IJPSR (2022), Volume 13, Issue 12 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 01 April 2022; received in revised form, 28 May 2022; accepted, 09 June 2022; published 01 December 2022

HEPATOPROTECTIVE ACTIVITY OF *RUNGIA PARVIFLORA* AGAINST THIOACETAMIDE INDUCED HEPATOTOXICITY IN WISTAR RATS

Santosh Kirtane [1], Vijendra Fulzele [*2], Vaibhav Uplanchiwar [3] and Sachin Hiradeve [3]

Noble College of Pharmacy [1], Parth Vatika, Bhesan Road, Bamangam, Junagadh - 362310, Gujarat, India.
School of Pharmacy [2], GH Raisoni University, Dhoda Borgaon, Tah. Saunsar, Chhindawara - 480106, Madhya Pradesh, India.
Nagpur College of Pharmacy Wanadongri [3], Hingna Road, Nagpur - 441110, Maharashtra, India.

Keywords:

Rungia parviflora, Acute toxicity, Hepatoprotective, Histopathology, SGPT, SGOT, Total Bilirubin, ALT

Correspondence to Author:
Vijendra Fulzele

Assistant Professor,
School of Pharmacy,
GH Raisoni University, Dhoda Borgaon, Tah. Saunsar, Chhindawara - 480106, Madhya Pradesh, India.

E-mail: vijendrabfulzele@gmail.com

ABSTRACT: *Rungia parviflora* belongs to the family Acanthaceae (Linn.). The dried and powdered leaves were extracted with ethanol in Soxhlet's apparatus for 12 hrs. The extract was subjected to toxicity studies at the doses of 5, 50, 300, and 2000 mg/kg under the observation of 24 hrs. An acute toxicity study of ethanolic extract was found to be toxic (1/2 of rats died) at a dose of 2000 mg/kg, orally. Hence, it was taken as LD_{50}. Further, the hepatoprotective activity of Ethanolic extract at the dose level of 250mg/kg and 500 mg/kg ware studied in Wistar male rats against thioacetamide (400mg/kg p.o.) induced hepatotoxicity. Silymarin 100mg/kg p.o. was taken as a standard hepatoprotective drug. The hepatoprotective activity was studied by determining the parameters like SGPT, SGOT, Total Bilirubin, ALT, Pentobarbitone induced sleeping time, Ascorbic acid content in urine, *in-vitro* Bromsulphalein uptake test and Histopathological observation of liver. Overall study indicated that ethanolic extract of leaves of *Rungia parviflora* at a dose level of 250mg/kg and 500mg/kg significantly protects the liver against hepatotoxicity induced by thioacetamide.

INTRODUCTION: Medicinal uses of many plants are yet scientifically proven even though their traditional uses have been mentioned for years. One of such plants, *Rungia parviflora* belongs to the family Acanthaceae. Traditionally it is used as aperient, febrifuge, Refrigerant, smallpox [1] as six spoonfuls of plant juice is taken three times a day [2]. The leaves are rubbed and extracted juice is applied to the wound and cut externally [3].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.13(12).4928-33

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.13(12).4928-33

Few drops of root extract together with honey are prescribed for fever, especially for children [4], and these traditional values direct toward the plant's medicinal potential. The current case was piloted to measure the liver protective capability of *Rungia parviflora* against the hepatotoxicity triggered by thioacetamide.

MATERIAL AND METHOD:

Plant Material: Leaves of *R. parviflora* were picked up in between the month November from "Girnar Forest, Junagadh town, Gujarat, India. The collected herb was acknowledged & legitimated by Prof. Vinod Kumar, Dept. of Botany, Rajasthan University, Jaipur, Rajasthan. "(Herbarium No. RUBL21134)"

Extract Preparation: The shelter dried leaves of *R. parviflora* were converted to courser particles and extraction was done using Soxhlet's apparatus using ethanol for 24 hrs. The solvent was removed from extract on a water bath and stored at 4°C. 8.49 % (w/w) yield of the extract was obtained.

