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Original Research Article

Safety study of Herbified® shilajit resin

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ABSTRACT

Background: Shilajit is an ancient herbo-mineral compound with numerous proven health benefits. In recent times, Shilajit has been extensively studied in preclinical and clinical models to validate its documented effects. However, there are very few preclinical safety studies from India, and they are scattered.
Methods: In the present study, we evaluated the safety of Herbified® Shilajit resin in a subacute oral toxicity study in Wistar rats. The study was conducted for 28 days for the treatment and control groups. For the satellite control and satellite reversal group, the study was extended for an additional 14 days. In the current manuscript, we documented body weight, biochemical and hematological parameters, and histopathological analysis of vital organs in male and female groups.
Results: The study showed that treatment with low-, moderate-, and high-dose Herbified® Shilajit was safe, and outcomes across all parameters were comparable to those of the control group. Also, the outcomes in the satellite control and satellite reversal groups were similar to those in the control group.
Conclusions: Based on the results of this study, 206.65 mg/kg, p.o., of Shilajit in rats is safe, equivalent to approximately 2000 mg of Shilajit for an adult human (60 kg).

Keywords: Shilajit, Preclinical, Safety, Acute toxicity, Pharmacology, Clinical trial, Quality of life

INTRODUCTION

Shilajit is an ancient herbal mineral compound with diverse pharmacological activity. Indeed, the health benefits of Shilajit are well-documented in ancient literature worldwide. Traditionally, Shilajit has been used to support strength, stamina, and overall well-being, and to reduce fatigue.[1] Later, in modern medicine, various preclinical studies demonstrated potent antioxidant, anti-inflammatory, anticancer, neuroprotective, cardio-protective, hepatoprotective, gonadoprotective, and endurance- and stamina-enhancing effects.[2-5]

Similarly, clinical trials and observational studies have demonstrated efficacy in strength, stamina, osteogenic effects, and fertility enhancement in both males and females.[4,6-8]

Moreover, in vitro studies have demonstrated the safety of this natural compound, and the LD50 of Shilajit in adults has been reported to be up to 2000 mg.[9,10] Hence, based on the previously published evidence, we aimed to evaluate the safety of Herbified® Shilajit in a 28-day subacute toxicity model in Wistar rats.

METHODS

The Shilajit used in the study was our-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 on and expiring 3 years from the date of manufacture.

Experimental animals

For this preclinical study, animals (n=60) were divided into six groups, where each group had five male and five female (weight 180-200 g). Animals were obtained from the central animal house facilities of NIET, Pharmacy Institute, Greater Noida, Uttar Pradesh, having 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 a temperature of 19.0–23.5°C, and relative humidity of 55–68%, with 12-hour l/day cycle, having access to conventional pelleted feed and filtered pure drinking water provided ad libitum to animals

Chemicals and reagents

All the chemicals and reagents used in the study were of analytical grade.

Experimental design and dose

The 28-day subacute toxicity study was conducted in accordance with the OECD guidelines no. 407, "repeated dose 28-day oral toxicity study in rodents". Animals were divided into 6 groups as shown in Table 1. Doses were selected based on the therapeutic dose. The therapeutic dose was converted to a human-equivalent dose (mg/kg) for humans and rats. We used doses of 206.65 mg/kg (high dose), 103.32 mg/kg (medium dose), and 51.66 mg/kg (low dose).[11] We also had satellite control and satellite reversal doses of 1 ml/kg and 206.65 mg/kg, respectively. All animals were treated p.o. for 28 days, and the satellite control and satellite reversal groups were treated for 14 days.[11] The details of the regimen are shown in Table 1.

Assessment of general health and mortality

Animals were monitored daily for signs of discomfort, changes in reflexes, diet, behavior, or locomotion, and responses to drugs. Additionally, the eyes, skin, fur, and paws were examined daily for changes.

Table 1: Showing the dose regimen of the subacute study.

Group	Dose	Route	Number of animals	Duration (days)
Control	1 ml/kg	p.o	10, 5-M, 5 F	28
High dose	206.65 mg/kg	p.o	10, 5-M, 5 F	28
Medium dose	103.32 mg/kg	p.o	10, 5-M, 5 F	28
Low dose	51.66 mg/kg	p.o	10, 5-M, 5 F	28
Satellite reversal	206.65 mg/kg	p.o	10, 5-M, 5 F	28
Satellite control	1 ml/kg	p.o	10, 5-M, 5 F	28

Feed consumption and body weight

Consumption of feeds and body weight were monitored and recorded weekly.

