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RESEARCH ARTICLE

Hematological, biochemical, and histopathological study of the plant extract of *Terminalia chebula* Reitz., *Aloe vera* Linn., and *Tamarindus indica* Linn. on animal model: A comparative study

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ABSTRACT

Background: *Terminalia chebula* Reitz., *Aloe vera* Linn., and *Tamarindus indica* Linn. are used in the treatment of different kinds of ailments. Their comparative toxicity profile is very important because all three drugs also affect on vital organ. **Aims and Objectives:** The foremost objective of the current study was relative evaluation of acute and subacute toxicity of the plant extracts of *T. chebula* Reitz., *A. vera* L., and *T. indica* L. in rats. **Materials and Methods:** Acute toxicity was conducted by the taking limit dose of 5000 mg/kg body weight. Utterance was completed and recorded for 24 h 1 time daily up to 14 days. Rats were supervised for mortality and behavioral changes 1 time daily throughout all days of study. Meant for subacute study, diverse group of animals was treated for all the three plant extracts at three distinct dose levels at 100, 200, and 400 mg/kg of freshly prepared extracts, respectively, every 24 h orally for 28 days. Control group only administered distilled water and normal feed. At the last moment of the study, biochemical parameters, hematological investigation, and histopathological examination of organs such as liver and kidney were examined. The relative analyses of histopathological investigation were done for all the three plant extracts in comparison to controls. **Results:** Not at all significant different ($P > 0.05$) or histopathological changes were detected in terms of hematological, biochemical parameters, and histopathological changes with respect to the control. None of any mortality and behavioral changes were noted. **Conclusions:** Overall, analysis of the results concluded that medium-term oral administration of all three plant extracts does not show toxicity for 28-day treatment.

KEY WORDS: Acute Toxicity; Subacute Toxicity, *Terminalia chebula* Reitz.; *Aloe vera* L., *Tamarindus indica* L.

INTRODUCTION

Terminalia chebula Reitz. is a flowering perennial tree belongs to the family Combretaceae. In Tibet, *T. chebula* is recognized as the "King of Medicine."[1] *T. chebula* is mainly used in traditional medicine for the management of different illnesses as antidiabetic, antimutagenic, antioxidant, antiulcer, antibacterial, antifungal, antiviral, and injury-curing properties.[2-5] It also impedes cardiac injury and is used for the management of kidney disorders.[5] *T. chebula* Reitz. consists almost all phytoconstituents, but it is properly ironic that tannins around 32%. Furthermore, availability of tannin in *T. chebula* mainly depends on its geographical site.[1] Glycosides and triterpenoids components of *T. chebula* have been sequestered from stem bark.[6]

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Scientifically *Aloe vera* named as *A. vera* Linn., *A. vera* belongs to *Liliaceae* family, and it is in nature xerophytic,

succulent, perennial, green pea color plant. Worldwide, well-known plant *A. vera* is a popular for its usefulness. *A. vera* applied externally in the cure of several skin disorder such as in burns, eczema, and cuts.[7] It is purported that juice from *A. vera* reduces inflammation and pain.[8] It has antibiotic and antiseptic stuffs which make it extremely valued in treating scratches and wounds. It has been frequently used in the treatment of 1st and 2nd degree burns, as well as suntans poison of oak, ivy, and sumac, infections, and eczema. Aloe has been promptly used as a therapeutic agent for wounds, ulcers, cancer, and many more disorders.[9,10] *A. vera* contains more than seventy active constituents such as minerals, sugars, enzymes, amino acids, and salicylic acids.[11-14]

Tamarindus is a member of family Leguminosae. *Tamarindus indica* Linn. mainly known as *Tamarind* tree. It is one of the another significant versatiles tropical fruit tree in the Indian subcontinent. *T. indica* is used as antimicrobial, antiseptic, antiviral, diabetes, diarrhea, eye inflammation, sore throat, sores, sprains, swelling (joints), urinary stones, etc.[15-17] *T. indica* is active components, such as cardiac glycosides, compounds phenolic,[18] L-(-) mallic acid,[19] mucilage, tartaric acid, arabinose, xylose, pectin, galactose, uronic acid, and glucose.[19,20] The ethanolic extract of *T. indica* contains fatty acids also and various trace elements such as cadmium, copper, iron, sodium, manganese, magnesium, potassium, arsenic, calcium, phosphorus, zinc, and lead which is important for our body in very low amount.[21]

