



Research Article

PROTECTIVE EFFECT OF HERBIFIED® *SHILAJIT*-HONEY COMPLEX AGAINST
COMPROMISED FATIGUE AND STAMINA IN WISTAR RATS: HISTOPATHOLOGICAL AND
BIOCHEMICAL-BASED STUDY

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Article info

Article History:

Received: 08-08-2026

Accepted: 10-09-2026

Published: 04-10-2026

KEYWORDS:

Fatigue, Strength,
Stamina,
Depression, *Shilajit*,
Safety and Quality
of life.

ABSTRACT

Shilajit and honey are among the oldest known natural compounds, well recognized globally for their health benefits. Both natural products possess substantial and diverse pharmacological properties. However, their combination has not been studied for its effect on strength and stamina. Therefore, in the present study, we explored the effect of the Herbified® *Shilajit*-honey complex on compromised fatigue and stamina in Wistar rats. Animals were divided into 5 groups: normal control, negative control, low dose, high/optimum dose, and per se. In the normal control group, normal saline was given orally for 28 days; in the negative control weight loaded forced swim test was used to induce fatigue; in the low dose and high dose group, 51.65mg/kg and 103.29mg/kg of Herbified® *Shilajit*-honey complex was administered orally for 28 days, before 1 hr of weight loaded forced swim test and in the per se group, only 103.29mg/kg of Herbified® *Shilajit*-honey complex was administered for 28 days. Swimming time was measured weekly, and immobility duration, as a measure of depressive-like behavior, was assessed on day 29. On day 29, animals were sacrificed, and blood, serum, and various organs were collected for histological, biochemical, and neurobehavioral studies. The study showed that the low dose was ineffective in reversing fatigue-induced biochemical, neurobehavioral, and histological alterations, whereas the high dose effectively restored these parameters to normal. Hence, this is the first published report of the *Shilajit*-honey complex for increased fatigue, reduced strength and stamina, and associated effects.

INTRODUCTION

Fatigue refers to the difficulty in continuing voluntary activities in day-to-day life.^[1] Clinically, fatigue is characterized by extreme physical and mental tiredness resulting from persistent oxidative stress and physical and mental work. Also, fatigue is directly related to general weakness. Another definition of fatigue is an exhausting feeling and an inability to continue routine work.

Physical fatigue, which compromises performance, is mechanistically associated with oxidative stress, inflammation, compromised immune function, altered gut microbiota, depleted glycogen stores, mitochondrial dysfunction, and other mechanisms.^[1] The well-known clogging theory states that exercise causes substantial accumulation of lactic acid, urea, and other metabolites, which eventually compromises homeostasis and impairs ATP production. This results in muscle cramps, respiratory distress (acute), and pain. Fatigue can also result from several chronic conditions, including cardiovascular dysfunction, hepatotoxicity and nephrotoxicity, autoimmune disorders, neurological or neurodegenerative disorders, respiratory dysfunction, reproductive issues, and others.^[1] Therefore, clinicians must manage or treat fatigue, as the focus often

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<https://doi.org/10.47070/ijapr.v14i9.4405>

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remains on the primary disorder while fatigue is ignored. However, in clinical practice, clinicians often prescribe multivitamins or certain nutraceuticals, but this support remains incomplete in most cases. Therefore, well-validated nutritional supplements are needed to address fatigue and potentially affect the disease.^[1]

Shilajit and honey are two important natural compounds, backed by traditional evidence of multiple health benefits. *Shilajit* is primarily a herbo-mineral compound rich in vitamins, minerals, and various bioactive compounds, including fulvic acid and 1,2,4-dibenzopyrene.^[2,3] Honey also contains significant amounts of vitamins, minerals, and other natural bioactive compounds. Both compounds have shown significant antioxidant, anti-inflammatory, anti-apoptotic, immunosupportive, cardiovascular, hepatoprotective, nephroprotective, neuroprotective, and gonadal-protective activities when tested individually.^[4,5] Both compounds have also been tested clinically and found safe for human consumption. However, to date, no studies have reported any biological activities associated with the combination of honey and *Shilajit*.^[2,3,5] Hence, this is the first study globally evaluating the effect of the combination of *Shilajit* and honey on Forced Swim test-induced fatigue and associated complications. We firmly believe this study will be a foundational pillar for this innovative combination for human consumption and a step toward scientific validation of our ancient/traditional Indian culture.

Therefore, given the unmet need for managing fatigue and its associated complications, and the diverse pharmacological attributes of these natural products, we planned to explore a blend of *Shilajit* and

honey in the weight-loaded, forced swim-induced fatigue model. To validate efficacy, we assessed changes in body weight and in biochemical markers (LFT, KFT, CBC), histopathology of the heart, liver, and brain, and neurobehavioral attributes.