Biochemical estimation

Blood was collected from the retroorbital plexus. For this, animals were anaesthetized using anaesthesia (ketamine 60 mg/kg + Xylazine 5 mg/kg). A blood sample was collected in an EDTA tube for hematological analysis, and a separate blood sample was collected in a non-EDTA tube for biochemical analysis.[12]

Animals were sacrificed using the Pentobarbital 150 mg/kg method.

Analysis of hematological parameters

For the hematological parameters, hemoglobin (Hb), red blood count (RBCs), total leukocyte count (TLC), acidophils, basophils, neutrophils, lymphocytes, monocytes, mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC), packed cell volume (PCV/hematocrit), and platelets were analyzed.[11]

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 test (LFT), kidney function test (KFT), lipid profile test, and electrolyte analysis.[11]

Necropsy

On day 28, after withdrawal of blood from the retro-orbital plexus, animals were sacrificed. The satellite control and satellite reversal were sacrificed after 14 days, i.e., on the 42nd day. Post-sacrifice, animals were carefully examined for the cranial, thoracic, and abdominal cavities, as well as for organs such as the brain, heart, thymus, lungs, adrenal glands, spleen, liver, kidneys, pancreas, stomach, intestine, and gonads. Afterward, organs were weighed, and the relative organ weights were calculated.

Histopathological analysis

Histopathological analysis (H&E staining) was performed on the brain (cortex), heart, lungs, liver, spleen, kidney, colon, and bone marrow. The tissue was fixed in 10% formaldehyde, then embedded in paraffin and sectioned at

4-5 µm. The section was stained and observed under a Motic microscope for histomorphological evaluation. We were unable to perform H&E staining on the thymus, stomach, small intestine, adrenal gland, testis, and ovary because the tissues were damaged during storage.[11]

Statistical analysis

Data were presented as mean±standard error of the mean (SEM), and analysis was performed using Tukey's multiple comparison test. GraphPad Prism version 8.1 software was used.

RESULTS

Assessment of general health and mortality

After 28 days of subacute study and an additional 14-day study in the satellite control and satellite reversal groups, there was no sign of toxicity in either group, nor was there any delayed toxicity. Also, none of the groups reported mortality.

Feed consumption and change in body weight

The weekly analysis of feed consumption showed no abnormal pattern. Neither a reduction nor a drastic increase in feed consumption was found, as seen in Figure 1. After 28 days and over the next 14 days all the treatment groups showed increased body weight. In fact, the pattern of body weight increase was comparable across all groups. The Increase in body weight was comparable across all groups, including the satellite control and satellite reversal groups.

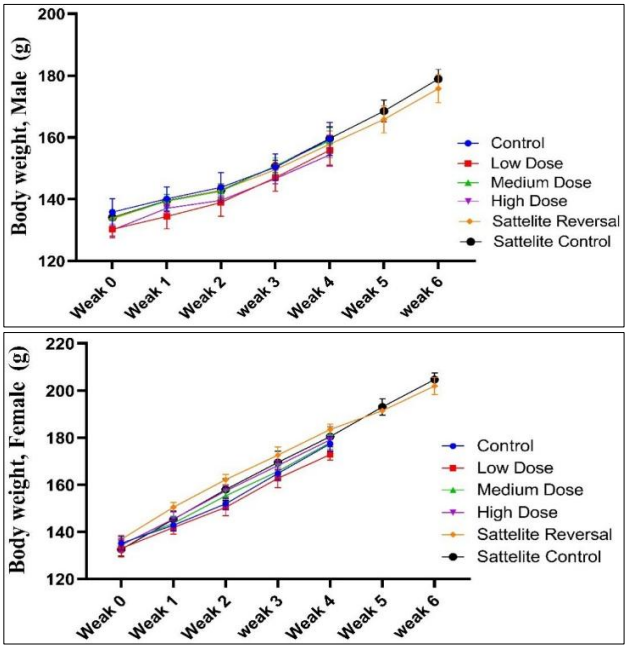

Figure 1: Showing the change in body weight in all the treatment groups weekly for 0-4 weeks in treatment groups and additionally 2 weeks in satellite control and satellite reversal groups.