Animals: Wistar male rats weighing 150 - 170 gm were selected for the experiment. Polypropylene cages were used to keep the animal in controlled temperature of 25 ± 2°C along with 45-55% humidity and twelve hours light and dark cycle. The pallet diet and water were provided to selected experimental animals and all tests were accompanied as per the "CPCSEA guidelines (Reg. No. 1239/a/08/CPCSEA)."

Acute Toxicity Study: According to OECD guideline No.425 [5], an acute toxicity trial was performed with a dose ranges from 5, 50, 300 and 2000mg/kg. The autonomical and behavioural changes were closely observed for 24 hours.

One out of two rats died when treated by 2000mg/kg, which was considered LD_{50}. Further 250mg/kg and 500mg/kg ($1/7^{th}$ and $1/5^{th}$ of LD_{50}) doses were selected for plant leaf extract's liver-protective efficiency.

Assessment of Hepatoprotective Activity:

Biochemical Analysis: Five groups of six rats in each were starved for twenty-four hours prior to Thioacetamide treatment as given below

Group I: Received normal saline 5mg/kg *p.o;* referred as normal control.

Group II - V: Treated with thioacetamide 400mg/kg.

Group II- Received only thioacetamide; referred as control group.

Group III and IV: Received 250mg/kg and 500mg/kg of ethanolic extract; referred as treatment group.

Group V: Received Silymarin 100mg/kg *p.o;* considered as a standard group. The experimental dosing was continued for 5 days with a normal diet and sufficient water, on 6^{th} day, all the animals were sacrificed to collect blood samples separately into centrifuge tubes and coagulated for thirty minutes. Biochemical analyses like SGPT (Serum Glutamate Pyruvate Transaminase), SGOT (Serum Glutamate Oxaloacetate Transaminase) [6], "Total Bilirubin [7] and ALP (Serum Glutamate Alkaline Phosphate) [8] were done by separating the serum by centrifugation at 3000 rotation per minutes from the blood sample. Simultaneously livers were collected and stored in 10% formalin solution for "Bromosulphalein uptake [9] and histopathological testing.

Pentobarbitone Induced Sleeping time: Five group of six rats in each were starved for twenty-four hours prior thioacetamide treatment as given below

Group I: Received normal saline 5mg/kg *p.o;* referred as normal control.

Group II – V: Treated with thioacetamide 400mg/kg.

Group II: Received only thioacetamide; referred to as control group.

Group III and IV: Received 250mg/kg and 500mg/kg of ethanolic extract; referred as treatment group.

Group V: Received Silymarin 100mg/kg *p.o;* considered as a standard group.

On first day, all the groups were treated with the drug and doses assigned to them and finally, after two hours received thioacetamide. On the second day, assigned doses were repeated along with Pentobarbitone sodium 40mg/kg *i.p.*loss of righting reflex was noted [10, 11, 12, 13].

2.3 Ascorbic Acid Content in Urine: In the first part of this study, all the animals in four different groups were retained in a metabolic cage with a regular diet and *ad libitum* to collect the urine samples in 5 ml oxalic acid. The amount of ascorbic acid in urine was considered as normal control. In the second part of the experiment, animals were divided in four groups and treated as:

Group I: Received Thioacetamide

Group II: Received Thioacetamide and ethanolic extract 250mg/kg.

Group III: Received Thioacetamide and ethanolic extract 500mg/kg**.**

Group IV: Received Thioacetamide and Silymarin 100mg/kg *p.o.*

After 7 days of treatment, urine samples were collected and tested for ascorbic acid content as per the modified method [14].

To 0.5 ml Urine sample, 0.5 ml DNPH reagent (2% DNPH) + 4% Thiourea in 9 N sulphuric acid was added and incubated for 3 hrs at room temperature.

Post incubation 2.5 ml of 85%, H_2SO_4 was added. Absorbance was measured by UV- visible spectrophotometer (Shimadzu 1800) at 530 nm) [15].