MATERIALS AND METHODS

The *Shilajit* used in the study was our in-house manufactured product. It is marketed as 'Herbified® *Shilajit* resin' (Mfd. Herbified® Healthcare, Okhla Phase-II, South Delhi, India), with batch no. (HHC-HHSR-11/25-26/001), manufactured and expiring 3 years from the date of manufacture.

Experimental Animals

Animals (n=25), of either sex, were divided into five groups (weight 180-220 g). Animals were obtained from the central animal house facilities of NIET, Pharmacy Institute, Greater Noida, Uttar Pradesh, under protocol no. IAEC/NIET/2025/01/22. Animals were kept in cages (polycarbonate) and acclimatized for 7 days prior to initiation of the study. Animals were kept at 19.0–23.5°C and 55–68% relative humidity, with a 12-hour L/day cycle, and had access to conventional pelleted feed and filtered, pure drinking water ad libitum.

Experimental design

Rats were given 1 week (week 1) to acclimatize to experimental housing conditions (temperature maintained at 23±1°C, humidity maintained at 45%-55%, 12/12-h light/dark cycle). As mentioned above, animals were divided into five groups: Normal control, Negative control (FST), Optimum dose, Low dose, and Optimum dose per se. Table 1 shows the dose, route, and duration details.

Table 1: Treatment details for the study

Group	Dose	Route	N	Duration
Normal control	1 ml/kg	P.o	5	28 days
Negative control (FST)	FST	NA	5	28 days
High dose (optimum dose)	103.29 mg/kg + FST	P.o	5	28 days
Low dose	51.65 mg/kg + FST	P.o	5	28 days
High dose/optimum dose per se	103.29 mg/kg	P.o	5	28 days

NA: Not applicable

Forced Swim Test (weight-loaded) [FST -WL]

The forced swim test (weight-loaded) was performed with slight modifications. The test was conducted in a 50cm × 50cm × 40cm tank with a depth of 30cm. The test was conducted for 28 days in the negative control and treatment groups. For the experiment, a fixed load (5% of body weight) was attached to the tails of all experimental animals, and they were allowed to swim for 60 min daily for 28 days after 1 hr of dosing. After 1 hr, the animals were removed, dried with a soft towel, and returned to the

cage. Loss of coordinated movement, weakness, or failure were considered signs of exhaustion. Endurance time was recorded when the rats failed to return to the surface or struggled to do so.^[6,7]

Biochemical estimations

On day 29, animals were sacrificed, and blood was collected from the retroorbital plexus. For this, animals were anesthetized using anesthesia (ketamine 60mg/kg + Xylazine 5mg/kg). Blood was collected in

an EDTA tube for hematological analysis and in a separate non-EDTA tube for biochemical analysis.^[8] Animals were sacrificed using the Pentobarbital 150mg/kg method.

Analysis of hematological parameters

For hematological parameters, we analyzed hemoglobin (Hb), WBCs, and platelets.^[9]

Analysis of biochemical parameters

For biochemical analysis, the collected blood sample was allowed to stand for half an hour, then centrifuged (4000 rpm for 10 min at 4°C) to obtain serum, which was stored at -20°C. Liver function tests (LFTs), kidney function tests (KFTs), and lipid profile tests were conducted.^[9]

Neurobehavioral analysis

The forced swim test was conducted as per the previously published article, and the duration of immobility was recorded. ^[10,11]

Histopathological analysis

Histopathological analysis (H&E staining) was performed on the brain (cortex), heart, and liver. The tissue was fixed in 10% formaldehyde, then embedded in paraffin and sectioned at 4-5µm. Sections were stained and observed under a microscope for histomorphological analysis.^[9]

Statistical analysis

Data were presented as mean ± standard error of the mean (SEM), and analysis was performed using

Tukey's multiple comparison test. We used GraphPad Prism version 8.1.

RESULTS

Effect on Forced Swim Test (weight loaded)

We observed a significant, gradual reduction in swimming time in the negative control group from week 1 through week 4. We then compared swim time among the optimum-dose-, low-dose-, and negative-control groups. The outcome showed no significant difference between the low-dose-treated group and the negative-control-treated group at weeks 0, 1, 2, and 3 ($p>0.05$), but a significant difference at week 4 ($p<0.05$). However, compared with the negative control group, the optimal dose showed no significant difference at week 0 ($p>0.05$), but significant differences at week 1 ($p<0.01$) and from weeks 2 through 4 ($p<0.001$). These results support the conclusion that the optimal dose is effective, whereas lower doses are insufficient to reverse fatigue induced by forced swim in rats. Also, at low doses, effects may take about a month to become apparent, whereas at high doses, effects are evident from week 1 onward, with maximum benefits expected by 14 days, as shown in Figure 1. Therefore, to manage fatigue or associated complications, patients should take a sufficient dose of *Shilajit-honey* for a sufficient duration. The drug-per se group had almost similar effects, as exhibited by the control group.