Phytochemical analysis of *T. chebula* Reitz. reveals the presence of tannins, flavonoids, saponins, and cardiac glycosides. Despite the numerous use of all three plants is mainly used as anti-inflammatory, analgesic, and antipyretic. Steroid narcotic and nonsteroidal anti-inflammatory drugs are used at the present time. However, the available drugs have decreased popularity against inflammatory conditions due to numerous adverse effects. As an example, steroidal drugs are used as anti-inflammatories due to their precise mechanisms of action and also the same mechanism of actions is responsible for their adverse effects as well. Leukotriene inhibition and other basal physiological function inhibiting phenomena are used for anti-inflammatories by steroidal drugs. Hypertension as adverse effects noted due to the resemblance of these drugs to the steroid hormones. The nonsteroidal drugs have fewer adverse effects than the steroidal, but it includes gastrointestinal bleeding and improper clotting of blood. Therefore, researchers are looking for alternative treatment options that have least adverse effects and full of efficacy and must be comparable to a standard drug. To fulfill this demand currently, researchers are looking for alternate source of drug apart from synthetics one. The best alternative is an herbal crude drug that is commonly used in rural part of India as a folk medicine. Research work plan in the present study for safety and potency after repeated dosing schedule has not been fully scrutinized in a comprehensive manner. As per World Health Organization 2008 there is huge demand of herbal medicinal products hence assessment and standardization is required of all kind of herbal preparations including *T. chebula Reitz*., *A. vera* L., and *T. indica* Linn. Investigation was projected to determine oral acute and subacute toxicity profile of the ethanol leaf extract of *T. chebula* Reitz., *A. vera* L., and *T. indica* L. in experimental rats. It was also investigated for their hematological, biochemical, and histopathological changes in liver and kidney tissues after the treatment.

MATERIALS AND METHODS

Plant Collection

On the basis literature appraisal, three plants are selected for the studies:

- *T. chebula* Reitz.
- *A. vera* Linn.
- *T. indica* Linn.

Afterward collection of all three plants and it was acknowledged and authenticated by a pharmacognosist and a letter receipt specimen (No. IU/PHAR/HRB/7/10) was submitted at the Department of Pharmacognosy, Integral University, Lucknow, Uttar Pradesh, India. Aqueous extractions of all these three plants were done according to standard procedure.

Preparation of Plant Extract

T. chebula Reitz.

T. chebula Reitz. fruit was utilized for the experimentation. *T. chebula* Reitz. shade-dried fruits were taken for the preparation of extract. A fine powder in an electric grinder was grinded. Approximately 890 g of crushed processed material was hauled out with 80% ethanol at a temperature of 60°C for near about 48 h. The mixture was segregated. Condensed solvents, transferred in watch glass in an abundant surface area to make it more compressed and permit the remaining solvent to vaporize. Whereas the compressed filtrate transferred into a viscid concentrate, it was observable that we create the crude ethanolic extract. The ethanolic extract was more vaporized to dehydration to obtain the dried out ethanolic extract. Harvested extract percentage obtained 12.1% weight/weight in compare to the actual air-dried powder.[22] The extract was lastly deposited in airtight holder at 2–8°C for more uses throughout the study.[23] The dose was accustomed as per dosing schedule following standard technique.

A. vera Linn.

For extraction with water of *A. vera* leaves first wash away in cold water then spikes round the leaves were removed using a blade after that the leaves were cut up by knife. 200 g of the cut up material were assorted in distilled water and blended in an electric blender for 3 min to obtain 400% (weight/volume)

extract. The melded material was pressed in a muslin cloth. The filtrate was freeze-dried at −50°C beneath vacuum using a lyophilizer and kept at −20°C till utilize. Numerous dose ranks of *A. vera* were prepared by reconstituting the extract at concentration of 1% (w/v).[24]

T. indica Linn.

The extraction was performed using softening technique.[25] The coarse powder of *T. indica* seeds (100 g) was imperiled to maceration for 72 h at room temperature using 500 ml methanol. After sieving of the extract, the solvent was removed by evaporation technique to acquire a powdered residue. After that during experiment dose was adjusted as per dosing schedule following standard procedure.

