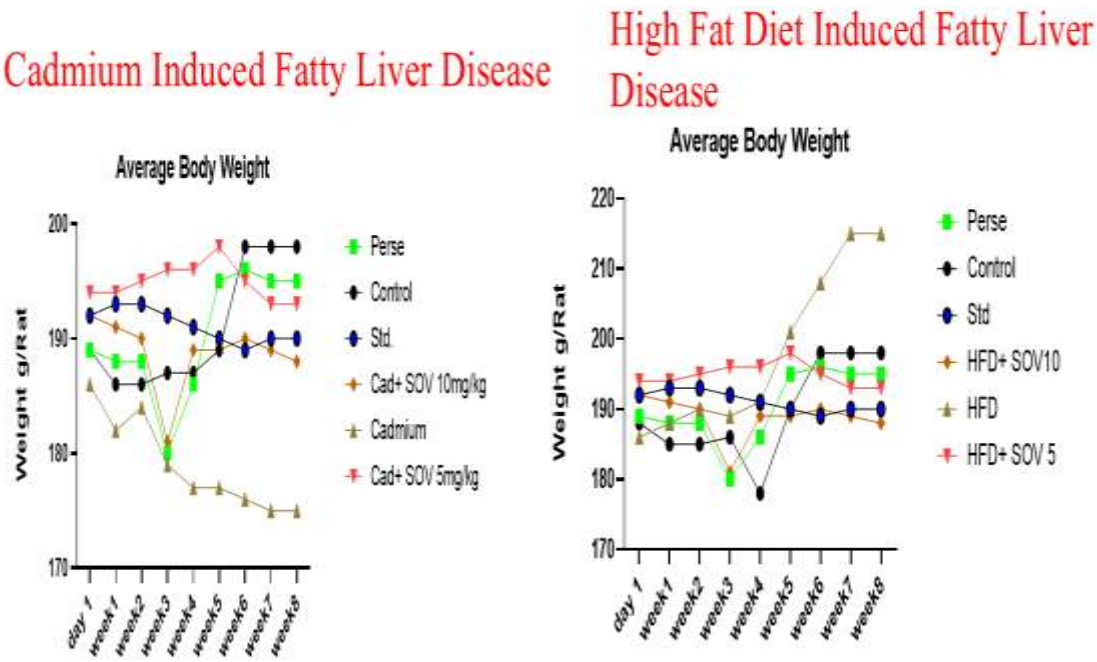

Figure 2: Effect of SOV (5, and 10 mg/kg) pre-treatment on mean body weight. After the rat was stabilised, its body weight (gm) was measured using a digital animal weighing equipment. On the first day (before to treatments) and every week after that (weeks 1, 2, 3, 4, 5, 6, 7, and 8) the mean body weights of each group were measured. The control group's mean body weight gradually increased in both the CAD and HFD - induced models, rats' body weights are considerably reduced by pre-treatment with SOV. Value are expressed as ±SEM (n=6).

Con: Control, Per: Perse, Cad: Cadmium, HFD: High Fat Diet, SOV5: Sodium Orthovenadate 5mg/kg, SOV10: Sodium Orthovenadate 10mg/kg.

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3.3 Effect of SOV on metabolic and liver serum biomarkers in CAD and HFD -Induced hepatotoxicity model Cadmium exposure in the CAD group significantly elevated serum levels of ALP, AST (SGOT), ALT (SGPT), LDH, total bilirubin, uric acid, cholesterol, and triglycerides compared to the control group. SOV treatment (5 mg/kg and 10 mg/kg) dose-dependently reduced these biomarkers, with SOV (10 mg/kg) showing a more pronounced effect. The standard drug effectively mitigated these biochemical alterations, with an enhanced effect in comparison with SOV(10 mg/kg), which exhibited comparable efficacy to the standard drug. Similarly, in the HFD group, high-fat diet consumption significantly increased serum biomarker levels, while SOV (5 mg/kg and 10 mg/kg) dose-dependently reduced these elevations, with SOV (10 mg/kg) showing greater efficacy. The standard drug demonstrated the most substantial reduction, especially when compared with SOV (10 mg/kg) **(Fig. 3)**. Overall, the CAD and HFD group showed a more effective response in reducing oxidative stress biomarkers and a high-fat diet may alter the metabolic response to treatment.