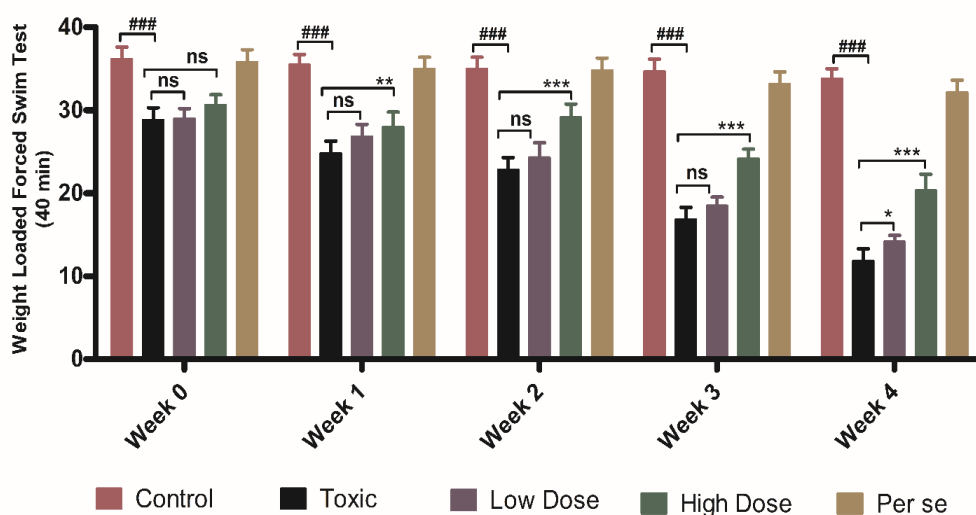


Figure 1: Effect of low and high doses of Herbified® Shilajit-honey complex after 28 days of weight-loaded forced swim test. One-way ANOVA and Tukey's multiple comparison tests were employed for statistical analysis.

Change in body weight

We found a significant difference in body weight between the negative control group and the other groups. We then compared body weight among the optimum-dose-treated, low-dose-treated, and negative-control groups. The outcome showed no significant difference between the low-dose-treated group and the negative-control-treated group ($p>0.05$). However, the optimal dose differed significantly from the negative control group ($p<0.01$), as shown in Figure 2. These results support the conclusion that the optimal dose is effective, whereas lower doses are insufficient to reverse weight loss induced by forced swimming in rats. This weight loss is due to excessive fatigue, and the loss of fat or protein is not compensated for by a normal diet, which may lead to several complications. However, consuming an optimal dose of Herbified® Shilajit-honey complex effectively

compensates for body mass loss, thereby providing protection. This should not be confused with the interpretation that consuming honey *Shilajit* will increase weight in routine use. In fact, studies have shown that a combination of these nutraceuticals in obese subjects causes a significant reduction when added with lifestyle modification. Therefore, to manage fatigue or associated weight loss, consume a sufficient dose of *Shilajit*-honey for a sufficient duration. The drug-per se group had almost similar effects, as exhibited by the control group.

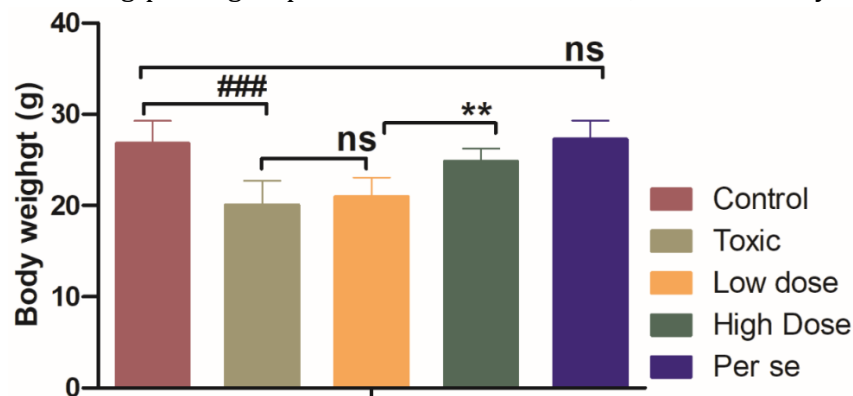


Figure 2: Effect of low and high doses of Herbified® *Shilajit*-honey complex on weight loss after 28 days of the weight-loaded forced swim test. One-way ANOVA and Tukey's multiple comparison tests were employed for statistical analysis.

