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Journal of Liver and Clinical Research

Research Article

Effects of *Withania somnifera* on High Fat Diet Induced Fatty Liver in Rat Model

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Submitted: 16 October 2025

Accepted: 17 November 2025

Published: 19 November 2025

ISSN: 2379-0830

Keywords

- Fatty liver; Withania somnifera; Rat model; Liver; Kidney; Biomarkers

Abstract

Liver is one of the vital organs of the body. Fatty liver is an excessive amassing of fats in the liver which causes anorexia, reduced milk production, recumbency, death and impairs the normal liver functions which is evident by the rise in level of liver enzymes. Conventional treatment has many side effects so the herbal medicines as an alternative therapy is grabbing the attention of the researchers. The present study aims to use *Withania somnifera*, a herbal plant rich in phytochemicals and possesses many benefits. In this study, rat model of fatty liver was used. There were four groups of rats and each group will have n=6. Group 1 was the the control group and fed with normal chow diet, group 2 was the negative control fed with High fat diet (HFD), group 3 was the positive control and group 4 was the treatment group for 12 weeks. Moreover, trial was terminated at 12th week and liver as well as kidney biomarkers were evaluated along with the histological analysis to see the effects induced by *Withania somnifera*. The herbal treatment depicted its beneficial hepatoprotective effects along with a betterment in biomarkers of liver and kidney.

INTRODUCTION

The liver is one of the most vital organs of the body that has an extensive functional reserve of 70-80% and keeps itself functional despite being damaged by any pathological conditions [1]. It plays multiple roles, such as the metabolism of medicines. It functions not only as an endocrine gland but also as an exocrine gland which plays a crucial part in the production of clotting factors, hormones, blood proteins, immune factors and enzymes [2]. Moreover, detoxification and excretion of waste materials are also some of the major functions of the liver [3]. The liver provides numerous benefits on one hand and gets damaged due to diseases and other medical conditions on the other hand. Among diseases, fatty liver is one of the major matters of concern. Both food [4], and companion animals suffer from it [5]. It is a metabolic disorder in which there is an amassing of fat in the hepatocytes of the liver [6].

In dairy herds, 50% of the transition cows suffer from this metabolic disorder [4]. Moreover, sheep also face such a disorder and it is generally called as pregnancy toxaemia in them [7]. Major risk factor negative energy balance (NEB) in early lactation period [6]. Hepatic lipidosis in cats and dogs occur due to obesity, lack of exercise as well as sedentary life style [8]. Whereas, horses develop fatty liver mainly due to equine metabolic syndrome (EMS) [9]. However, conventional treatments are not that beneficial as they should be because they do not eliminate the root cause of the condition and cause reduced milk production in cattle, immunosuppression and lipolysis [10].

As far as dairy cattle is concerned, it affects 50-60% of cows in the initial 3 weeks after parturition [4]. It is an aggregation of fats in the liver cells called hepatocytes. Based on the accumulation of fats in the liver, the ailment is categorized as mild, moderate and severe form of hepatic lipidosis [6,11]. Moreover, cows with TGs amassing exceeds from 4-7% are more prone to deteriorated health, survival and productive ability [12].

Various risk factors contribute to the onset of such a disorder and one of them is obesity. Excessive and uncontrolled feeding of dairy cows during the dry period and late gestation leads to make an animal obese. It leads to an increase in the body condition score (BCS) >3.5 on the scale from 1 to 5 [13]. Obese cows have reduced feed intake in their postpartum period which in turn leads to NEB and hike in lipolysis [11]. The clinical signs of fatty liver in cattle are anorexia, lethargy, reduced milk production. Moreover, if an animal does not eat for more than three

days, it may lead to recumbency, coma and eventually death. Cats which remain anorexic for more than 2-7 days also suffer from electrolyte deprivation. Consequently, they suffer from ptyalism and neck ventroflexion. Moreover, hepatic lipidosis is also a wide spread health disorder in companion animals such as cats, dogs and horses. In cats it is a quite common condition among hepatobiliary diseases. Fatty liver is generally represented by an unreasonable of aggregation TAGs in more than 80% of hepatocytes. Resultantly, there is more than 50% increase in overall liver weight [14]. Furthermore, hepatic lipidosis is the most frequent metabolic condition which leads to the liver failure in obese equine e.g. horses, donkeys, ponies. Apart from obesity, NEB also leads to fatty liver which is associated with an increase in lipolysis of already stored fats. For the purpose of the diagnosis of hepatic lipidosis in cattle several methods are used such as performing blood test, liver biopsy and ultrasonography [7]. Blood test indicates the presence of elevated level of liver enzymes. The prognosis of fatty liver is unfavourable. Diagnosis of fatty liver in cats and dogs is done by various methods and procedures for instance abdominal ultrasound, radiograph, bilirubin level in blood and through the profile of particular liver enzymes e.g. ALT, AST, GGT [15].