***In-vitro* Bromsulphalein (BSP) Uptake Test:** The Bromsulphalein uptake test is a supremely profound and trustworthy process to evaluate the functional position of the liver.

Bromosulphalein like bile, undergoes storage, breakdown and elimination by liver. Withholding the Bromosulphalein by the liver (uptake and retention) indicates abnormal functions of the liver caused by thioacetamide. Liver slices of 60mg weight were kept in icy- cold Phosphate buffer (0.2 M) at pH 7.4 containing 30µg BSP/ml at 38°C. The concentration of Bromosulphalein was determined at 580 nm spectrophotometrically (SHIMADZU 1800) [16, 17].

Histopathological Preparation: The slides for histopathological studies were prepared from the liver previously preserved in 10 % formalin.

The slides were prepared by using a microtome, stained suitably, and observed under a microscope for histological alteration caused by thioacetamide in each group.

Statistical Analysis: The mean ± SEM was measured for every parameter, and one-way analysis of variance (ANOVA) was done to assess total variations, followed by Dunnett's test. $P<0.05$ was measured as statistically significant when matched with the control group.

Observations:

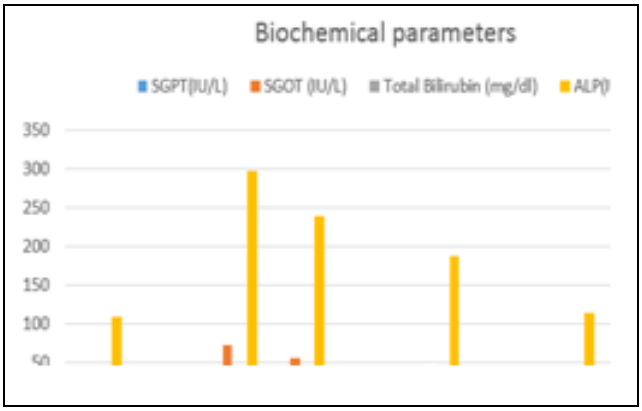


FIG. 1: BIOCHEMICAL PARAMETERS

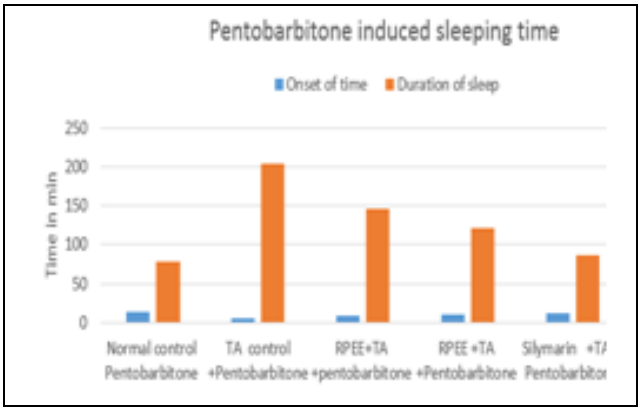


FIG. 2: PENTOBARBITONE INDUCED SLEEPING TIME

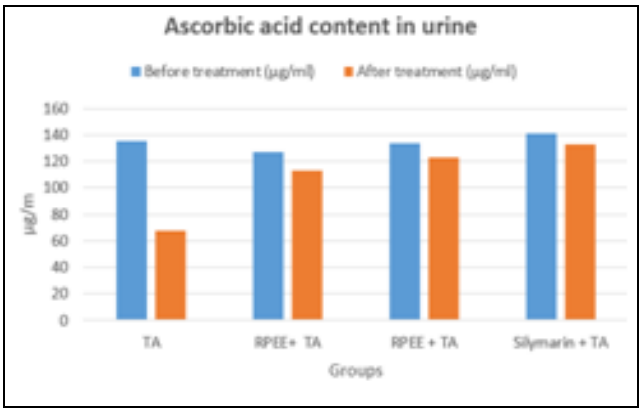


FIG. 3: ASCORBIC ACID CONTENT IN URINE

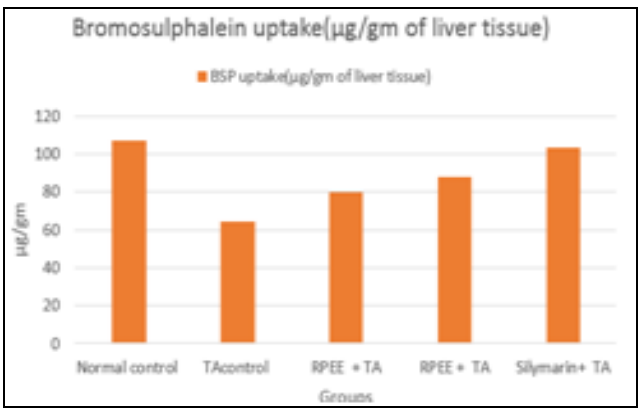


FIG. 4: BROMOSULPHALEIN UPTAKE

Histopathology:

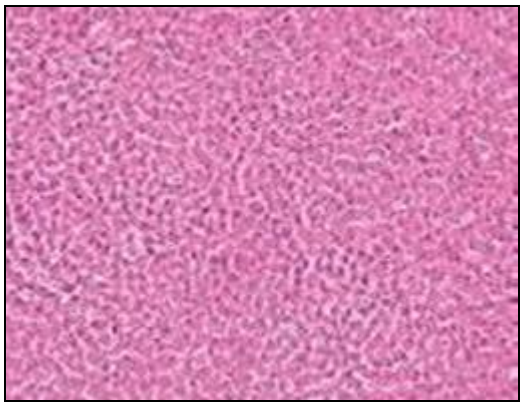


FIG. 2A: NORMAL CONTROL

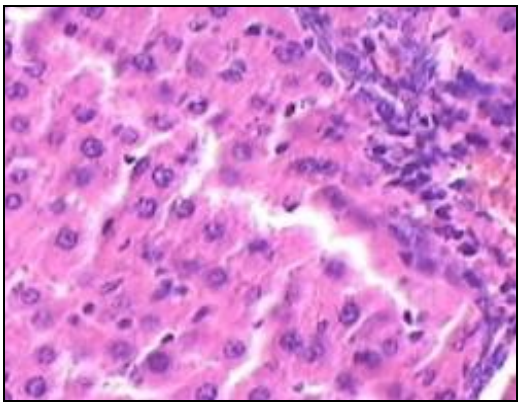


FIG. 2B: THIOACETAMIDE

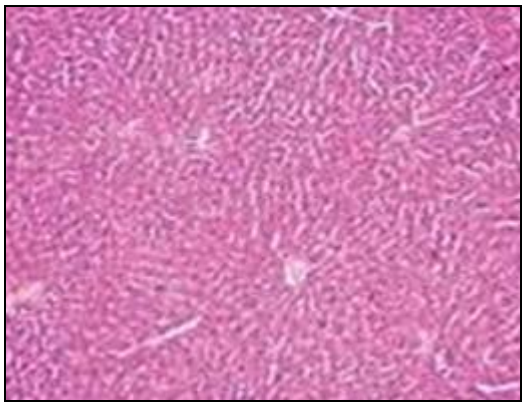


FIG. 2C: SILYMARIN + THIOACETAMIDE

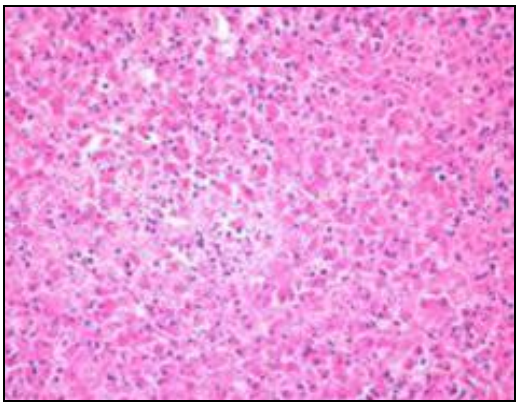


FIG. 2D: *RUNGIA PARVIFLORA* +THIOACETAMIDE

RESULT:

