

Antioxidant and hepatoprotective potentiality of *Trapa natans* L. leaf extract in liver damage

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ABSTRACT

The antioxidant and hepatoprotective potentialities of the methanolic extract of *Trapa natans* L were investigated in this study. The DPPH, H₂O₂, and phosphomolybdate test methods were used to assess antioxidant activity. The paracetamol-induced hepatotoxic model in rats was used to investigate the hepatoprotective potentiality. The level of liver biochemicals such as ALT, AST, bilirubin, and total protein was examined to determine hepatoprotective potential. The *Trapa natans* L. (T.N) extract in different dose (200 & 400. mg/kg) were induced once a day orally. Serum enzymes like AST, ALT, ALP, and total bilirubin were detected for the animal treated in the test drug (T.N extract). Silymarin, a widely used drug used for liver damage that significantly protects the liver from damage caused by paracetamol, has been used. The histological analysis of rat liver reinforces the biochemical investigation. From the *in-vitro* and *in-vivo* antioxidant study, it was concluded that the plant extract has a potent free radical scavenging capacity. This investigation conclusively demonstrated that the *Trapa natans* L. plant extract has an excellent hepatoprotective effect in paracetamol-induced hepatotoxic rats. The free radical inhibition potentiality of polyphenolic chemicals found in the plant extract could be the mechanism of action of *Trapa natans* L.

Keywords: *Trapa natans* L, antioxidant potentiality, liver protective effect, serum enzyme

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1. INTRODUCTION

The liver, which is the largest organ in the body, is crucial to digestion and metabolic function. The liver cell, liver tissues, and liver structure were affected when the liver was affected by the hazardous chemicals. The number of patients with liver disorders is steadily rising today; according to the report, approximately 2 million people die worldwide from liver problems each year (17). Reactive oxygen species or free radicals are thought to be the primary cause of a number of diseases, according to numerous research studies. One of the most typical causes of liver disease or damage to the liver is ROS, which is produced when the liver is exposed to unsafe substances or when it is under excessive stress. Although paracetamol is a commonly used drug, a high dose and long could damage the liver (18). The liver converts paracetamol to the reactive metabolite (N-acetyl-p-benzoquinone imine), which forms a covalent link with cellular proteins that destroy liver cells (4).

People have relied on herbal medicine for their health care system since ancient times. Still, 80% of the developing world's population relies on herbal remedies. By addressing the many negative aspects of synthetic medicine, developed countries have been encouraged to use natural medicine. Due to their few side effects, low cost, and high potential, herbal medicines are becoming increasingly popular in the treatment of liver diseases. It has been noted that herbal medicines with antioxidant potential are good hepatoprotective agents, and this has been supported by numerous *in-vivo* and *in-vitro* studies (14).

T. natans is a water plant that can be located all throughout India. The drug is used to treat liver disease, kidney-related problems, and stomach disorders in conventional medicine. All of this plant's parts contain substantial quantities of flavonoids (11). This plant is rich in polyphenols like, p-hydroxybenzoic acid, coumaric acid, caffeic acid, gallic acid, p-hexoside, as well as flavonoids like quercetin, morin, naringenin, rhamnetin, and pinobanksin. It also contains flavonoid glycosides like naringenin-7-O-hexoside, rutin, and kaempferol which have more bioavailability than that flavonoids (3,7). It has been found that substances containing significant amounts of polyphenols and flavonoids tend to block free radicals and act as powerful antioxidants, making them potent hepatoprotective agents (13). Due to the abovementioned reason, we decided to use this plant in our investigation. In this investigation, biochemical analysis *in-vitro*, *in-vivo*, as well as histological examination were carried out to examine the antioxidant and hepatoprotective effects of *T. natans* extract in a paracetamol-induced oxidative stress paradigm.

2. MATERIALS AND METHODS

All of the are analytical-grade chemicals perches from SRL and standard medication silymarin acquired from Sigma-Aldrich. Plant parts were collected in the morning of July 2017 from Dadri, Uttar Pradesh. After proper plant material washing, the leaves were separated out and shade dried for 15 days while maintaining a relative humidity of 31 to 40%. In order to select the solvent, a series of extractions were carried out gradually raising the solvent's polarity. Methanol has been selected as a suitable solvent based on the extract's stability and yield percentage. The power drug was extracted with methanol in hot percolation method, and evaporated by the rotary evaporator.

Using the technique detailed by Maizura et al., (2011) the total phenolic content of the extract was established, which was crucial for its antioxidant activity (12).

2.1. Experimental animal

The male Wistar albino rats utilized in this examination had an average weight of 200 ± 6.9 g. Animals were obtained from the Central Animal Facility, AIIMS, Ansari - Nagar, New Delhi, India, with permission from the institutional animal ethics committee (Protocol no - IAEC/N.I.E.T/2018/05/03).