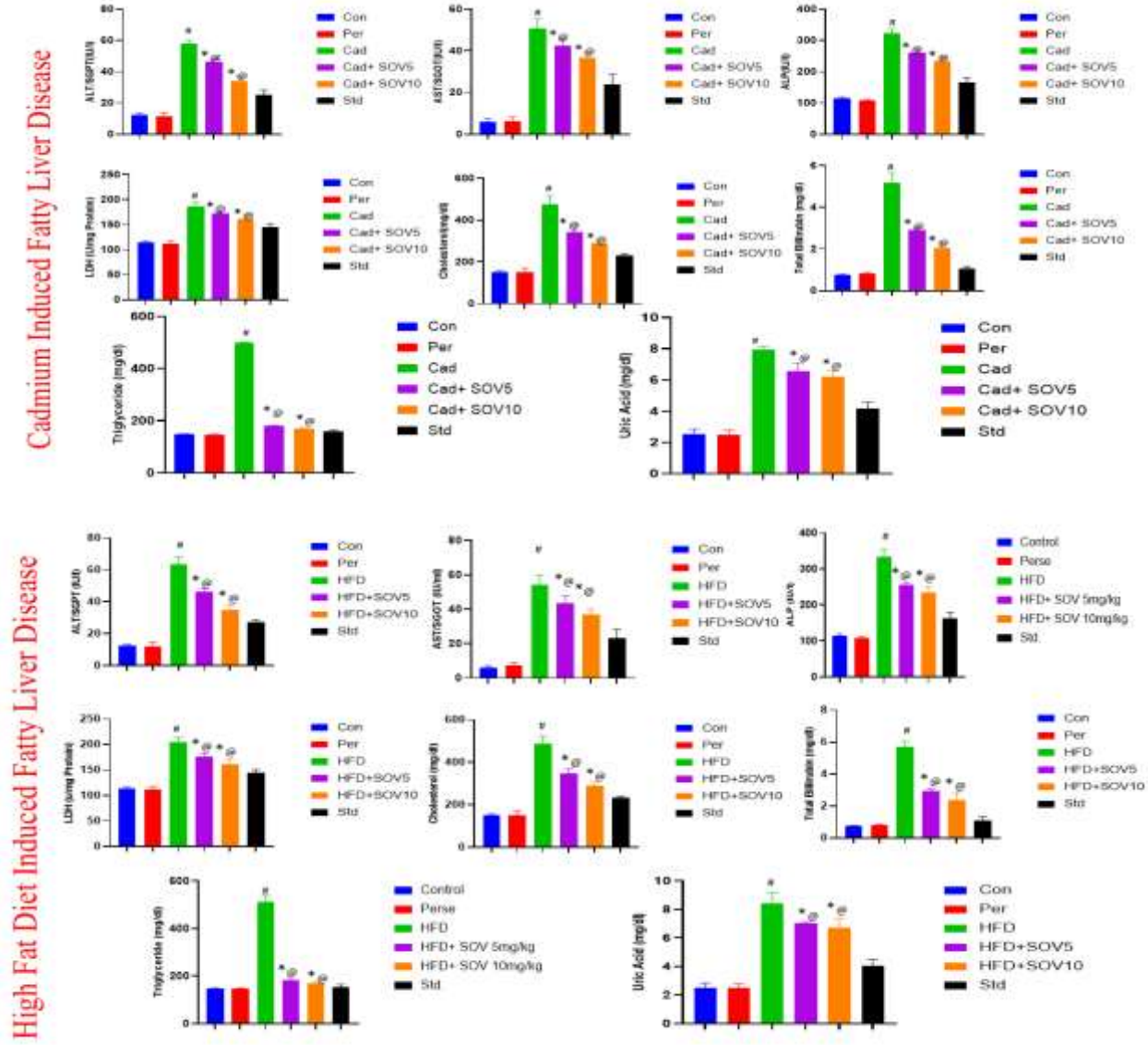

Figure 3: Assessment of metabolic and liver function parameters in rat serum.