Biochemical Analysis: On the 5th day of the study, the blood from the thioacetamide-treated group showed a significant rise in SGPT, ALP, and SGOT concerning normal control groups. Prior treated groups with *R. parviflora* extract at 250 mg/kg & 500 mg/kg revealed a major reduction in SGPT, ALP and SGOT. The dose of 500mg/kg shows Maximum protection **Fig. 1.** As shown in Fig.1, there was a noteworthy rise in total Bilirubin levels in the rats exposed to thioacetamide as compared to control. The prior treated animals with *R. parviflora* extract showed a remarkable drop in total and direct bilirubin.

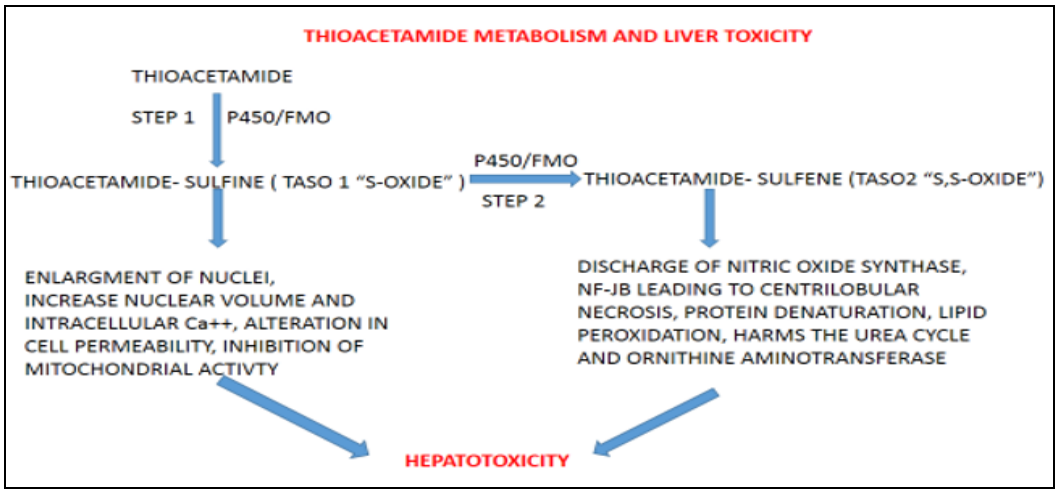
Pentobarbitone Induced Sleeping time: Ethanolic extract of *R. parviflora* showed significant liver protection (65.47%) by decreasing the napping duration of 122±3.12 min compared to 204.05.±4.64 min of thioacetamide (500mg/kg) control as shown in **Fig. 2.**

Ascorbic Acid Content in Urine: Ethanolic extract of *R. parviflora* showed considerable liver protective (82.75%) activity by preventing lessening in daily elimination of ascorbic acid in urine 123.33±1.38 µg/ml compared to 67.33±2.53 µg/ml of thioacetamide (500mg/kg) control as indicated in **Fig. 3.**

Bromsulphalein up take test: The ethanolic extract of *R. parviflora* treated liver slices showed statistical significant liver protective (55.75%) activity by 88.09±3.21 µg of BSP uptake per gm. of liver tissue related to 64.13±3.40 µg of thioacetamide control at 500mg/kg as shown in **Fig. 4.**

Histopathological Observation: The regular cellular structures with distinct central vein, sinusoidal spaces, and hepatic cells were detected in the control group **Fig. A**. Though, histological alteration like centrilobular liver cell necrosis, kupffer cells ballooning and generation, infiltrating lymphocytes and fatty changes were noticed in animals treated with thioacetamide groups **Fig. B**. While no clinical changes in liver structure were seen in groups treated with Silymarin drug and plant extract **Fig. C, D.**