Qualitative Analysis of Phytochemical Constituents

Primary chemical tests were conducted for all plant extracts to distinguish diverse phytochemical compositions present in the plants extract as per the protocol described.[26]

Experimental Animals

Acute and Subacute toxicological study were conducted on male and female Wistar albino rats. Animals were familiarized adapted to laboratory surroundings for 14 days before the investigation. Throughout familiarization, accommodation rats were kept in cages with pellet diet and clean water. All methods in the present work were done as per the Institutional Animal Ethics Committee guidelines. Working protocols were approved by the Institutional Animal Ethics Committee with reference no. SUG/Pharm/2015/II/22.

Acute Toxicity Study

The Organisation for Economic Co-operation and Development (OECD), 2002 method was considered. Either sex wistar rats 180–200 g weight were taken for the same study divided into 2 groups. Three sets of three rats in every one cage were administered 100, 600, and 1000 mg/kg all three plant extracts, respectively, through oral route. Surveillance for indications of toxicity and mortality was done for 24 h with exceptional care given to the first 4 h. 2000, 3000, and 5000 mg/kg dose administered for the three plant extracts and monitored to all three groups of three rats as earlier stated and daily for 7 days for indication of late toxicity.

Subacute Oral Toxicity Study

OECD, 1995 guidelines were considered for this study. A total of 24 rats (12 each as male and female) weighed and grouped in four of three each as male and female rats in each group (the ♂ rats were separated from the ♀ rats) for mentioned experiment. The animals were assembled depend on three different treatment doses of the leaf extract and one control (distilled water) group. The rats were orally given daily with extract of *T. chebula* at doses of 100, 200, and 400 mg/kg and distilled water for 28 days. Same dosing schedule and number of groups of animal taken for rest two plant extracts of *T. chebula* Reitz. and *A. vera* L. on experiment rats. The treatment doses *in lieu* of the therapeutically active dose which was the most effective dose detected in the evaluation of the analgesic, anti-inflammatory, and antipyretic.[27] Finally, after 28 days treated period, all the rats were destitute of pellet but given free access to water for 24 h before being sacrificed under inhaled standard (isoflurane, dose - 4–5% for induction and 1–2% for maintenance) anesthesia. Cardiac pinhole into ethylenediaminetetraacetic acid and non-heparin containing test tubes used for blood samples for hematological and biochemical investigation correspondingly. The organs were cut down, measured and macroscopic study was done.

Hematological Analysis

Heparinized test tubes were used for the collection of blood sample. Estimation of white blood cells (WBC), red blood cells and platelets performed by visual methods (Dacie, 1991).

Biochemical Analysis

The blood sample has been collected in the non-heparinized tubes. Blood has been centrifuged at 3000 rotation per min for 10 min. The separated serum and ultimately liver and kidney homogenates (20%) were investigated for enzymes. Reitman and Frankel (1957) method is used for alanine aminotransferase and aspartate aminotransferase. Alkaline phosphatase was analyzed by Bessey *et al*., 1946. Protein and creatinine by Gornall *et al*., 1949, and Barterls *et al*. (1972) techniques used, respectively.

Histopathological Studies

Conventional hematoxylin-eosin technique is used for histopathological examines of the liver and kidneys.

Statistical analysis was articulated as mean. The mean values also expressed along with ± standard error mean. One-way analysis of variance (ANOVA) was executed to compare the variances between two or more means. A mean dissimilarity was considered statistically significant when $P < 0.05$.

RESULTS

Acute Oral Toxicity

The extract of all three plants did not produce any untoward effect in animal at the different dose levels. No changes in their behavior and stool texture of the animals. No mortality detected after 7 days of treatment. The LD_{50} values for all the plants were found to be >5000 mg/kg for oral administration.