Blood was collected before sacrificing of animals of both the models (CAD and HFD) and were subjected to determine the metabolic as well as liver biomarkers (ALP, AST, ALT, LDH, Total bilirubin, uric acid,

cholesterol, and triglyceride,). One-way ANOVA was used for analysis, and all values were displayed as mean ± SD (n = 6). In cadmium induced fatty liver disease #p<0.05 vs control, @p<0.05 vs Cadmium, *p<0.05 vs Std and Cadmium was compared, on the other hand high fat diet induced fatty liver disease # p<0.05 vs control, @p<0.05 vs HFD, *p<0.05 vs Std and HFD was compared. Con: Control, Per: Perse, Cad: Cadmium, HFD: High Fat Diet, SOV5: Sodium Orthovenadate 5mg/kg, SOV10: Sodium Orthovenadate 10mg/kg.

3.4 Anti-oxidant effect of SOV in CAD and HFD Induced hepatotoxicity model

Cadmium exposure in the CAD group significantly increased hepatic TBARS levels while decreasing GPx, SOD, catalase, and GSH activity compared to the control group. SOV treatment (5 mg/kg and 10 mg/kg) dose-dependently reduced TBARS levels and mitigated Cadmium-induced oxidative stress, with SOV (10 mg/kg) showing a more pronounced effect. Additionally, SOV treatment prevented the cadmium-triggered decline in SOD and GPx activity while significantly increasing catalase levels. The standard drug also effectively reduced oxidative stress, with SOV (10 mg/kg) displaying comparable efficacy. Similarly, in the HFD group, high-fat diet exposure significantly increased TBARS levels and decreased GPx, SOD, catalase, and GSH activity. SOV treatment dose-dependently reversed these changes, with SOV (10 mg/kg) being more effective. Compared to the standard drug, SOV (10 mg/kg) demonstrated a similar ability to reduce oxidative stress (**Fig. 4**). Overall, the CAD and HFD group showed a more effective response in reducing oxidative stress biomarkers and a high-fat diet may alter the metabolic response to treatment.