DISCUSSION: *R. parviflora* at the dose intervals of 250mg and 500 mg orally is found to decrease the SGPT, SGOT, and ALT, which was raised by thioacetamide-induced hepatotoxicity. The finding of the study has resembled *Rungia repens* belonging to family Acanthaceae, *Rungia repens* shows hepatoprotective activity by inhibiting oxidative damage to the liver induced by CCl_4 [18]. *R. parviflora* reduces Pentobarbitone-induced sleeping time as it improves the function of the liver by preventing damage caused by thioacetamide. Thioacetamide metabolism and its role in hepatotoxicity are summarized in graphical chart below.



GRAPHICAL CHART: THIOACETAMIDE METABOLISM AND ITS ROLE IN HEPATOTOXICITY

As revealed by biochemical and histopathological studies, ethanolic extract of R. parviflora probably prevents liver damage. Ethanolic extract of *R. parviflora* retains the ascorbic acid by preventing its elimination in the urine. Ascorbic acid plays an important role in liver functions. Serum markers for hepatocyte damage such as alanine aminotransferase and aspartate aminotransferase increase in case of Vitamin C deficiency. The defective transport mechanism into the liver cell and impaired elimination or metabolism decreases the Bromsulphalein uptake [19, 20]. The ethanolic extract improves the uptake of Bromsulphalein by the liver cell as compared to hepatotoxicity caused by thioacetamide. The presence of centrilobular hepatic toxicity, fatty changes, and changes in Kupper cells in histopathological studies in thioacetamide treated group indicates liver toxicity which is absent in groups treated with the extract.

CONCLUSION: From the overall study, it is concluded that the doses of 250 mg/kg & 500 mg/kg of ethanolic extract considerably protect the liver against liver toxicity produced by thioacetamide. Further, the ethnobotanical and present study suggests that the mentioned plant can be explored for other pharmacological activities.

ACKNOWLEDGEMENT: The authors are thankful to Shree. Nilesh Dhulesia, Chairman, Noble Group of Institution, Junagadh, Gujarat, for providing the infrastructure to complete this project, and Mr. Prof. Vinod Kumar, Department of Botany, Rajasthan University, Jaipur, Rajasthan, who help us in identifying the plant.

CONFLICTS OF INTEREST: The authors whose names are listed immediately below certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

REFERENCES:

1. Khare CP: Indian Medicinal Plants. Springer, New York, NY 2007 https://doi.org/10.1007/978-0-387-70638-2 1389.
2. Taylor RSL, Edel F, Manandhar NP & Towers GHN: Antimicrobial activities of southern Nepalese medicinal plants. Journal of Ethnopharmacology 1996; 50(2): 97-102.