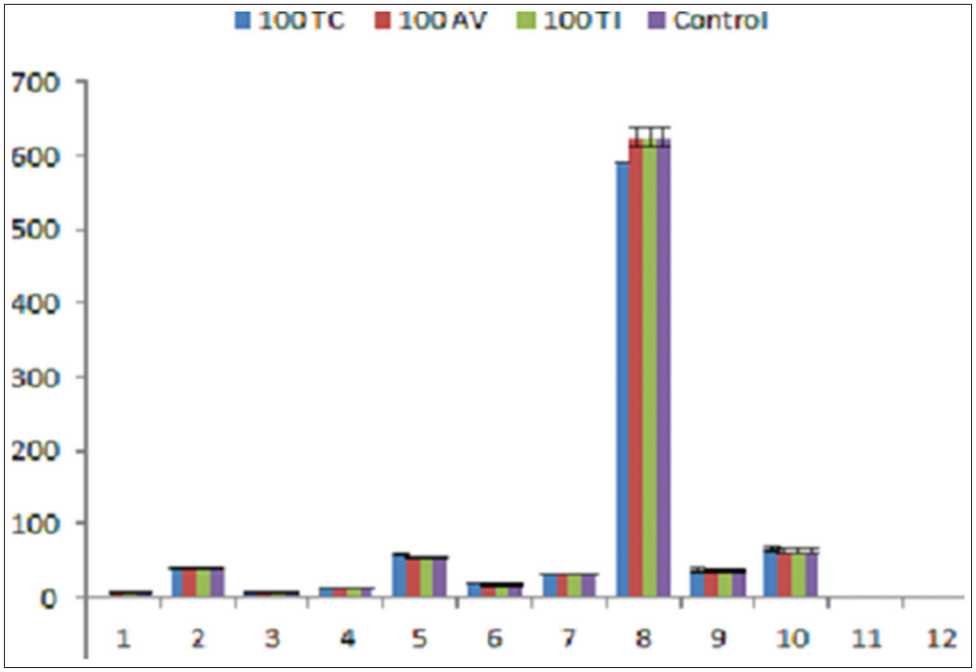

Figure 1: All parameters at 100 mg/kg body weight dose

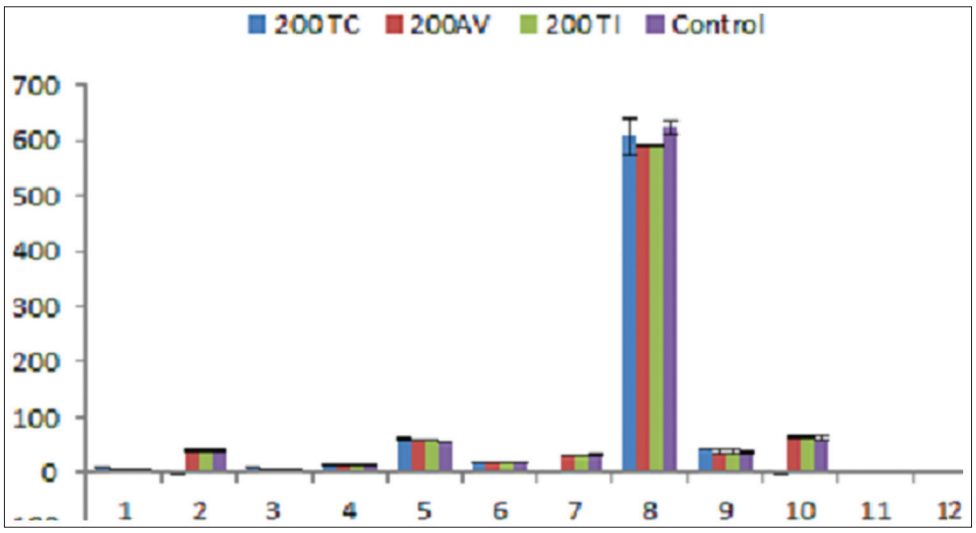

Figure 2: All parameters at 200 mg/kg body weight dose

Effect of the Extract on Hematological Parameters

Figures 1-3 show the effects of all the three plant extracts on the blood parameters during subacute study. Non-significant variation noticed in all other hematological parameters and persists within normal physiological norms after the 28 days treatment period.

Effect of the Extract on Biochemical Parameters

The effect on biochemical parameters is presented in Figures 4-6 after oral administration of subacute dose of all the three plant extracts. All the biochemical parameters were found to be normal levels in all the cases including control treatment.

Histopathological Studies

The histological investigation of the sections of liver tissue and kidney tissue of control and treated groups indicated a normal histological structure in Figure 7. No gross pathological lesions were observed even for higher dose treatment. It also showed no deformities in the color or texture when compared these organs in comparison to control.