2.2. DPPH assay method

For the preparation of DPPH stock solution (SS), DPPH (4 mg) was dissolved in methanol (100 mL). The SS was subsequently preserved at 20°C in a dark area for one hour. *T.N.* plant extracts were subsequently incorporated with 5 mL of the SS at different strengths (10 to 100 g/mL). The produced mixture had been kept in darkness for a further 15 minutes, and then the absorbance at 517 nm wave-length was recorded. using the standard drug, ascorbic acid (16).

2.3. Hydrogen peroxide assay method for determination of antioxidant potentiality

The scavenging power of plant extract (*T. natans*) against hydrogen peroxide was measured by the method Yen and Duh (2000). The solvent used to create the H₂O₂ solution (40 mM) was phosphate-buffer (pH 7.4) and absorbance was determined at 230 nm wavelength with the help of UV spectrophotometer. The of plant extract solution prepared in water in the concentration range (10 -100 µg/mL) was added to the previously prepared H₂O₂ solution and preserved in the shadow for at least for 15 minutes duration. The absorption was measured at 230 nm considering the phosphate buffer (pH 7.4) as blank and using ascorbic acid as the standard compound (10,20).

The free radical scavenging capacity was determined following the given calculation: Percentage of inhibition = $(1 - \text{Absorption of sample} / \text{Absorption of control}) \times 100$

2.4. Determination of antioxidant potential of the plant extracts using the phosphomolybdate assay method

The total antioxidant capacity of each of *T. natans* extracts was evaluated by the phosphomolybdenum assay method. The plant extract solution was prepared using distilled water in the concentration range 10 - 100 µg/mL and was mixed with a phosphomolybdate mixture. The combination was kept in a closed-mouth test tube and incubated for 90 minutes at a temperature of 95°C. After cooling, the value of absorbance at 765 nm was estimated using ascorbic acid as the reference substance (1).

2.5. Hepatoprotective activity of the *T. natans* extract

The animals have been split into five sections, every six animals, for hepatoprotective activity. Paracetamol was given to selected animal once at a dose of 3 gm/kg to test for hepatotoxicity. Before administering the hepatotoxic drug, batch -1 and 2 of the experimental rats received water (2 ml/kg), batch -3 received silymarin (30 mg./kg), and batch-4 and 5 received T.N extract (200 & 400 mg./kg) correspondingly. The above mention treatments were provided to the animals for a period of nine days. On the ninth day, every batch received paracetamol p.o. with one exception of batch I. For 12 hours earlier paracetamol was given, and food was restricted. The rats were killed by cervical dislocation, which occurred 24 hours after ingesting paracetamol (8).

For the biochemical analysis, obtained blood from the animals being studied was stored in a dry and clean tube was centrifuged for a period of ten minutes at two thousand revolutions per minute. The serum residual sample was then put into the Eppendorf container. Using a readily accessible biochemical analyzer in the kit (Roche -Diagnostics-GmbH, Mannheim, Germany), a biochemical study was conducted using a biochemical analyzer obtained from Microlab, model no MC300 for the measurement of the enzymes in the liver such as AST, ALT, ALP, and serum-bilirubin (19,6).

The liver from the treated animal was taken out and retained in freshly prepared 10% formalin solution for histological investigations. After the liver tissues were adequately embedded in the melted paraffin, sections were cut using a microtome to a thickness of 5–6 μm . Every section of the liver tissues was stained with the staining agent (hematoxylin & eosin) and covered with a cover slip for optimal visualization. The prepared slides were examined under a light microscope at a magnification of 40X for histopathological change. All of the collected experimental results were provided as mean \pm SED. Graph-Pad- Prism-5 software was applied to analyze the data statistically, first using one-way ANOVA and then Dunnett's test. At $p < 0.05$, statistical significance was recognized.

3. RESULTS AND DISCUSSION

The percentage yield of TN extract was found 20.5%. The total-phenolic contents of the methanolic extract of TN were found 55.25mg/g GAE.

The percentage of DPPH inhibitory activity increased in dependence upon the dose in the case of the test drug (*Trapa natans* L) and the standard drug (Ascorbic Acid) in the range of 10-100 g/ml and the median inhibitory concentration (IC_{50}) of standard drug and the *T. natans* L. extract were found respectively 38.59 \pm 2.15 $\mu\text{g/ml}$ and 70.15 \pm 3.15 $\mu\text{g/ml}$. In the H_2O_2 assay method, IC_{50} value of ascorbic acid was found 41.98 \pm 2.15 whereas the IC_{50} value of *T. natans* L. extract was founded 69.28 \pm 3.5. In the case of the phosphomolybdenum assay method, the IC_{50} value of ascorbic acid was found 45.12 \pm 2.1 whereas the IC_{50} value of *T. natans* L. extract was found 81.35 \pm 3.5.