Hematological parameters

The analysis of hematological parameters such as hemoglobin (Hb), red blood count (RBCs), total leukocyte count (TLC), acidophils, basophils, neutrophils, lymphocytes, monocytes, mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC), packed cell volume (PCV/hematocrit), and platelets were made after 28 days and also for extended period of 14 days. All parameters were within normal limits, and statistical analysis revealed no significant difference between the groups (Figure 2).

Liver function test

Analysis of liver function tests, including ALT, AST, ALP, total bilirubin, albumin, globulin, and protein, showed no significant differences between males and females and between males and females and the control group. Additionally, in the satellite control and satellite reversal groups, no significant differences between males and females were observed relative to the control, as shown in Figure 3.

Kidney function test

Analysis of kidney function tests, including blood urea nitrogen, urea, uric acid, creatinine, calcium, sodium, potassium, and chloride, showed no significant differences between males and females and were not statistically significant.

Additionally, in the satellite control and satellite reversal groups, no significant differences between males and females were observed relative to the control, as shown in Figure 4.

Lipid profile

Analysis of the lipid profile, i.e., triglycerides and total cholesterol, showed no significant differences between males and females compared with the control group. Additionally, in the satellite control and satellite reversal groups, no significant differences between males and females were observed relative to the control, as shown in Figure 5.

Histopathological analysis

The histopathological analysis of the brain cortex appears normal. As shown in Figure 6, neurons are healthy, with no evidence of necrosis, apoptosis, hemorrhage, or other structural distortion.

The histopathological analysis of the cardiac tissue demonstrates normal histopathological features. No evidence of cellular disintegration, pyknosis, fibrotic changes, congestion, or inflammatory infiltrates is seen (green arrow), as seen in Figure 7.