DISCUSSION

Since long time, human being has used different medicinal plants. All over the world accepted for the management of different types of ailment.[28] For the evaluation of the therapeutically active plants for their activity and finding of

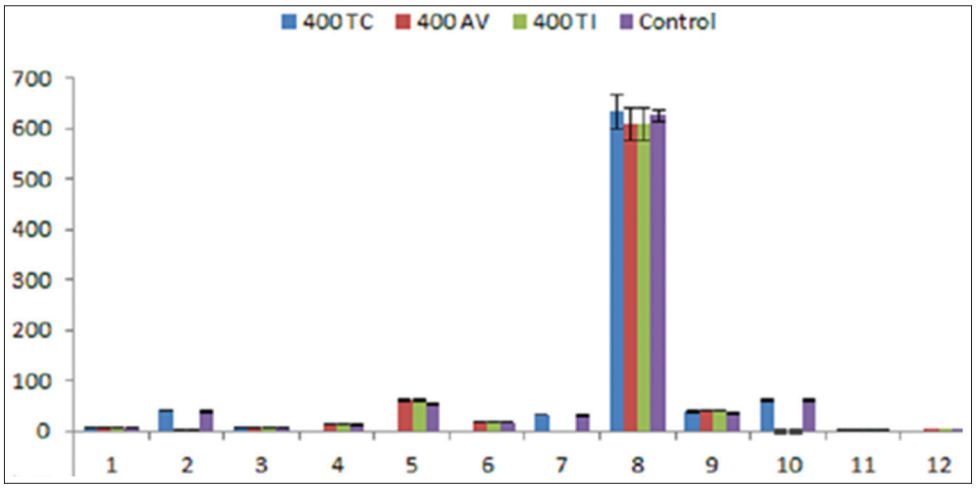

Figure 3: All parameters at 400 mg/kg body weight dose. White blood cells (×109/L), 2: Packed cell volume (%), 3: Red blood cells (×1012/L), 4: Hemoglobin (g/dL), 5: Mean corpuscular volume (fl), 6: Mean corpuscular hemoglobin (MCH) (pg), 7: MCH concentration (MCHC) (g/dl), 8: Platelet (×109/L), 9: Neutrophils (%), 10: Lymphocytes (%), 11: Monocytes (%), 12: Eosinophils (%), TC: *Terminalia chebula* Reitz., AV: *Aloe vera* L., TI: *Tamarindus indica* L., There was not significantly different in comparison to control at $P < 0.05$ and means ± standard error mean ($n = 6$)