Effect on FST-induced cardiac damage

In the current study, histopathological analysis was conducted to evaluate the impact of the forced swim test on cardiac tissue integrity. Our study showed significant cardiac tissue damage in the negative control group compared with the normal control group. In the normal control group, we found no cellular disintegration, pyknosis, inflammation, or fibrosis (green arrow). There was marked tissue disintegration, pyknosis (red arrow), vacuolation (white arrow), inflammatory infiltrates (black arrow), and fibrotic changes (yellow arrow) seen in the negative control group. When comparing the low and optimum doses for improvement in cardiac tissue, the low dose showed no significant improvement, whereas the optimum-dose group showed a significant reversal of cardiac damage. Notably, the optimal dose showed no signs of toxicity and was comparable to the normal control, as shown in Figure 3.

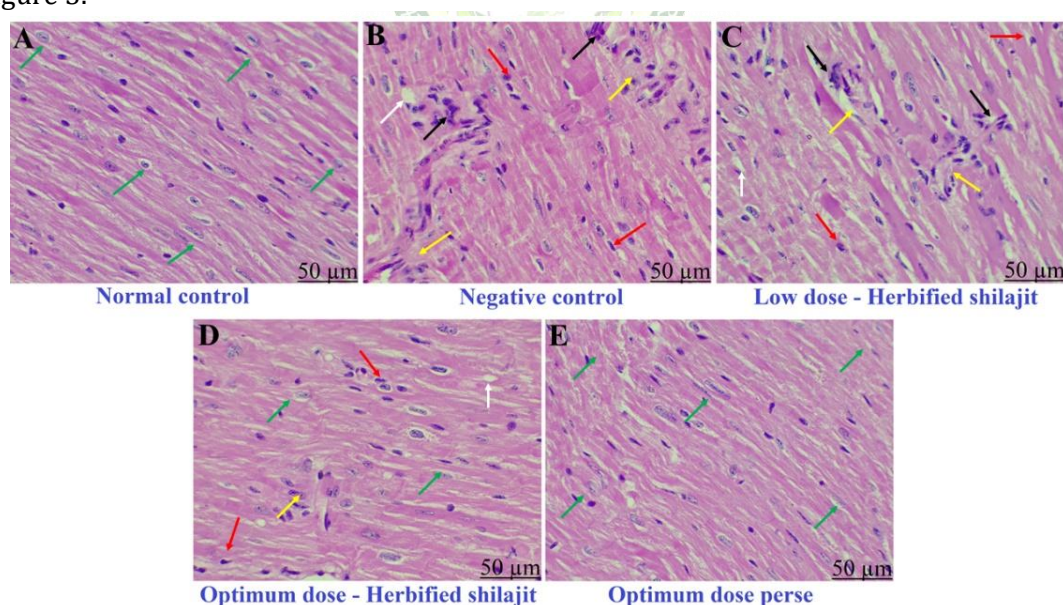


Figure 3: Effect of low and high doses of Herbified® *Shilajit*-honey complex on histopathological damage induced after 28 days of weight-loaded forced swim test. The low dose of the Herbified® *Shilajit*-honey complex failed to reverse structural damage, whereas the high dose effectively reversed it and normalized biochemical parameters. H&E; 50 μm; 400x

Effect of FST-induced Altered Hematological Parameters

In this study, hematological parameters were evaluated after 28 days of the forced swim test. The findings showed a significant reduction in hematological parameters such as Hb, an increase in WBC levels, and no substantial change in platelet levels. When the normal control group was compared to the negative control, and the low dose and the optimum dose were compared for the improvement of Hb and WBCs against the negative

control group, there was a significant difference ($P < 0.001$) between the normal control and the negative control, but no significant improvement was observed for the low dose. However, the optimum-dose-treated group showed a significant reversal of altered hematological parameters, notably Hb and WBCs, as shown in Figure 4. The optimal dose alone did not alter hematological parameters and was comparable to the control group.

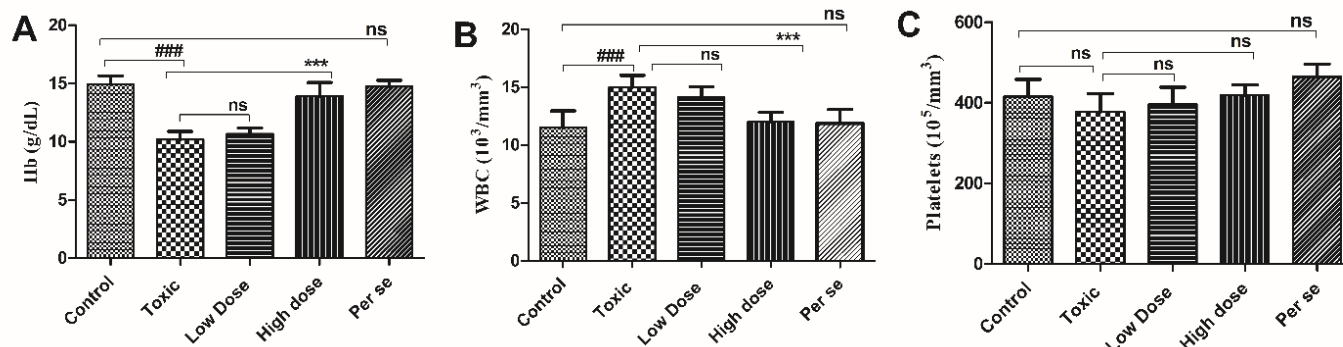


Figure 4: Effect of low and high doses of Herbified® shilajit-honey complex on altered hematological parameters caused after 28 days of weight-loaded forced swim test. One-way ANOVA and Tukey's multiple comparison tests were employed for statistical analysis.