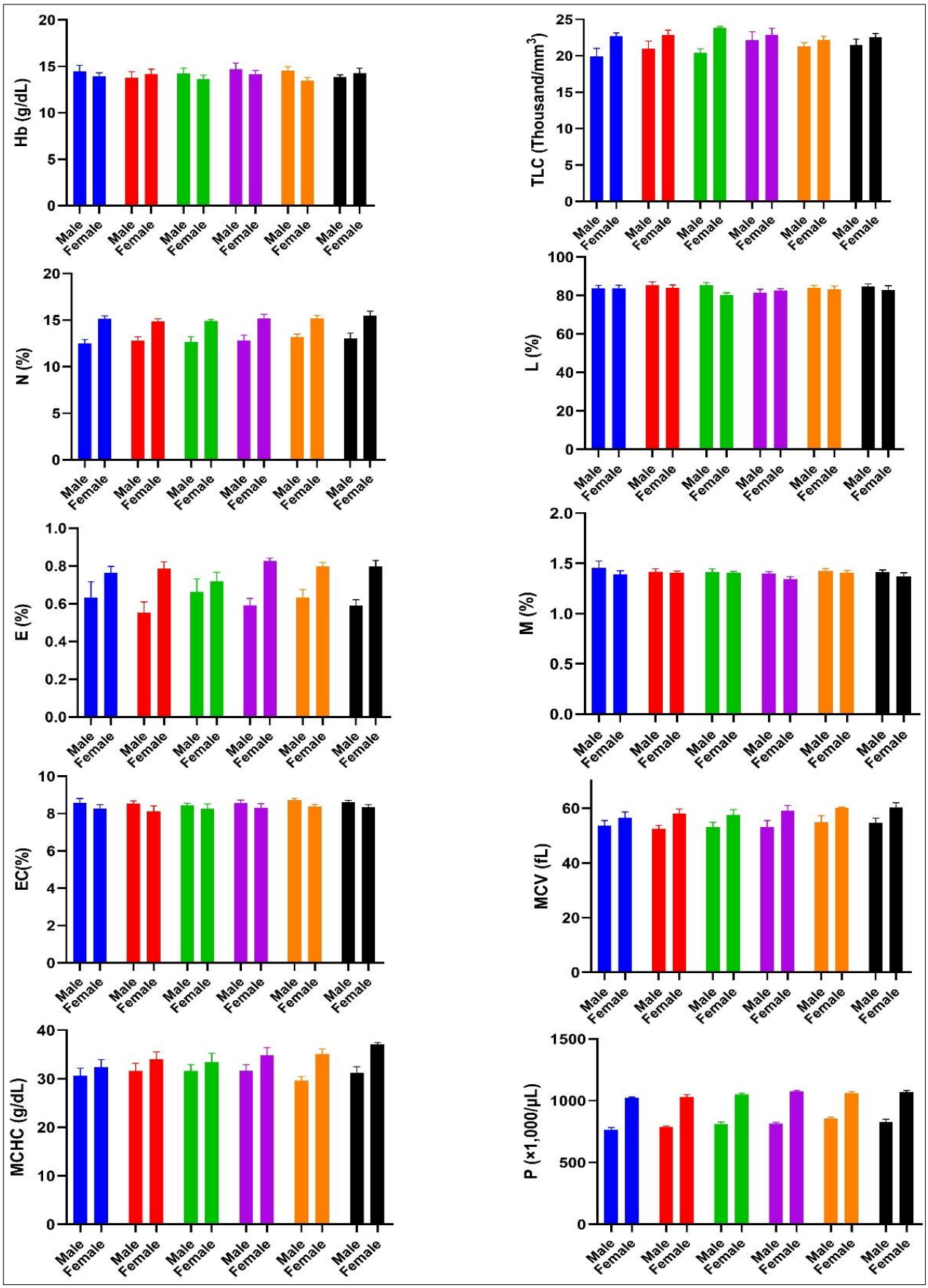

Figure 2: Showing the hematological parameters in all the treatment groups over 28 days, additionally for 2 weeks in satellite control and satellite reversal groups.

