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# A pharmacological audit and advancement on the recent trend of research on *Ficus benghalensis* L. including its in vitro hepatoprotective activity

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## Abstract

**Background:** Trend analysis, graphical representation, and summarization of pharmacological research trends were carried out to act as guidance for the future. The main objectives of the research are to find out the complete research trend on the national tree of India so far and to validate its traditional uses along with the pharmacological hepatoprotective activity of *Ficus benghalensis* L. by developing three in vitro experimental models.

**Methods:** The fruit of the said plant (*F. benghalensis*) was extracted with different solvent (petrochemical ether, chloroform, ethyl acetate, ethanol, water) system and the yield value was determined. Phytochemical screening was also done with a different solvent. In an in-vitro study, the liver of freshly slaughtered goat (Capra Capra) was used for various investigational tests, and the hepatotoxicity was induced by carbon tetrachloride ($CCl_4$) at a dose of 2 ml/kg, Acetaminophen at a dose of 7 g/kg, and with Erythromycin at a dose of 1.4 g/kg. The ethanol extract of fruits of *F. benghalensis* at the doses of 100 mg/kg, 250 mg/kg, and 500 mg/kg were used to observe its hepatoprotective effect against drug/chemical-induced in vitro hepatotoxicity as the model developed here with against a standard molecule, Silymarin.

**Results:** Amongst all solvents, ethanol was considered to be a universal solvent and resulted in a yield of 2.96%, which is maximum. Phytochemical screening of ethanol extract of fruit also showed the presence of alkaloids, steroids, flavonoids, carbohydrates, and glycosides. The protein concentration of liver homogenate based on comparison with standard protein concentration was found to be 1.6 mg/mL as measured at $\lambda_{max}$ of 750 nm. About the protein concentration, catalase (enzymatic) activity was also measured using the standard curve of $H_2O_2$, to calculate the specific activity of different models to compare the study results.

**Conclusion:** The significant effect of the reduction of hepatotoxicity was found at a dose of 500 mg/kg of fruit extract against Silymarin. Evaluation of Hepatoprotective activity of fruit in terms of catalase activity with different models flourishing the new research scope to fulfill the shortage of availability of a new, efficient, safe hepatoprotective agent in upcoming days.

**Keywords:** *Ficus benghalensis* L., Phytochemical screening, In-vitro model, Hepatotoxicity, Catalase activity

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## Highlights

- Analysis and summarization of pharmacological research trends on *Ficus benghalensis* L. with time.
- Fruit of *F. benghalensis* was extracted with different solvent systems and phytochemical screening was performed to confirm the basic chemical moiety.
- Hepatoprotective activity was evaluated based on different chemical/drug-induced goat liver models.

**Statement of novelty:**

Graphical representation with trend analysis and summarization of pharmacological trends will act as a guide for the future, and further development was carried out using phytochemical screening/analysis with different solvent extracts of fruits, as well as evaluation of Hepatoprotective activity following the biological model will lead to the specific extraction of phytochemical constitutes, the basic skeleton of a chemical moiety, identification of lead molecules, structure halogenation, pharmacological activity and therapeutic molecule(s).

## Introduction

The liver is the largest organ in humans; even for animals also liver is considered to be the largest organ. Physiologically the key role of the liver is to detoxify (metabolic) any materials including foods, drugs, and any other toxic components, which has been administered into the body either purposefully or unintentionally. Its metabolites the clone into a more polar or soluble component(s) which can be conveniently evacuated by excretion other than omission mentioned distinctly (non-hepatic site of metabolism as well as components excreted by other means like, bile, sweat, etc.) [1].

Toxicity cajoled liver injury when formed by the drugs and chemicals may virtually mimic any mode of the naturally developing liver disease(s) including iatrogenic disease. The harshness of hepatotoxicity is enormously expanded if the drug is pursued after symptoms development. Medicinal operators both from natural and synthetic sources are large statistics used as therapeutic operators in our day-to-day activity which may cause Predictable or Unpredictable including acute and continual liver disease [2]. Besides, the organic agents or operators and chemicals, Reactive Oxygen Species (ROS) can be achieved by all aerobic organisms from both sources related to exogenous and endogenous [3].

ROS, as described above, are usually produced by cellular metabolism and are capable of damaging biomolecules, distressing immune response, and also influencing the aging process [4].

As the liver is muddled in various biochemical reactions and metabolism processes, thus, its highly adhered to by this type of free radicals or ROS, which attribute to cell necrosis or hepatotoxicity. Furthermore, the dominance effect of ROS generation over natural antioxidant aegis systems like catalase, etc., and oxidative stress [5]. These effects lead to a comprehensive incremental in the intracellular ROS level.