Effect of FST-related hepatic damage

In this study, histopathological analysis and liver function tests were performed to evaluate the impact of the forced swim test on the structural integrity and biochemical status of hepatic tissue. Our study showed marked hepatic tissue damage in the negative control group compared with the normal control group. There was marked tissue disintegration, pyknosis, and fibrotic changes along with inflammatory infiltrates. Additionally, ALT and AST levels increased significantly in the negative control group. When comparing the low and optimum doses for hepatic tissue improvement, the low dose showed no significant improvement, whereas the optimum-dose group showed a significant reversal of hepatic damage. Notably, the optimal dose per se showed no signs of toxicity and was comparable to the normal control, as shown in Figure 5.

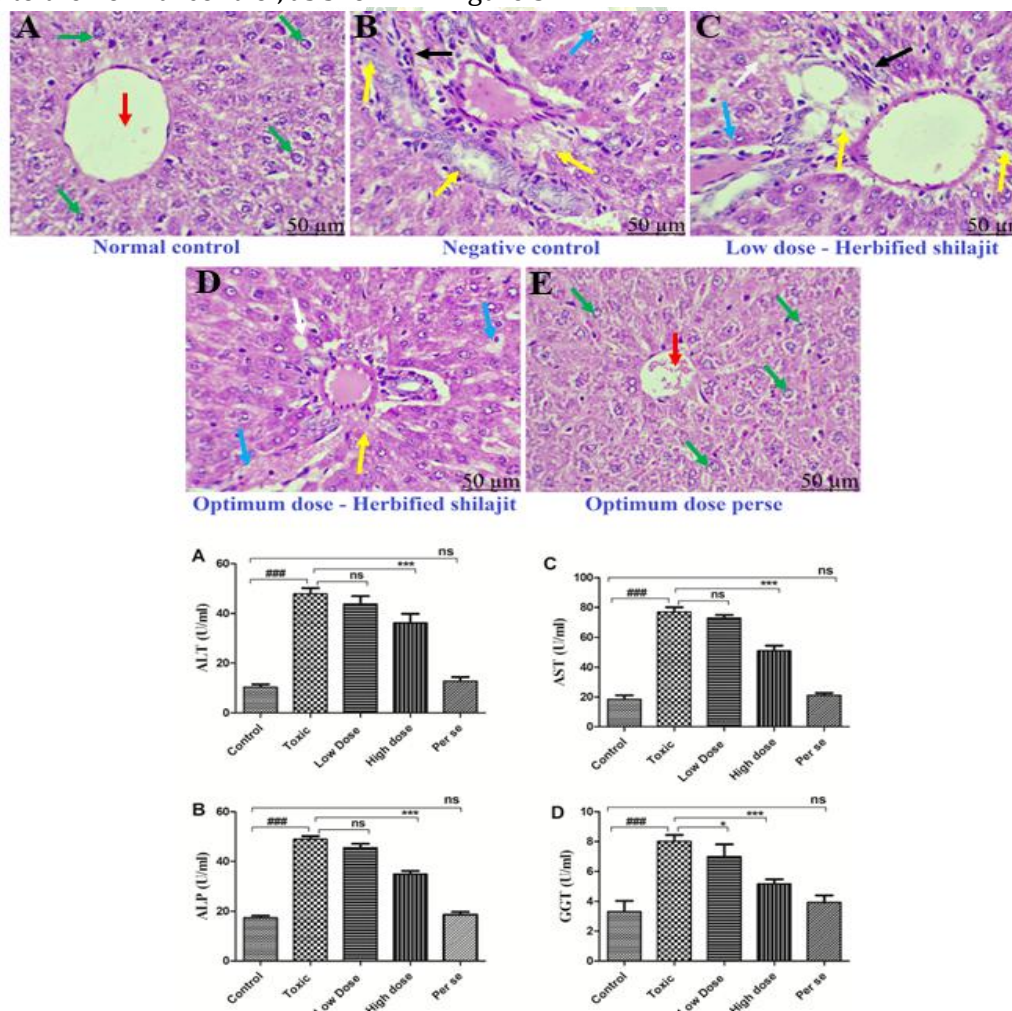


Figure 5: Effect of low and high doses of Herbified® shilajit-honey complex on histopathological damage in hepatic tissue and liver function parameters altered after a 28-day weight-loaded forced swim test. The low dose of the Herbified® Shilajit-honey complex failed to reverse structural damage, whereas the high dose effectively reversed it and normalized biochemical parameters. H&E; 50 µm; 400x.