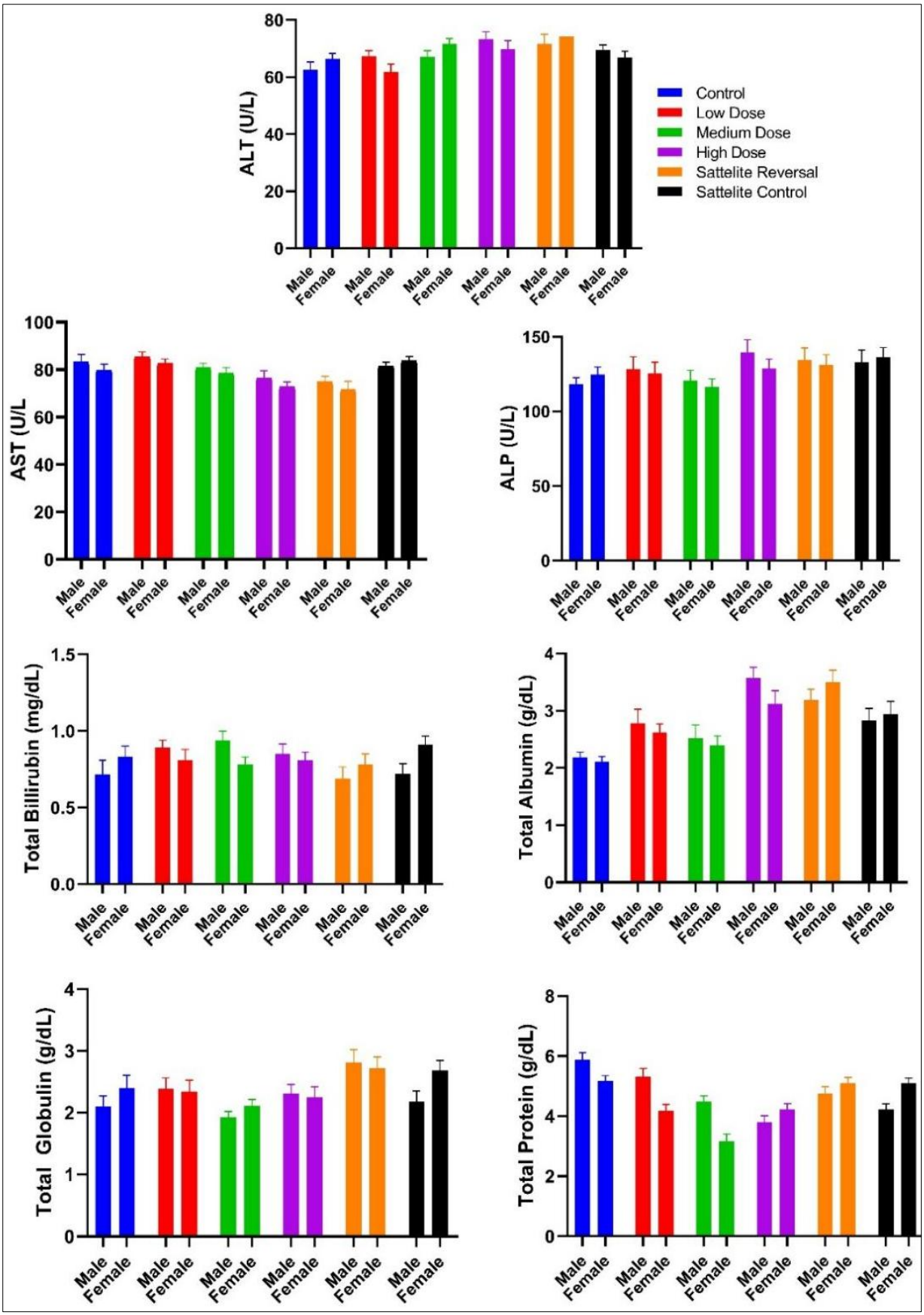

Figure 3: Showing the parameters for the liver function test in all the treatment groups over 28 days, and additionally for 2 weeks in satellite control and satellite reversal groups.