As prevention is always better than treatment so hepatic lipidosis can also be prevented. In dairy cattle, it can be avoided by getting rid of the risk factors such as controlling obesity, offering them proper and well formulated diet to manage NEB, daily exercise etc. In equine, it can be prevented by managing stress (factors associated with stress), feeding practices and NEB [13]. Additionally, plants have always been used for the treatment of many medical conditions in humans because of their minimum side effects than synthetic medicines [16]. Out of various herbal plants, *Withania somnifera* (WS) is one of the magical herbs which possesses countless gains. It belongs to the family Solanaceae genus Withania and also known as ashwagandha, winter cherry, Indian ginseng and suranjan. It is extensively found in Pakistan, India, Sri Lanka, Mediterranean and Africa [17]. It exhibits various advantageous impacts due to the presence of phytochemicals including steroids, alkaloids, phenolics, saponins, trepenoids etc. [18,19].

Furthermore, it is being used abundantly for its anti-inflammatory, adaptogenic and analgesic effects. It revealed its anti-inflammatory effects by regulating the expressions of interleukins (IL-1β, IL-6) and tumor necrosis factor [20]. Various studies demonstrated its antibacterial and antifungal properties due to the presence of certain compounds in it [21]. Besides, WS also in holds anti-cancer, hepatoprotective, anti-oxidant, adaptogenic, neuro-regeneration and cardioprotective characteristics [18]

The anti-diabetic effects of *Withania somnifera* (WS) along with *Berberis asciatica* (BA) had been studied in induced type II diabetes mellitus in wistar rats [22]. Besides, the valuable consequences of WS were also established as a hepatoprotective agent. The groups treated with WS were first had an administration of Carbon tetrachloride ($CCl_{4)}$ daily in order to induce hepatotoxicity. WS extract was dispensed to two groups at the dose rate of 500 and 1000 mg/kg. The results demonstrated that when WS was given at higher dose rate then there was a reduction in the elevation of ALT and ALP levels and results were similar to the effects induced by Liv. 52 [23].

MATERIALS AND METHODS

Sample procurement and preparation

WS extract was prepared by grinding 500 g of WS roots into a powder. After that, 100 g of powder was added into 1000ml of ethanol in each of the flasks. Flasks containing the mixture were placed on hot plates simultaneously and heated at 180°C for 1 hour and then at 130°C for the next 3 hours with a magnetic stirrer in each flask. After the period of 4 hours, the flasks were taken off the hot plates and were allowed to cool for 15 min.

Procurement of rats

Five weeks old male wistar rats (n=24) weighing around (100-150g) were procured from the National Institute of Health (NIH) Islamabad. They were kept at an ambient temperature (22 ± 1°C) with 12:12 h light–dark cycles and free access to water and rat feed. They were acclimatized for the period of one week.

Group identification

At six weeks of age, rats were randomly divided into four groups (G) (n=6), i.e. G1, G2, G3 and G4. G1 served as a control group. G2 was a negative control group. G3 was a positive control group and G4 being the treatment group. This trial was conducted for the period of 12 weeks.

Formulation of high fat diet

HFD for the rats was prepared through the composition mentioned in Table 1. Feed and water were offered to them *ad-libitum.*

Table 1: HFD for the rats was prepared through the composition

Ingredients	g/100g
Rat chow feed	60.0
Maize oil	12.0
Ghee	10.0
Milk powder	18.0

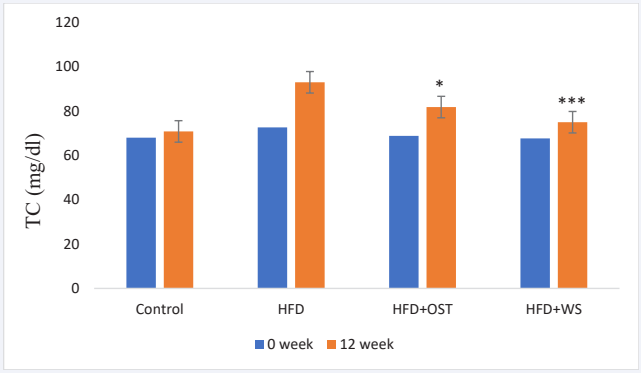

Figure 1 Graph showing the total serum cholesterol level.