Effect of FST on renal damage

The current study also explored the effect of a weight-loaded forced swim test on histopathological changes in renal tissue and on renal function parameters, such as BUN, urea, and creatinine levels. The histological findings showed normal PCT and DCT structures (green arrow), as well as normal glomeruli and basement membrane (red arrow), in the normal control and per se groups. In the negative control group, PCT and DCT were markedly damaged and disintegrated (blue arrow), along with damaged glomeruli and basement membrane (red arrow). Inflammatory infiltrates (black arrow), fibrosis (yellow arrow), and pyknosis (white arrow) were also seen in the negative control groups. Treatment with a low dose of Herbified® Shilajit-honey complex showed no significant or promising reversal of histological damage, whereas a high dose showed marked reversal of histological damage induced by the weight-loaded forced swim test, as seen in Figure 6. Considering renal function parameters, we found that in the negative control group, renal function parameters were markedly increased compared with the normal control group ($P < 0.001$). The low-dose treatment group showed no marked improvement in reversing urea and creatinine levels ($P > 0.05$), but BUN levels showed a mild reduction ($P < 0.05$). However, the optimal dose significantly improved and restored the deranged renal functional parameters toward normal ($P < 0.001$) for BUN, urea, and creatinine. Notably, the optimal dose per se showed no signs of toxicity and was comparable to the normal control, as shown in Figure 6.

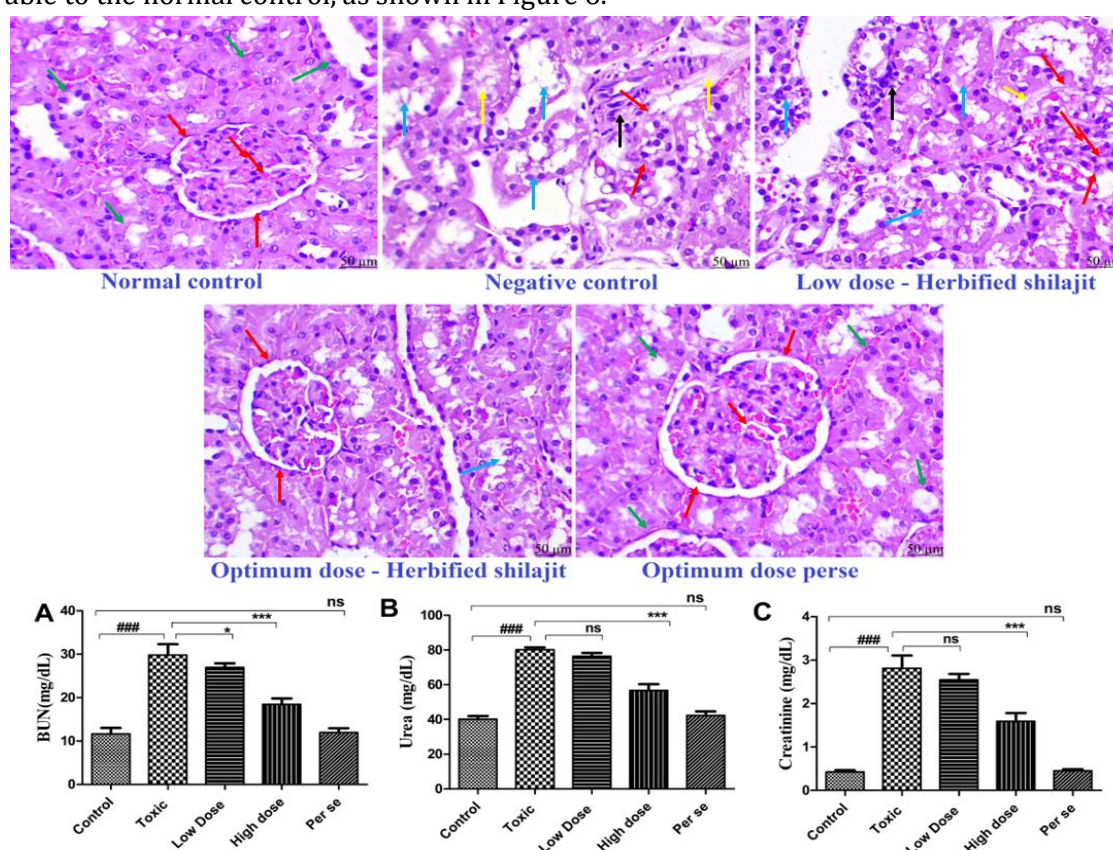


Figure 6: Effect of low and high doses of Herbified® shilajit-honey complex on histopathological damage in renal tissue and renal function parameters altered after a 28-day weight-loaded forced swim test. The low dose of the Herbified® Shilajit-honey complex failed to reverse structural damage, whereas the high dose effectively reversed it and normalized biochemical parameters. H&E; 50 µm; 400x.