3. Shrestha PM and Dhillion SS: Medicinal plant diversity and use in the highlands of Dolakha district, Nepal. Journal of Ethnopharmacology 2003; 86(1): 81-96.
4. Saha D, Sarma TK and Mukherjee SK: Some medicinal plants of north 24 Parganas District of West Bengal (India). Int J Pharm Biol Sci 2016; 6: 191-206.
5. OECD (2002), Test No. 423: Acute Oral toxicity - Acute Toxic Class Method, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris
6. Rasyid SA and Lio TM: Analysis of serum glutamic pyruvic transaminase and serum glutamic oxaloacetic transaminase levels in tuberculosis patients who are undergoing oat treatment in Kendari City General Hospital, Kota Kendari, Indonesia. Infectious Disease Reports 2020; 12(1): 75-7.
7. Cao SY, Li BY, Gan RY, Mao QQ, Wang YF, Shang A, Meng JM, Xu XY, Wei XL and Li HB: The *in-vivo* antioxidant and hepatoprotective actions of selected Chinese teas. Foods 2020; 9(3): 262.
8. Arunachalam K and Sasidharan SP: Animal experiments of hepatoprotective activities. In Bioassays in Experimental and Preclinical Pharmacology Humana New York NY 2021; 157-166.
9. Srinivasarao M, Mangamoori Lakshminarasu TM and Ibrahim M: Research article hepatoprotective potential of total anthraquinone fraction of rheum emodi: *in-vitro* and *in-vivo* studies. Journal of Medical and Pharmaceutical Innovation 2015; 2(11): 15-22.
10. Begum R, Papia SA, Begum MM, Wang H, Karim R, Sultana R, Das PR, Begum T, Islam M, Manwar N and Rahman M: Evaluation of Hepatoprotective Potential of Polyherbal Preparations in CCl_4-Induced Hepatotoxicity in Mice. Adv in Pharmacological and Pharma Sciences 2022.
11. Chale-Dzul J, de Vaca RP, Quintal-Novelo C, Olivera-Castillo L and Moo-Puc R: Hepatoprotective effect of a fucoidan extract from Sargassum fluitansBorgesen against CCl_4-induced toxicity in rats. International Journal of Biological Macromolecules 2020; 145: 500-9.
12. Geresu GD, Umer S, Arayaselassie M, Ashebir G and Makonnen E: Hepatoprotective effects of crude stem bark extracts and solvent fractions of *Cordia africana* against acetaminophen-induced liver injury in rats. Canadian Journal of Gastroenterology and Hepatology 2022; 2022.
13. Liu M, Zheng B, Liu P, Zhang J, Chu X, Dong C, Shi J, Liang Y, Chu L, Liu Y and Han X: Exploration of the hepatoprotective effect and mechanism of magnesium isoglycyrrhizinate in mice with arsenic trioxide induced acute liver injury. Molecular Medicine Reports 2021; 23(6): 1-3.
14. Sajid M, Khan MR, Shah NA, Shah SA, Ismail H, Younis T and Zahra Z: Phytochemical, antioxidant and hepatoprotective effects of Alnus nitida bark in carbon tetrachloride challenged Sprague Dawley rats. BMC Complementary and Alternative Medicine 2016;16(1): 1-7.
15. Rather AH & Rao R: Evaluation of antioxidant and hepatoprotective activity of biomass and crude astaxanthin extract of green alga Haematococcuspluvialis. Research Journal of Pharmacy and Technology 2021; 14(4): 1933-1937.
16. Dwijayanti A, Frethernety A, Hardiany NS & Purwaningsih EH: Hepatoprotective effects of *Acalypha indica* and *Centella asiatica* in rat's liver against hypoxia. Procedia Chemistry 2015; 14: 11-14.
17. Ahmed AA & Fatani AJ: Protective effect of grape seeds proanthocyanidins against naphthalene-induced hepatotoxicity in rats. Saudi Pharmaceutical Journal 2007; 15(1): 38.
18. Jeffery GH, Bassett J, Mendham J, Denny RC, Murugesan T, Manikandan L, Suresh KB, Pal M, Saha BP, Bauer AW, Kirby WM. Sür-Altiner D, Gürkan E, Mutlu G, Tuzlaci E and Ang Ö: The antifungal activity of *Pancratium maritimum*. Fitoterapia 1999; 70: 1879. J Pharm Sci. 2000; 62: 150-.
19. Kim JH, Jeong YJ, Hong JM, Kim HR, Kang JS, Lee WJ & Hwang YI: Chronic vitamin C insufficiency aggravated thioacetamide-induced liver fibrosis in gulo-knockout mice. Free Radical Biology and Medicine 2014; 67: 81-90.
20. Maddison JE and Page SW: Adverse drug reactions. Small Animal Clinical Pharmacology 2008; 2: 41-57.

How to cite this article:
Kirtane S, Fulzele V, Uplanchiwar V and Hiradeve S: Hepatoprotective activity of *Rungia parviflora* against thioacetamide induced hepatotoxicity in Wistar rats. Int J Pharm Sci & Res 2022; 13(12): 4928-33. doi: 10.13040/IJPSR.0975-8232.13(12).4928-33.