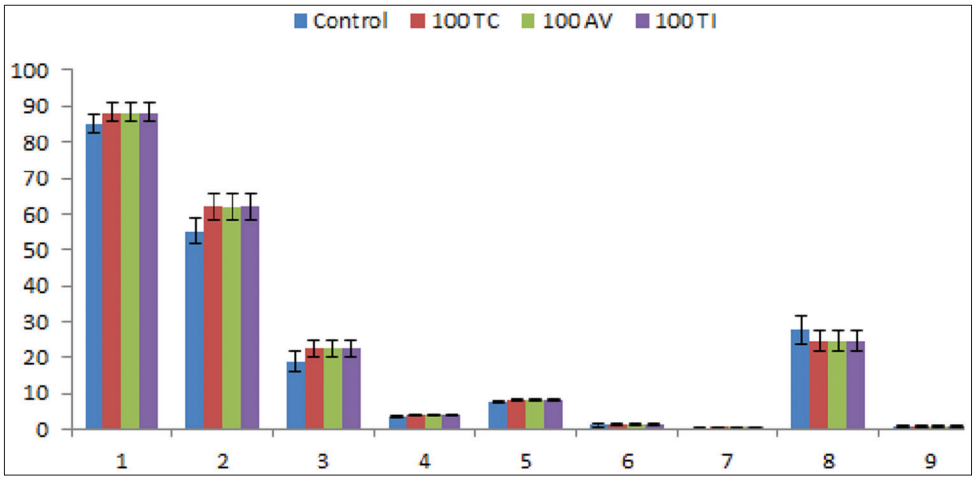

Figure 4: All parameters at 100 mg/kg body weight dose

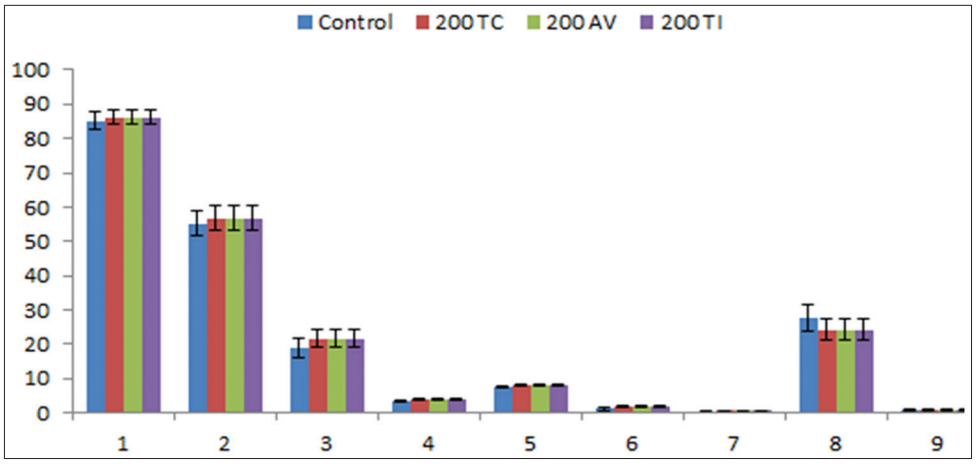

Figure 5: All parameters at 200 mg/kg body weight dose

any toxic effect of the substances is taken into consideration. Besides number of therapeutic usefulness of all the three plant extracts, a comparative knowledge related to the toxicological effect not been screened. So that, the acute and subacute toxicity of all three plant extracts in rats is conducted to rule out toxicological effects.

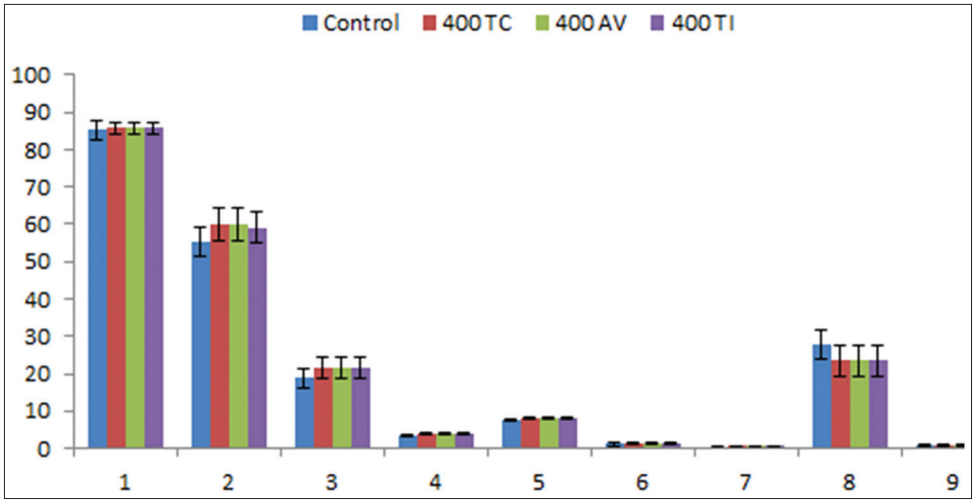

Figure 6: All parameters at 400 mg/kg body weight dose. Alkaline phosphatase (U/L), 2: Aspartate transaminase (U/L), 3: Albumin (mg/dL), 4: Total protein (mg/dL), 5: Total protein (mg/dL), 6: Total bilirubin (U/L), 7: Direct bilirubin (U/L), 8: Urea (mg/dL), 9: Creatinine (mg/dL), TC: *Terminalia chebula* Reitz., AV: *Aloe vera* L., TI: *Tamarindus indica* L. There was not significantly different in comparison to control at $P < 0.05$ and means ± SEM (n = 6).