Effect on W-FST neuronal damage

In this study, histopathological analysis evaluated the effects of the forced swim test on neural tissue integrity and neurobehavioral parameters, including those measured at the end of the study. Our study showed significant damage to neural tissue (cortex) in the negative control group when compared to the normal control group. The negative control group showed marked neuronal damage and degeneration, along with a longer duration of immobility, compared with the normal control group ($P < 0.001$). We compared the low and optimal doses for improvements in neuronal tissue and neurobehavioral attributes. The low dose showed no significant

improvement ($P>0.05$), but the optimal dose significantly reversed neuronal damage and reduced immobility duration ($P<0.001$) compared with the W-FST group. Notably, the optimal dose showed no signs of toxicity and was comparable to the normal control, as shown in Figure 7.

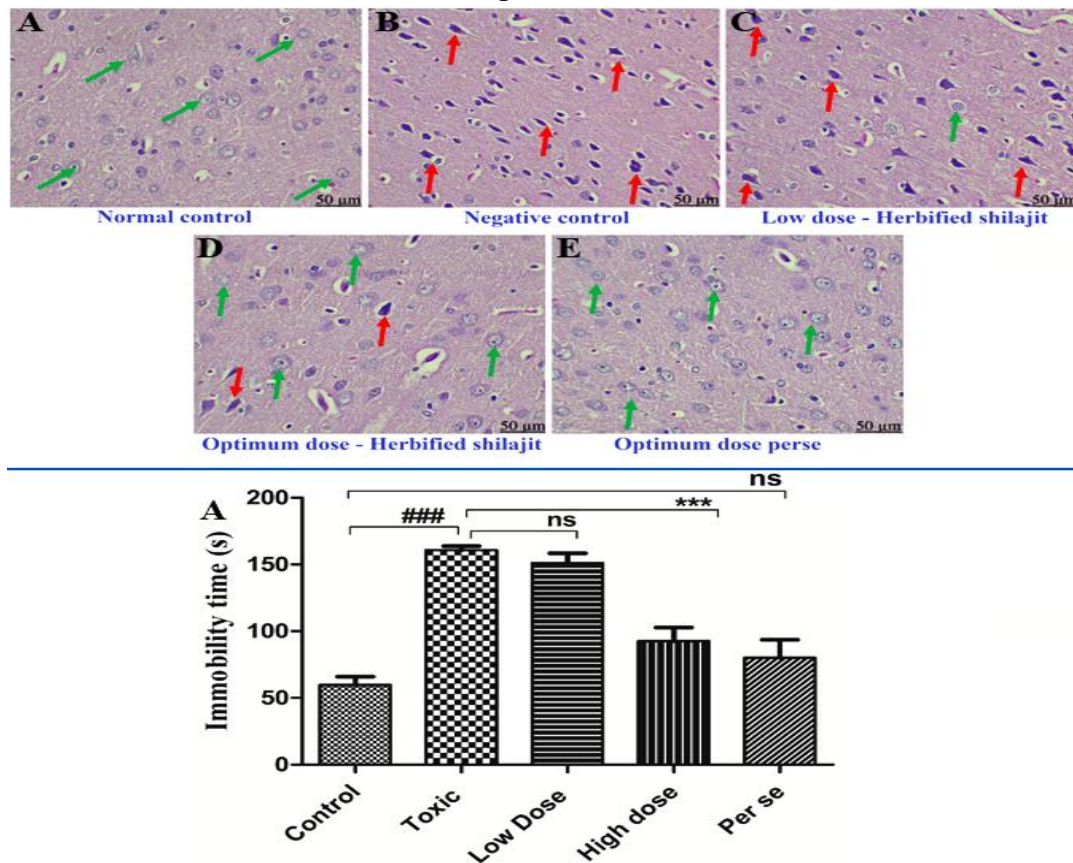


Figure 7: Effect of low and high doses of Herbified® Shilajit-honey complex on histopathological damage induced in the cortex tissue after a 28-day weight-loaded forced swim test and immobility time to study depressive like behavior. The low dose of the Herbified® Shilajit-honey complex failed to reverse structural damage, whereas the high dose effectively reversed it and normalized biochemical parameters. H&E; 5µm; 400x.