Blood sample and tissue preparation

After 12 weeks of treatment, rats were euthanized by following the eutahnization guidelines of the American Veterinary Medical Association (AVMA) and blood was collected. Serum was obtained by centrifugation at 1000 g for 10 min (hemolyzed serum was discarded). Liver and kidneys tissues were collected in neutral buffered formalin for histological analysis.

Haematoxylin and eosin staining

Right after the rats were euthanized, samples from liver and renal tissue were taken from each rat and immediately fixed in 10% phosphate buffered formalin. Fixed samples were incorporated in paraffin blocks and sections of 5 µm were prepared, stained with Haematoxylin and Eosin (H&E).

Statistical analysis

Statistical analysis was performed by using the one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Body weight calculation of rats

It was observed in this study that the rats fed with HFD had a significant increase in body weight as compared to rats fed with normal chow diet. Whereas, the rats fed with HFD+WS showed a significant decrease in their body weight likewise positive control group of HFD+OST during the period of 12 weeks.

Estimation of blood glucose level of rats

It was demonstrated that rats fed with HFD showed a marked increase in blood glucose level. On contrary, those who were treated with HFD+WS exhibited a reduction in their blood glucose level similar to the control group which was fed with normal chow diet and positive control group.

Evaluation of biochemical lipid profile of rats

Total cholesterol: It was exhibited from the study that those rats which were fed with HFD showed a noticeable increase in serum TC level. Whereas, those fed with HFD+WS depicted a reduction in their serum TC level likewise positive control group.

High-density lipoprotein cholesterol: In the present study it was shown that in the negative control group where the rats were fed with HFD had a marked reduction in serum HDL-C level. While those fed with normal chow diet had the high levels of serum HDL-C. Moreover, rats fed with HFD+WS and HFD+OST revealed a significant rise in HDL-C level during the period of 12 weeks.

Low-density lipoprotein cholesterol and Total triglycerides: In this study it was observed that the rats fed with HFD had a significant elevation in serum LDL-C and TG level. However, rats fed with HFD+WS and HFD+OST revealed an observable reduction in LDL-C and TG level during the period of 12 weeks.

Evaluation of serum liver biomarker

Alanine transaminase: In this research work it was observed that rat fed with HFD had a significant rise in serum level of ALT as compared to the treatment and positive control group which showed a significant decline in ALT level during the period of 12 weeks.

Evaluation of serum renal biomarkers

Urea: In the present study it was shown that rats in the negative control group fed with HFD had a significant rise in serum level of urea. While rats fed with HFD+WS showed a reduction in the serum level of urea during the period of 12 weeks.

Liver and Kidney weight

In the present study it was observed that rats fed with HFD had a marked increase in the weight of liver and kidney when the rats were euthanized. While rats fed with HFD+WS showed a reduction in the liver and kidney weight likewise positive control group.

DISCUSSION

Fatty liver is one of the metabolic disorders of the body in which there is an accumulation of TGs in the hepatocytes. The liver becomes unable to oxidize the excessive amount of fat and resultantly leads to an increase in blood level of liver enzymes such as AST, ALT. WS is a miraculous herb which possesses various phytochemicals including phenolics, steroids, saponins, trepenoids, and

glycosides. In addition to this, it is also known for its multiple advantageous effects for instance antibacterial, neuroprotective, adaptogenic, anti-inflammatory, anticancer, antioxidant and cardioprotective [18].

In this research work, the beneficial effects of WS on fatty liver of the rat model induced by HFD are observed through assessing the changes in biochemical markers of liver and kidney. Further, blood level of TGs, HDL and LDL is also observed. In addition to this, ameliorative effects of WS in liver and kidney through histopathology are also examined.

It is revealed from the present study that rats fed with HFD have more body weight due to fat accumulation. Whereas, rats fed with HFD+WS show a reduction in their body weight. It shows consistency with the results of the study where WS was used to treat the hyperlipidaemia in wistar rats and exhibited reduction in their body weight. Moreover, the rats which were on HFD have high level of blood glucose level due to insulin resistance. On the contrary, groups fed with HFD+WS and HFD+OST showed an obvious reduction in blood glucose level. This aligns with the results of a study where WS was used to treat the hyperlipidaemia in wistar rats and demonstrated a reduction in blood glucose level [24,25].

CONCLUSION

Consequently, it is demonstrated from the present study that WS has a significant potential to use as a treatment for fatty liver. It not only reduced the blood glucose level but also alleviated the serum levels of liver and kidney biomarkers. Moreover, histopathological analysis of the liver and kidney also revealed the marked results of WS. Therefore, the results of this study can be used as evidence for future to use WS as a successful treatment for fatty liver.

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