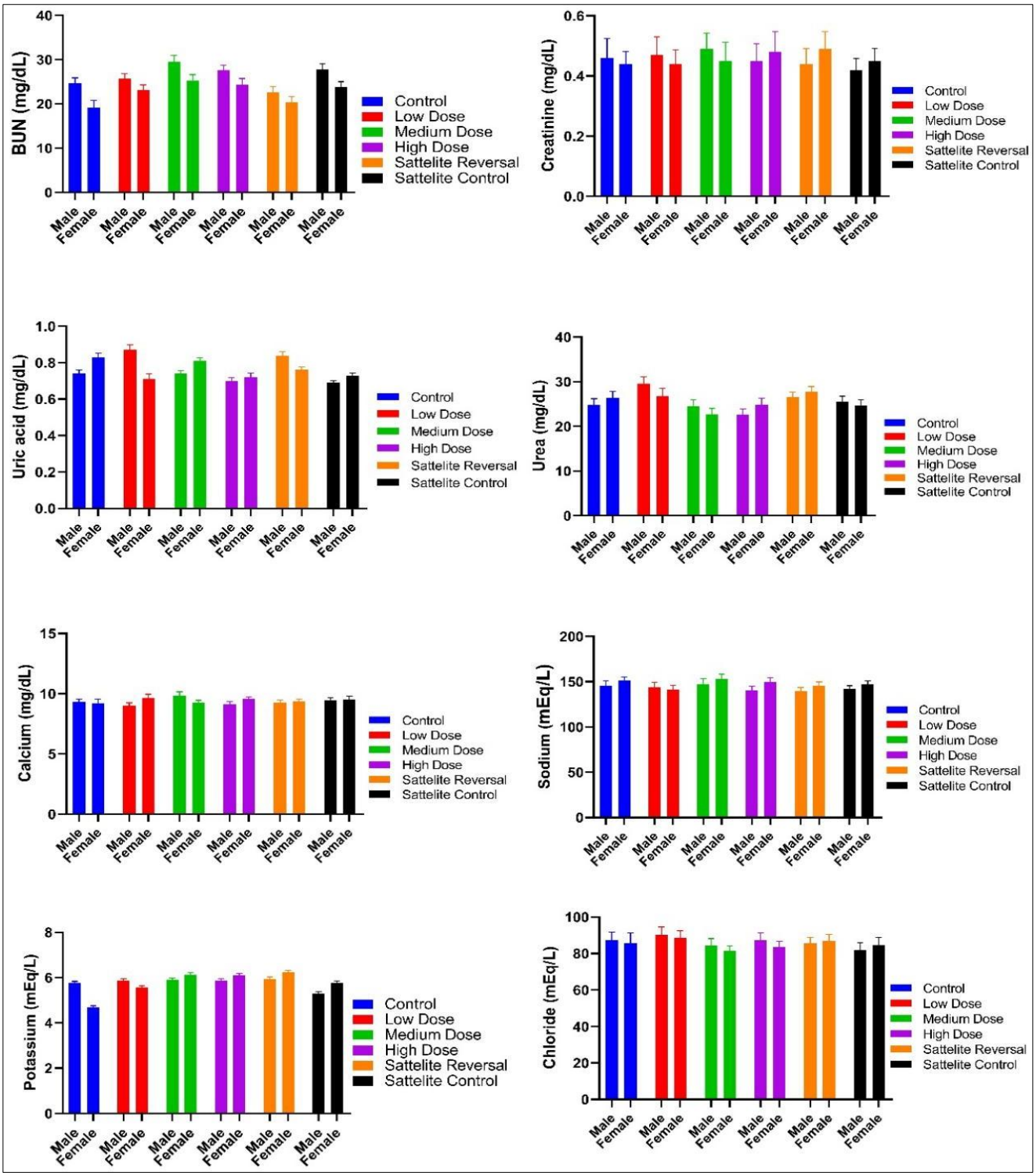

Figure 4: Showing the parameters for the kidney function test in all the treatment groups over 28 days, and additionally for 2 weeks in the satellite control and satellite reversal groups.

The histopathological analysis of the liver shows a normal histological appearance. No evidence of cellular disintegration, pyknosis, apoptosis, central vein congestion (CV), and nuclear morphology was found (red arrow). The section also shows normal and trabecular-arranged hepatocytes, which are polygonal in shape (blue arrow). Clear cytoplasm, normal/round/oval nuclei, and no fatty changes, inflammatory infiltrates, and congestion in the central vein were seen, as represented in Figure 7.

The histopathological analysis of the kidney section examined shows normal renal histopathology. As seen, no evidence of cellular disintegration, necrosis, pyknosis, vacuolation, damaged PCT, or DCT is seen (red arrow).

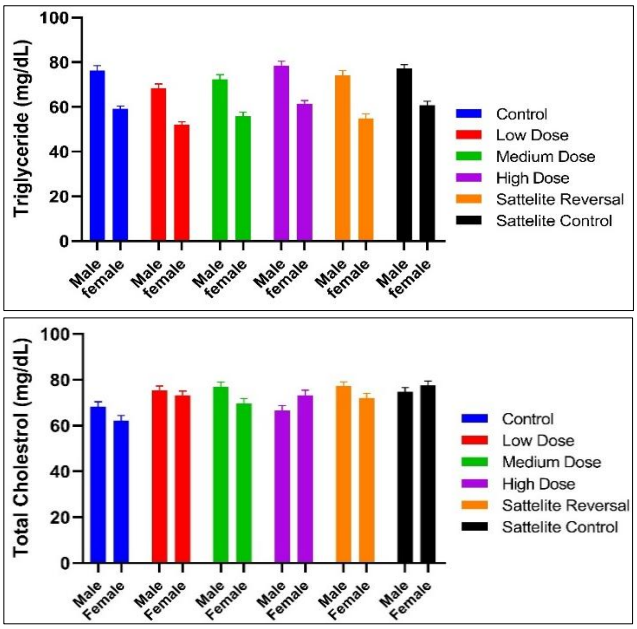

Figure 5: Showing the parameters for the lipid profile in all the treatment groups over 28 days, and additionally for 2 weeks in the satellite control and satellite reversal group.

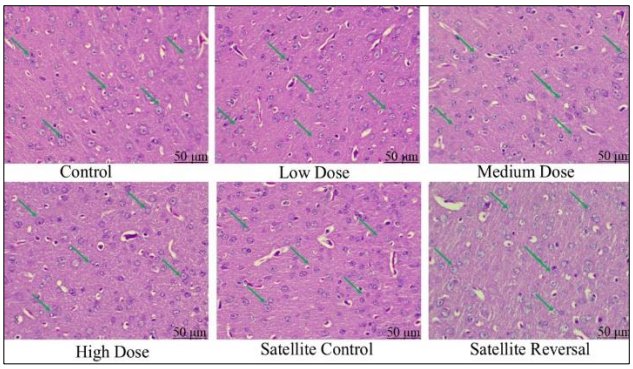

Figure 6: Showing the histopathological analysis of the cortex region of different treatment groups.