DISCUSSION

As it is well established that chronic fatigue or exposure to stress often leads to alterations in physiological homeostasis and histopathological aberrations, it has been reported that excessive exhaustion, stress, depression, along with oxidative stress and inflammation, are involved in fatigue, compromised endurance, and associated complications.^[12] Hence, in the present study, we have used the forced swim-induced fatigue model to evaluate the protective effect of *Shilajit*. Accordingly, we found that a high-dose Herbified® Shilajit-honey complex treatment significantly enhanced endurance capacity over four weeks compared with a low dose. Moreover, alterations in hematological parameters in response to fatigue are positively correlated with fatigue and associated complications. Hemoglobin plays a decisive role in maintaining optimal energy levels through efficient oxygen-carrying capacity. Hence, Hb levels are directly associated with energy metabolism, exercise capacity, and fatigue.^[13] Hb levels may also be an important parameter for assessing fatigue. Therefore, in the current manuscript, we estimated Hb, WBC, and platelet levels. Lamou *et al.*

(2016) found that levels were reduced in the negative control group, except for platelets, and that a high dose effectively reversed these levels.^[1] However, administration of a low dose of *Shilajit* did not significantly affect deranged hematological parameters.

Fatigue is also strongly correlated with hepatic function, which directly or indirectly affects nutrient metabolism. Published evidence has shown that exhaustive exercise is associated with hepatocyte hypoxia, leading to hepatic inflammation, oxidative stress, hepatic necrosis, and elevated liver enzymes such as ALT and AST.^[13] Therefore, in the current manuscript, we measured serum ALT and AST levels. We found that exhaustive exercise increases ALT and AST levels and induces histological aberrations in hepatic tissue. Treatment with a high dose of *Shilajit* and honey significantly reversed the altered biochemical and histological parameters, whereas a low dose was ineffective.

Beyond the liver's role, renal injury markers are also important for assessing fatigue and associated complications. Blood urea nitrogen (BUN) directly

reflects protein metabolism; therefore, an elevated BUN level indicates fatigue.^[14] Similar to BUN, uric acid, creatinine, and urea also reflect the extent of fatigue. Therefore, in the current manuscript, we have estimated the serum levels of UA, urea, creatinine, and BUN. We found that exhaustive exercise increases levels of these markers of renal injury and induces histological aberrations in renal tissue. Treatment with a high dose of Herbified® Shilajit-honey complex significantly reversed the altered biochemical and histological parameters, whereas a low dose was ineffective. To strongly validate the impact of fatigue on vital organs, we also performed a histopathological analysis of the heart. Our findings showed marked histopathological aberrations in the heart; treatment with a low dose of Herbified® Shilajit-honey complex was ineffective, whereas a high dose effectively reversed the histopathological damage toward normal. One of the critical impacts of fatigue is depressive like behavior and dementia. Therefore, in the current study, we assessed neurobehavioral parameters related to depression. We found a reduced increase in immobility duration in the forced swim test, which reflects depressive-like behavior. We also analyzed the histopathological changes in brain tissue and found marked histological damage. Treatment with a high dose of Herbified® Shilajit-honey complex significantly reversed the altered neurobehavioral and histological attributes, whereas a low dose was ineffective.

CONCLUSION

The present study demonstrated that chronic fatigue alters the levels of Hb, WBCs, ALT, AST, ALP, GGT, BUN, urea, and creatinine, as well as histopathological alterations in the liver, heart, and brain. There was also reduced swimming endurance time and increased duration of immobility, which signifies depression like behavior as reported in various preclinical studies. A high dose of Herbified® Shilajit-honey complex, by virtue of its potent antioxidant, anti-inflammatory, and other multifactorial mechanisms of action, effectively mitigated fatigue-induced biochemical, histological, and neurobehavioral changes. In conclusion, *Shilajit* at doses up to 103.29mg/kg (equivalent to 1000mg in humans; average human weight: 60kg) is safe and effective for managing and reducing fatigue and associated complications. However, a more detailed, cellular, and molecular study that we are conducting would substantially enhance our findings

Conflict of Interest: The authors declare no potential conflict of Interest

Acknowledgments: The authors are thankful to the Noida Institute of Engineering and Technology (Pharmacy Institute), Greater Noida, Uttar Pradesh, for providing the necessary facilities to conduct this work.

Funding: The study was funded by Herbified® Research Foundation

Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as:

Ashif Iqbal, Nausheen Khan, Mohd. Aqil, Priyanka Bansal, Abdul Qadir. Protective Effect of Herbified® Shilajit-Honey Complex Against Compromised Fatigue and Stamina in Wistar Rats: Histopathological and Biochemical-Based Study. *International Journal of Ayurveda and Pharma Research*. 2026;14(9):1-9.

<https://doi.org/10.47070/ijapr.v14i9.4405>

Source of support: Nil, Conflict of interest: None Declared

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