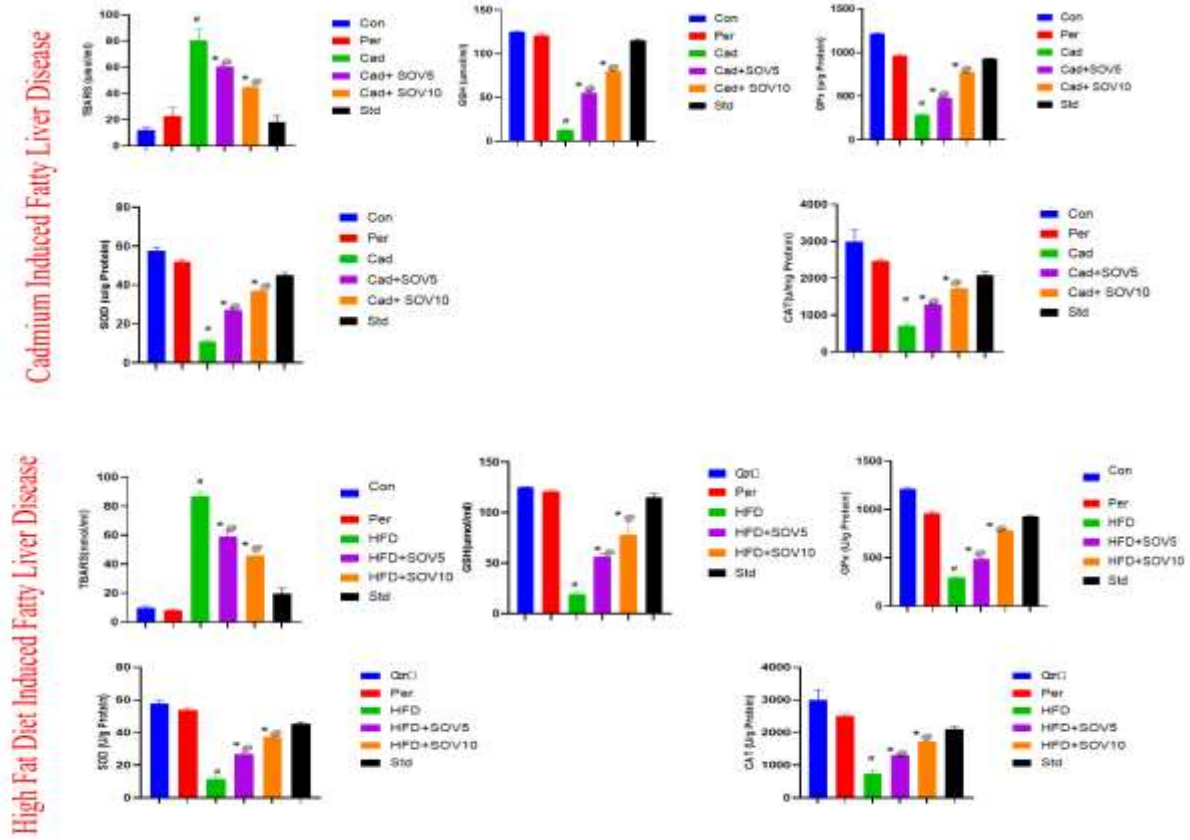

Figure 4: Assessment of anti-oxidative effect of SOV in CAD and HFD induced NAFLD model.

Effect of SOV (5 and 10 mg/kg) pre-treatment for 8 weeks on hepatic oxidative stress biomarkers in both CAD and HFD induced FLD model were measured by Thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH) content, superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase

(CAT) activity. Pre-treatment with SOV (5, and 10 mg/kg) significantly prevents hepatotoxicity by increasing TBARS content and decrease in GSH, SOD, CAT, and GPx activity in hepatic tissues lysate. All values were presented as mean ± SD (n = 6) and analysed using one-way ANOVA. In cadmium induced fatty liver disease #p<0.05 vs control, @p<0.05 vs Cadmium, *p<0.05 vs Std and Cadmium was compared, on the other hand high fat diet induced fatty liver disease #p<0.05 vs control, @p<0.05 vs HFD, *p<0.05 vs Std and HFD was compared.Con: Control, Per: Perse, Cad: Cadmium, HFD: High Fat Diet, SOV5: Sodium Orthovenadate 5mg/kg, SOV10: Sodium Orthovenadate 10mg/kg

1.5 Histopathological Evaluation of Liver Tissues

Histological analysis of liver sections revealed distinct structural differences among the experimental groups. The control group exhibited normal hepatic architecture with well-preserved hepatocytes and sinusoidal structures. In contrast, rats exposed to Cadmiun (CAD) or high-fat diet (HFD) showed significant hepatic damage, characterized by vacuolar and granular degeneration of hepatocytes, sinusoidal dilation, and widening of the portal triad (PT), as indicated by the presence of marked histopathological alterations. SOV treatment (10 mg/kg) for 8 weeks demonstrated a protective effect against CAD-induced liver injury than 5mg/kg dose. In the CAD model, SOV administration notably mitigated hepatic damage, with a marked reduction in portal triad widening and overall tissue degeneration. However, in the HFD model, although SOV treatment improved liver histology, the extent of portal triad widening remained more pronounced compared to the CAD-only group, suggesting a potentially greater challenge in reversing liver damage in the presence of both CAD and HFD-induced metabolic stress **(Fig. 5)**.