Alluringly, it had been recorded [6] from time to time by epidemiological examination that hepatotoxicity may be interrupted or cured by the utilization of phytonutrients or phytochemicals enriched plant(s) having potential anti-oxidant properties. Limitation of the authorities of synthetic chemicals as the hepatoprotective agent had persuaded motivation for the concoction of any potential lead substance from natural resources with the least toxicity or adverse effects. The use of plants as medicinal agents has been started in ancient. On other hand, the conventional use of medicinal plants enlightens scientists or researchers to work hard in certifying the use, scientifically. Amongst the 2000 species of plants, many have been left for their scientific legalization. Although, they have been used traditionally or therapeutically among most of the common population for a long period.

Traditionally, all parts of the *F. benghalensis* L. (Moraceae) are used as astringent, acrid, sweet, refrigerant, anodyne, vulnerary, depurative, anti-inflammatory, ophthalmic, styptic, anti-arthritic, diaphoretic, anti-diarrheal, anti-emetic, and tonic [7]. It's also used in Ayurveda for the treatment of diarrhea, dysentery and piles, teeth disorder, rheumatism, skin disorders like sores and to boost the immune system and hypoglycaemic [8].

Pharmacological appraisal for different extracts of various parts of *F. benghalensis* already investigated and showed its anti-oxidant activity in in-vivo [9] and in-vitro model [10], but so far as our knowledge goes, the ethnomedicinal use of the fruits of *F. benghalensis* as the hepatoprotective agent has not been investigated scientifically by experimental model. Therefore, the present investigation was designed or performed to determine the hepatoprotective activity of the ethanol extract of fruits by developing a biological model.

### Plant description

Indian Banayan tree, *Ficus banayan*, *Ficus Indica*, vada tree, all are the synonyms of *F. benghalensis* L [11]. It consists of the entire plant (Seeds, Fruits, Leaf, Bark, Steam, Latex, and Root) of *F. benghalensis* L. and belongs to the Moraceae family [12]. The genus *Ficus* is made up of about 1000 species from pan-tropical and sub-tropical origins [13] and all are woody, ranging from trees and shrubs to climbers [14].

## Research trend on pharmacological activity

As being a national tree [15], different parts of '*F. benghalensis* L.' have lots of potential character and

properties. The detailed usage from ancient times as conventional medicine and modern research applications are enlisted in Table A1. With time, researchers and the modern research era were in full phase to explore and validate the traditional usage. Moreover, the current research trend also indicates the depth of focus on this. For better understanding, we have made a break up for this research trend in two possible ways, initially 'part of plant' and secondly, 'type of pharmacological activity' wise.

In the first case, observation and research visions were more focused on bark, root, and leaves and mostly studied too, almost ` ~ 30%, 26%, and 27% of research works were based on these parts. Whereas, fruit (~ 7%), latex (~ 5%), seed (~ 1.4), and whole plant extract (~ 2.5%) were studied less competitively to evaluate the pharmacological activity.

In the very next case, maximum research activity was performed to evaluate, anti-oxidant activity, followed by anti-microbial, anti-diabetic, anti-inflammatory, anti-cancer, immunomodulatory, analgesic, wound healing, anti-stress, and anti-arthritic activity. Further, pharmacological activities likes, anxiolytic, antidepressant, memory enhancement, anti-allergic, anti-coagulation, anti-diarrheal, anti-helminthic, anti-HIV, anti-pyretic, anti-rheumatic, anti-tumor, cholinesterase inhibition, cytotoxic effect, hepatoprotective, anti-hypertensive, larvicidal, neuro-protective, pain management, renal impairment, seizure, and anxiety were studied less by the scientist.

## Materials and methods

### Plant collection and identification

The fruits of *F. benghalensis* were collected from the roadside at Sugandha, Hooghly. The collection was made in August 2015. The identification and authentication were done by a Taxonomist of the Botanical Survey of India, Shibpur, Howrah, India, and a voucher specimen (Herbarium No. HS-2/BST-15), the certification is shown as supplementary as A1. and deposited at the host institution for future references.

### Instruments and chemicals used to perform the study

Chemicals and reagents used for the current investigations were laboratory graded. Ethanol, mercuric chloride, ferric chloride, glacial acetic acid, pyridine, sodium chloride, potassium dihydrogen phosphate, disodium hydrogen phosphate, sodium bicarbonate, sodium hydroxide, tween 80, and hydrogen peroxide (30%) were obtained from Merck & Co, US. Petroleum Ether, chloroform, ethyl acetate, hydrochloric acid, and sulphuric acid were purchased from Quest Chemical Corporation, India. Bismuth nitrate, picric acid, biuret reagent, ninhydrine solution (2%), carbon tetrachloride, and potassium dichromate were procured from Sigma Aldrich, India. Tartaric acid, α-naphthol, and gelatin were obtained from Sisco research laboratories Pvt. Ltd., India. Dichloromethane, potassium iodide, iodine, ammonia solution, potassium chloride, and calcium chloride were procured from Finar Limited, India. Zinc powder, bovine serum albumin, lowry reagent, folinciocalteu's phenol, sodium nitroprusside, lead acetate (10%), and magnesium sulfate were procured from Thermo Fisher Scientific, Fisher Scientific, and Loba Chemie of Indian division respectively. Magnesium turnings, glucose, were obtained from Spectrum Chemical Manufacturing Corporation and HiMedia Laboratories.