The level of liver enzymes rises in people with fatty liver illnesses, when the liver is affected by a specific hazardous chemical, or when the liver is affected by a viral infection. Therefore, the liver enzyme level was evaluated for the test of any liver condition. It was found that when the liver is damaged, in the blood AST and ALT enzyme levels rise (5,9). After paracetamol is introduced, the liver was affected, which causes an abnormal rise in the amount of liver enzymes. On the other hand, it was additionally found that the level of total protein was reduced when hepatotoxic medications were introduced. The liver produces a variety of proteins, however when the liver is affected by something, the protein synthesis is interrupted. As a result, the level of ALT, AST, and ALP was found to be high and the amount of total protein was decreased in the negative control group.

Silymarin was used as the standard treatment in this experiment because it has a strong antioxidant capacity and can successfully hinder the damaging radical moiety. Silymarin has the ability to protect the liver's outer membrane from toxins, stabilise the liver's cell walls, and promote liver cell regeneration (15).

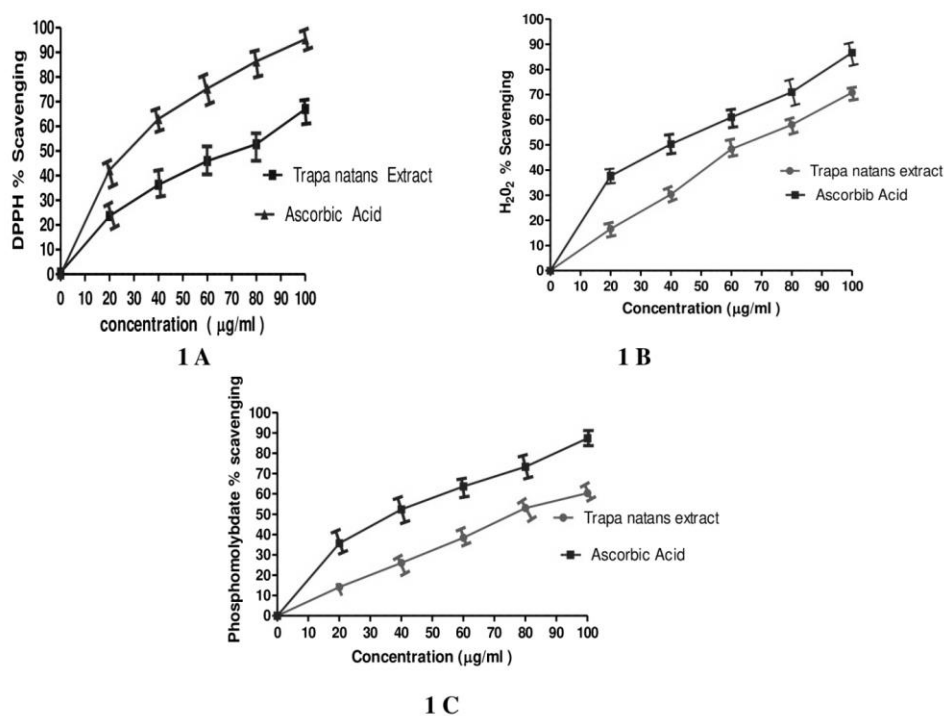


Figure 1. (1A) DPPH radical scavenging activity (1B) Hydrogen peroxide (H₂O₂) radical scavenging (1C) Phosphomolybdate scavenging capacity of *Trapa natans* L. extract compare to the standard compound ascorbic acid. The results presented mean \pm SEM where n=3acid. The results presented mean \pm SEM where n=3

In our study, it was determined that the group who took silymarin had much lower levels of liver enzyme than those who received any conventional medication. The test TN extract has a very good antioxidant- potential as this drug has lots of polyphenolic compounds. When the animal received treatment with TN extract at a dose of 200 mg/kg, the levels of ALT, AST, and ALP were determined to be 95.00 ± 1.42 , 203.6 ± 5.19 , and 124 ± 2.21 correspondingly. The liver enzyme levels for the test drug in high doses (400. mg/kg) were 77.60 ± 3.14 , 173.0 ± 3.92 , and 87 ± 1.91 . As a result of this, it came to light that a higher dose has a better effect.

The normal hepatic cellular structure was visible over the majority of the tissue segment in the normal control group (only water 2ml/kg), with a clear visible nucleolus. In the negative control groups rats liver, major fatty change, cell necrosis in some locations, increased sinusoidal gaps that were possibly filled with blood or edematous fluid, and elimination of cellular walls were found (treated with simply paracetamol).