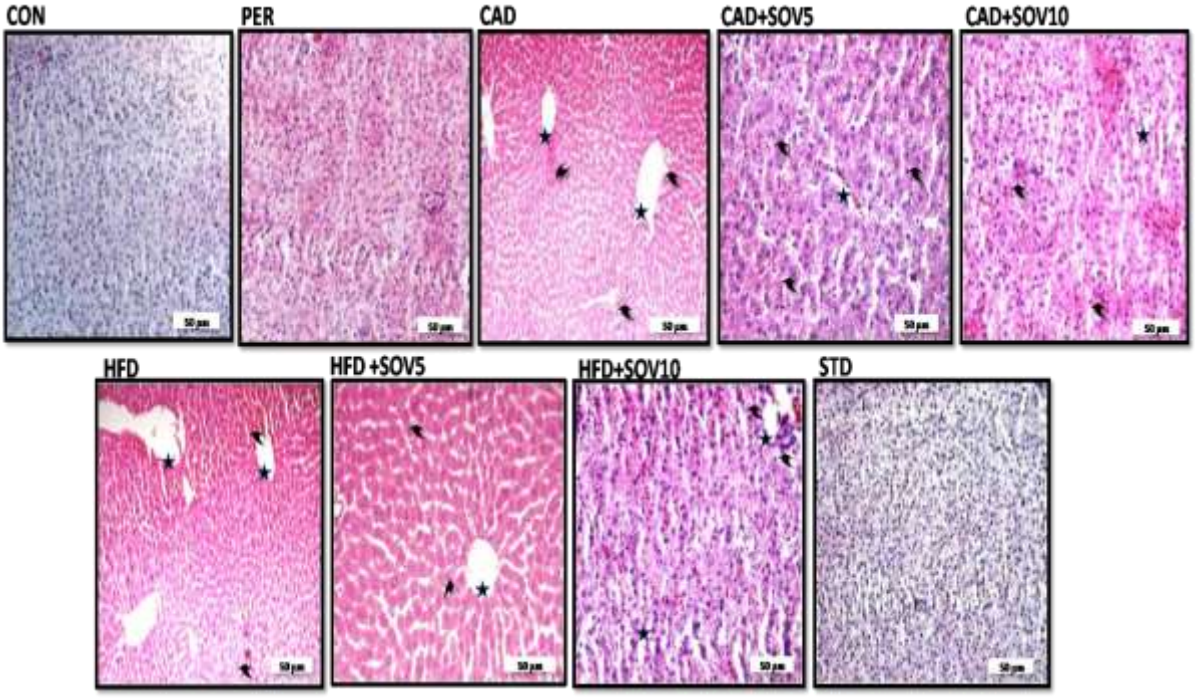

Figure 5: Histopathological analysis of liver tissues following SOV treatment in cadmium and HFD-induced fatty liver disease models.

The liver sections (50 µm) were stained with haematoxylin and eosin (H&E) after sacrifice and analysed under using a light microscope. Representative liver sections from different experimental groups are shown. The control group exhibited a normal hepatic structure, while Cadmium (CAD) and high-fat diet (HFD) exposure led to vacuolar and granular degeneration, sinusoidal dilation, and widening of the portal triad (PT, marked

by a star and inflammatory cell infiltration in the central vein marked by arrow). Notably, SOV treatment (10 mg/kg) for 8 weeks alleviated liver damage, with a more pronounced reduction in portal triad widening observed in the cadmium induced model compared to the HFD model.
Con: Control, Per: Perse, Cad: Cadmium, HFD: High Fat Diet, SOV5: Sodium Orthovenadate 5mg/kg, SOV10: Sodium Orthovenadate 10mg/kg.