Also, the glomerulus, basement membrane, or podocytes are intact, and evidence of damage was observed (blue arrow). Additionally, no evidence of fibrosis, vacuolation, or hemorrhage was seen, as shown in Figure 8. The histopathological analysis of the lung section shows a nest of polygonal cells with pink cytoplasm and well-defined borders (red arrow), without fatty changes or keratin pearls. There are no hyperchromatic, polymorphic, or angular nuclei. No congestion of blood vessels and interstitial hemorrhage were also seen (blue arrow), or with the presence of inflammatory cells, as shown in Figure 8.

Histopathological analysis of bone marrow shows a normal structural appearance, with no evidence of bone marrow depression, damage, or disorganized trabecular architecture, or apoptotic changes, across all treatment groups, as shown in Figure 9. The histopathological analysis of the spleen shows a normal histological appearance. As shown, red and white pulp are clear, with no signs of disintegration, necrosis, congestion, or inflammatory infiltrates, as shown in Figure 9.

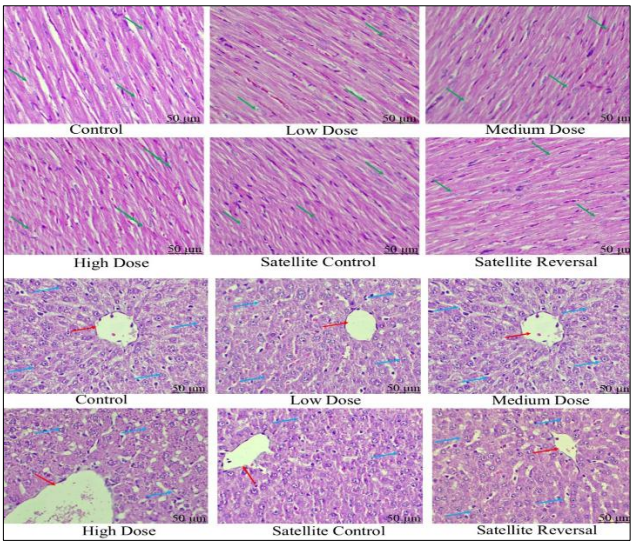

Figure 7: The upper panel shows the histopathological analysis of cardiac tissue of different treatment groups, and the lower panel shows the histopathological analysis of liver tissue of different treatment groups (H and E stain, 400×, scale bar 50 µm).

Figure 10 shows the histopathological analysis of the intestinal section, which reveals a normal structural architecture with no signs of inflammation, apoptotic changes, or cellular disintegration, as indicated by the red and yellow arrows, as shown in Figure 10.

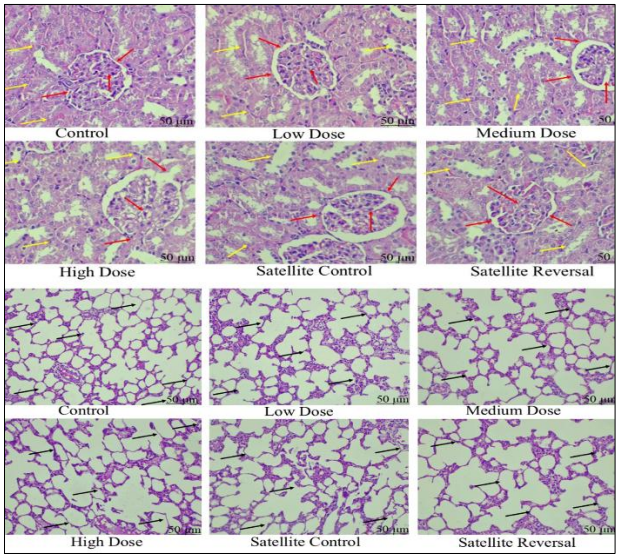

Figure 8: Upper panel showing the histopathological analysis of renal tissue of different treatment groups, and the lower panel shows the histopathological analysis of renal tissue of different treatment groups (H and E stain, 400×, scale bar 50 µm).