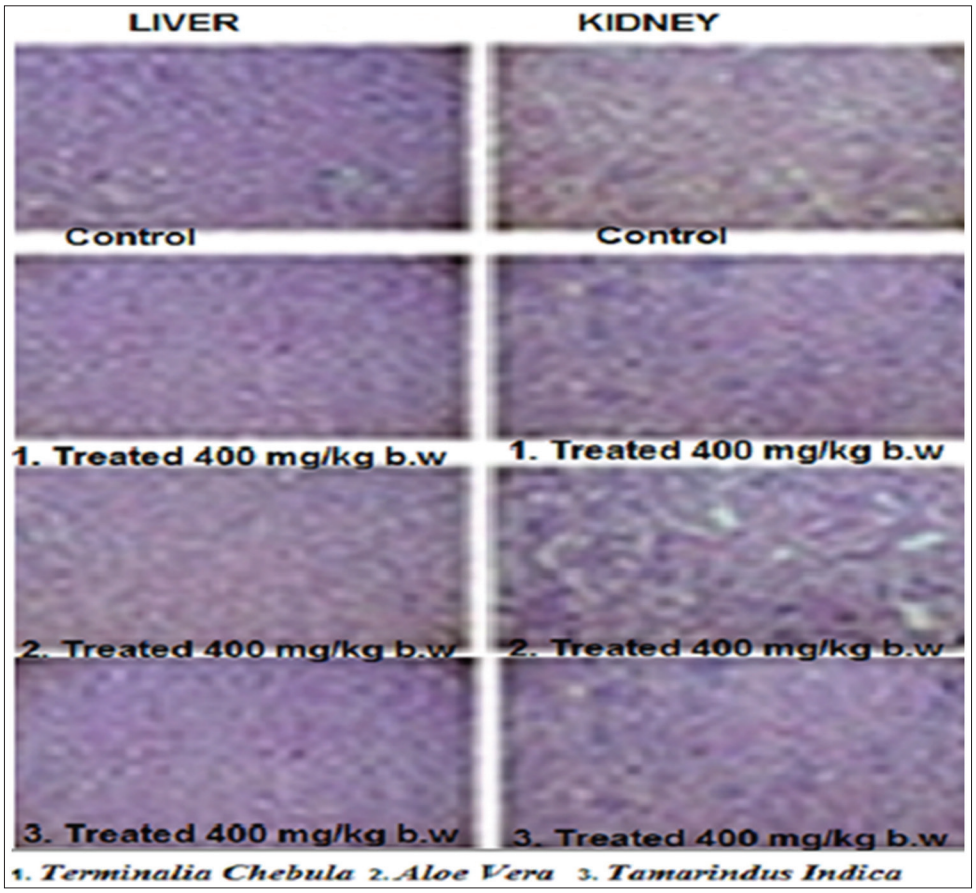

Figure 7: Histopathological investigation of liver and kidney in rat at subacute oral dose level of 400 mg/kg body weight

After oral treatment >5000 mg/kg, no any mortality was observed for all the three plant extracts. The food, water taken, and behavior were did not change throughout the study period in comparison to control group. It was also observed that the meal-taking behavior such as food and water was freely taken by the investigating animals. When exposed to all the three plants extract which recommend that the extract was did not caused any changes in food metabolism in these experimental animals. Hematological analysis was performed for all the three plant extracts treated and their outcome indicates no any significant effects when compared with control group. No any alterations in renal and hepatic functions were observed for any possibility of pathological changes after biochemical analyses of serum. Furthermore,

it was observed that slight increase in blood volume and hemoglobin content. These changes may be due to the nutritional substances in the extract as previously reported by other researchers also.[28]

The blood parameters evaluation usually carried out to find out the adverse effect of any foreign molecule, along with therapeutic constituents in plantsextract.[29,30,31] Meanwhile, it is also mandatory to examine as alteration in the blood composition. These alterations are mainly indicative to toxicological effect to human being. Here, results are derived from animal studies.[32] The liver and kidney function exploration is an important factor for the toxicological assessment of drug and plant extracts as it is well-known that both are vital organs for the survival of animal.[33] WBC is the indicator of defense which fights against infection, inflammation, or organ damages. Moreover, no any such countable alteration observed in the neutrophils, lymphocytes, and monocytes when studied for all the three plant extracts treated animals. All the three plant extracts were not displayed any countable alterations for all the biochemical values at different dose level. It means no toxicological effect on rats liver and kidney function.[34] The results of the present study indicated all the vital organs such as kidney, liver, lungs, spleen, and heart were not altered throughout the 28-day treatment period. Therefore, it could be concluded that all three plant extracts are not toxic to vital organs of the said groups in comparison to control group in rats.

CONCLUSIONS

Major outcomes from this present study suggest that all the three plant extracts do not having any such toxic effects which will lead to compromise their therapeutic application. Long-term use of these herbal drugs showed the effects which recommended all three plant extracts do not exhibit any toxic effects in terms of hematological and biochemical parameters of rats. The outcomes also indicated that *A. vera* extract may have immune system modulating effect. It too recommended that all the three plant extracts having defensive potential to hepatic and nephrotic cell. All our above findings indicated that all the three plant extracts not showed adverse effects and are non-toxic for therapeutic purposes.

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