4. DISCUSSION

Significant physiological changes brought on by cadmium exposure included decreased body weight, irregular feed and water intake, metabolic abnormalities, oxidative stress, and organ damage. Body weight significantly decreased in the cadmium-exposed group when compared to the control group, most likely as a result of appetite suppression and metabolic abnormalities brought on by poisoning. Though the higher dose had a more noticeable effect, co-administration of SOV (5 mg/kg and 10 mg/kg) resulted in a partial recovery of body weight. Effective toxicity mitigation was demonstrated by the standard treatment (Std.) group, which showed body weight restoration comparable to the control. The cadmium group's intake of water and feed was also negatively impacted, indicating metabolic stress linked to poisoning. In a dose-dependent way, both metrics improved when SOV was administered. Indicating its potential to help with hydration imbalance and appetite suppression brought on by cadmium. Increased levels of triglycerides, cholesterol, and uric acid were indicative of metabolic abnormalities, which also showed renal impairment and disruptions in lipid metabolism. These indicators were dramatically decreased by SOV therapy, with the 10 mg/kg dose demonstrating the highest efficacy. Rats exposed to cadmium showed increased levels of hepatic and renal toxicity indicators, such as ALT, ALP, bilirubin, and LDH, which indicate liver and kidney damage. These markers decreased when SOV was co-administered, demonstrating its hepatoprotective and renoprotective properties. Further evidence of cadmium-induced damage was provided by oxidative stress indicators, such as reduced levels of glutathione (GSH), catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD). In a dose-dependent manner, SOV treatment restored these antioxidant defenses, indicating that it may be useful in preventing oxidative damage. Overall, our results indicate that exposure to cadmium results in severe toxicity, but therapy with SOV reduces its effects by strengthening antioxidant defence, preserving critical organs, and increasing metabolic function. In order to establish its potential as a therapeutic agent against cadmium-induced toxicity, the 10 mg/kg dose of SOV showed superior protective effects. Statistical analysis (one-way ANOVA, $p < 0.05$) validated substantial differences between groups, confirming that SOV is beneficial in reducing physiological disruptions caused by cadmium. When lactate dehydrogenase (LDH) levels are analyzed, it is shown that a high-fat diet (HFD) causes cellular damage and substantial metabolic stress. LDH levels in the Perse group remain comparable to those in the control group, indicating a protective effect. In comparison to the HFD group, SOV administration at 5 and 10 mg/kg lowers LDH levels, suggesting a dose-dependent hepatoprotective effect. Additionally, lower LDH levels are seen in the standard therapy (Std.) group, confirming the effectiveness of the treatment. Similar trends are shown in triglyceride levels, which significantly increased in the HFD group, indicating lipid build up and dyslipidemia. Triglyceride levels in the Perse group remain similar to those in the control group, suggesting a protective effect. Triglyceride levels are noticeably lower in the "HFD + SOV 5 mg/kg" and "HFD + SOV 10 mg/kg" groups than in the HFD group, suggesting that SOV helps regulate lipid metabolism. The Std. group's ability to lower triglyceride levels further supports its function in reducing hyperlipidemia. These results imply that SOV supplementation lessens lipid metabolic abnormalities and liver damage brought on by an HFD. Higher SOV concentrations offer more protective advantages, according to the dose-dependent response, which suggests that SOV may be used as a therapeutic agent to treat metabolic dysfunctions brought on by HFD.

5. CONCLUSION

Significant physiological and metabolic abnormalities, such as weight loss, irregular feed and water intake, oxidative stress, and organ damage, were brought on by cadmium exposure as well as by HFD. Cadmium exposure and HFD exposure in rats resulted in decreased body weight, most likely as a result of metabolic disturbance and appetite suppression. Nonetheless, weight restoration was enhanced by SOV therapy (5 mg/kg and 10 mg/kg), with the higher dose demonstrating stronger effectiveness. Furthermore, cadmium-induced as well as HFD Induced metabolic stress and hydration imbalance were reduced by SOV. ALT, ALP, bilirubin, and LDH, markers of renal and hepatic toxicity, were increased in the cadmium group but decreased with SOV treatment, suggesting hepatoprotective effects. Additionally, SOV mitigated oxidative stress by restoring antioxidant enzyme levels (GSH, CAT, GPx, SOD). Likewise, increased LDH and triglyceride levels were observed in response to a high-fat diet (HFD), suggesting cellular damage and lipid metabolism disturbances. Administration of SOV decreased these markers, indicating its potential to prevent hyperlipidemia and liver damage. In general, exposure to cadmium and HFD causes severe metabolic dysfunction; nevertheless, SOV (especially at 10 mg/kg) offers considerable protective effects. This implies that SOV could be a viable therapeutic agent against metabolic disease associated with HFD and cadmium-induced toxicity.

2. Ethical Issue

All the performed experiments were following the rulings of The Institutional Animal Ethics Committee (IAEC) of Central Animal House at Desh Bhagat University, Mandi Gobindgrah and gave its approval to the study's research protocol.

7. Conflict Of Interest:

Authors declare no conflict of interest.

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