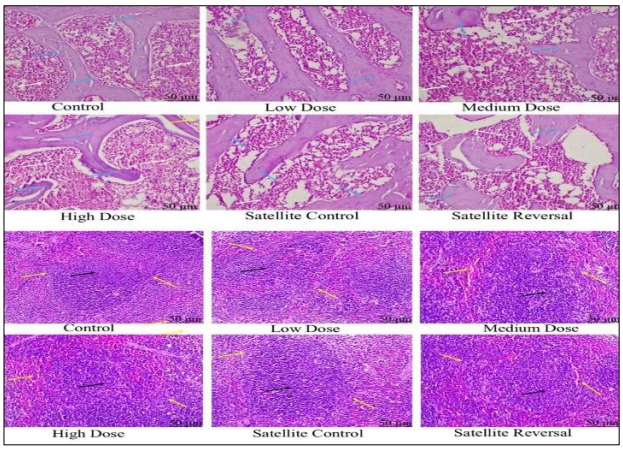

Figure 9: The upper panel shows the histopathological analysis of the marrow of different treatment groups, and the lower panel shows the spleen of different treatment groups (H and E stain, 200×, scale bar 50 µm).

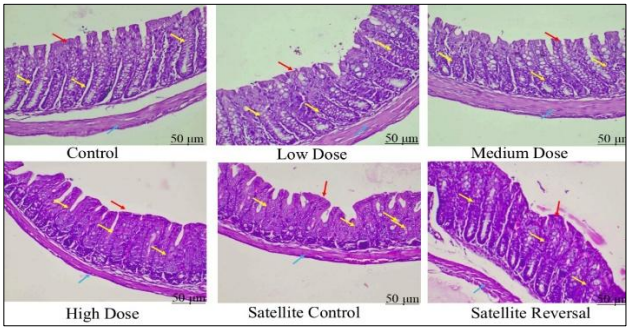

Figure 10: Showing the histopathological analysis of colon tissue of different treatment groups (H and E stain, 100×, scale bar 50 µm).

DISCUSSION

To the best of our knowledge, this is the first sub-acute oral toxicity study or safety assessment of Shilajit resin (Herbified® Shilajit) from India. In addition to this study, a 91-day chronic survey was previously conducted to assess iron content, and another subacute study was conducted on a polyherbal formulation that also contains Shilajit.[13] When the animals were observed for changes in body weight over 28 days and an additional 14 days, we observed an exponential increase in body weight in both males and females, consistent with previous reports in both sexes. We evaluated the effects of low-, moderate-, and high-dose Shilajit on hematological parameters, hepatic and renal profiles, lipid profiles, and histopathological changes. The results of our study showed that hematological parameter values were similar or nearly similar across treatment groups compared with the control group. These findings are consistent with previously published reports on Shilajit.[4,13] Additionally, this finding clearly demonstrates that all doses are well tolerated and not hematotoxic. Going forward, we estimated the marker of liver injury and the histopathological findings of the liver. The outcome showed that no markers of liver injury exceeded the threshold and were comparable to those of the control group. A similar trend was observed in the histopathology of hepatic tissue, with no evidence of hepatic toxicity; the findings are consistent with previously published reports.[4,13] Renal function tests or markers of renal injury were also evaluated, along with kidney histopathology. Findings clearly demonstrated that markers of renal injury were well within reference limits and comparable to those of the normal control group. Histopathological analysis demonstrated the safety of renal tissue across all treatment groups, and the findings were consistent with the previously published report.[4,13] The lipid profiles of the various treatment groups also showed no significant deviation compared with the control group. Histopathological examination of the heart, lungs, spleen, bone marrow, and intestine also showed no evidence of toxicity, and the structural appearance was similar to that of the control-treated group. The histopathological findings are also consistent with previously published reports.[4,13]

In summary, this study found that Shilajit at a dose of 206.65 mg/kg is safe in rats. This dose in rats is equivalent to 2000 mg of the human dose (average weight: 60 kg). In the current study, we evaluated weekly changes in body weight for 4 weeks in the control and treatment groups, and satellite control and satellite reversal groups were studied for an additional 14 days. In addition to body weight, feed consumption was also recorded (not reported in the manuscript). After completion of the study, blood and serum samples were used for biochemical analyses, including CBC, LFT, KFT, lipid profile, electrolytes, and histopathological examination of multiple organs.

CONCLUSION

The outcomes of the observational, biochemical, and histopathological analyses showed no significant differences between the groups and the control group. In other words, there was no significant difference between the treatment groups and the control group. In conclusion, Shilajit at doses up to 206.65 mg/kg in rats (equivalent to 2000 mg in humans) is safe for consumption, according to our study protocol. However, a more detailed, cellular, and molecular study that we are conducting would substantially enhance our findings.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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