In the model control groups rats liver, massive fatty change, cell necrosis in some areas, enlarged sinusoidal gaps that were either filled with blood or edematous fluid, and loss of cellular boundaries were identified (treated with only paracetamol). The histogram of the liver in the control group (silymarin 30 mg/kg) showed natural cellular and

Table 1. Effect of *T. natans* extract on liver biochemicals

Study Group	level of liver biochemical				
	ALT-(U/L)	AST-(U/L)	ALP-(U/L)	Total Bilirubin (mg/dl)	Total Protein (mg/dl)
Batch I (Control Group)	40.90±1.63	132.0±2.36	96.60±1.89	0.34±0.018	6.72±0.12
Batch II (Negative Control)	214.0±1.31	298.0±6.12	136.0±1.87	0.62±0.007	5.51±0.12
Batch III (Standard Drug)	74.20±1.45***	145.2±3.19***	90 ±1.91***	0.53±0.01***	7.15±0.32***
Batch IV (TN extract 200 mg/kg)	95.00±1.428** *	203.6±5.19***	124±2.21***	0.41±0.005***	6.2±0.25***
Batch V (TN extract 400 mg/kg)	77.60±3.14***	173.0±3.92***	100±2.19***	0.47±0.005***	6.82±0.17***

Results are represented as mean ± SME, where n=6; ** $p < 0.05$; *** $p < 0.001$ compare with model control

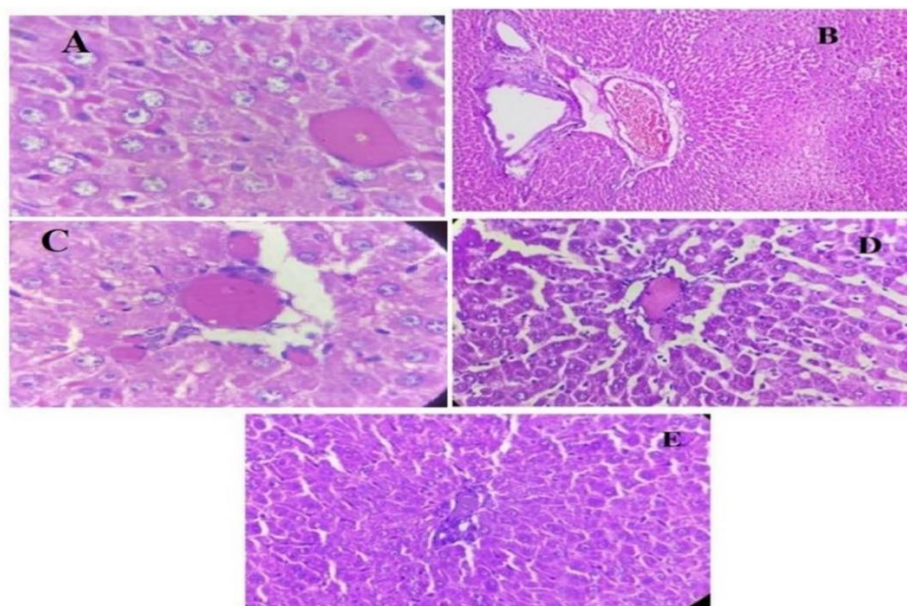


Figure 2. A. Liver section of normal control B. Model control, C. Standard (treated with silymarin 30 mg/kg) D. Test group (Treated with *T. natans* extract - 200 mg/kg). E. Test group (Treated with *T. natans* extract - 400 mg/kg)

architectural structure throughout the majority of the tissue segment, with moderate fatty changes in some areas. Hepatocyte binucleations were also seen in a few cases. The group given *T. natans* L. extract (200 mg/kg) showed numerous changes in the liver tissue, including bile duct hyperplasia and enlarged sinusoidal gaps filled with blood or edematous fluid (2). The liver histology of the group treated with TN extract (400 mg/kg) showed a prominent cell structure, there was no change in the cellular arrangement, and the outer layer of the cell wall normal was normal. Mild fatty alterations in hepatocytes were found in a few multifocal areas, combined with increased sinusoidal space and congestion.

4. CONCLUSION

The above experimental results have shown that the methanolic extract of *Trapa natans* possesses very good hepatotoxic activity which may be due to the hepatoprotective potentiality of the methanol soluble active phytoconstituents mainly polyphenolic and flavonoid compounds. However, more research with *T. natans* is needed to understand the cellular mechanism as well as to determine the structural components of active substances in order to standardize the plant. Selectivity for cytochrome P450 inhibition should also be examined.

5. ACKNOWLEDGMENT

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6. AUTHOR CONTRIBUTION

In the present research article, C. Majee performs the lab work and analyses the result, R. Mazumder and A.N. Choudhary designed the experiment, analyze the data and help in writing the paper. All authors read and approved the final manuscript.

7. ETHICAL APPROVAL

For animal studies the approval of the animals for this experiment obtained was obtained from the institutional animal ethics committee and the Protocol no was IAEC/N.I.E.T/2018/05/03.

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