Penicillin (Pencom 12 Inj.), Gentamycin (Gentamicin), Fluconazole (Forcan), Paracetamol (Calpol), Erythromycin (Erythrocin), Silymarin (Silybon) were manufactured by Alembic Pharmaceuticals Ltd., Abbott limited, Cipla Limited, GlaxoSmithKline plc., Pfizer India, and Micro Labs Ltd., purchased from the local pharmaceutical store (pharmacy).

Analytical instruments required for the investigations were tray drier, mechanical grinder/mixer grinder, analytical balance, rotary vacuum evaporator, deep refrigerator, laminar airflow, water bath, which were procured from Rima Engineering Concern, Bajaj Electricals Ltd., Sartorius AG, IKA India Private Limited, Blue Star Limited, Klenzaids Contamination Controls Pvt. Ltd., Biotech Laboratories of Indian division, respectively. Venturimeter vacuumed desiccator, BOD incubator, hot air oven were obtained by Remco Products Corporation, India. Homogenizer, centrifuge, and vortex mixer were procured by Remi sales & engineering LTD, India. Borosil glass apparatus used for investing the observations of the experiment were supplied by S.C. Dey and Company, India. Lastly, UV-VIS Spectrophotometer with model number UV-1800 was obtained from Shimadzu, Japan.

### Extraction and fractionation

*Maceration Extraction:* Collected samples were washed thoroughly, shed dried in a single day, and then dried in a tray dryer (at 40 °C) followed by pulverization in a mechanical grinder. A portion of 100 g of powder material (both coarse and fines) is directly extracted by soaking in 600 mL of ethanol at room temperature overnight. The solvent was filtered by vacuum filtration. Another portion of 100 g of powder material is soaked in 500 mL of distilled water overnight and then filtered, finally, the solvent was collected.

A portion of 100 g of powder material is indirectly fractionated (successive fractionation based on solvent polarity) with petroleum ether, chloroform, ethyl acetate, ethanol, water (300 mL of each solvent as previously) according to the increasing grade of polarity. After extraction, solvents were removed by rotary vacuum

evaporator followed by drying in a Tray drier at 40 °C for 5 to 6 h to get sticky mass and stored in vacuumed desiccators for further use. The whole extraction and fractionation procedure were done according to the previous researchers [16]. The observed percentage yield was found to be 1.75–2.96% w/w in the case of the single solvent system, whereas 0.22–0.84% w/w in multiple solvent systems (details of % yield was elaborated in Table 4).

### Phytochemical screening

Qualitative analysis for the detection of phytoconstituents in the extract of *F. benghalensis* was carried out by standard methods as mentioned by previous researchers [17–21].

*Alkaloids* were identified by employing four reagents viz. *Mayer's reagent*, *Dragendorff's reagent*, *Hager's reagent,* and *Wagner's reagent* separately in a triplicate manner. The test sample was prepared by dissolving ~ 2 g of crude extract in 2 mL of 10% ammonia solution followed by 10 mL methanol with reflux for 10–12 min [18].

In the *Mayer reagent* test [20], a few mL of the test sample was thoroughly mixed with potassium mercuric iodide solution, the formation of cream color precipitate will confirm the presence of alkaloids.

The test solution was treated with a few drops of potassium mercuric iodide solution in *Dragendorff's reagent* test [20] for alkaloids. On that, the development of reddish-brown precipitate will identify the same.

A saturated solution of picric acid was added with a few drops of test solution under *Hager's reagent* testing procedure [20]. Yellow precipitate on test tube leads to indicate the presence of alkaloids.

In the *Wagner reagent* test [20], a few mL of test sample along with iodine-potassium iodide solution was mixed and shaken well; the formation of reddish-brown precipitate will confirm the presence of alkaloids.

*Glycosides* were detected by *Borntrager's*, *Modified Borntrager's*, *Killer-Killiani*, *Legal's, Baljet's*, and *Forth formation* tests. Each test was performed in a triplicate manner [21].

*Borntrager's* and *Modified Borntrager's* test was performed for anthraquinone glycoside, the test sample was prepared by, dissolving 0.3 g of crude extract in 3 mL of methanol for ~ 5–6 min of reflux underwater bath [18].

In *Borntrager's* test [20], the sample was added with dilute sulfuric acid under the boiling condition followed by filtration. An equal volume of chloroform was added with cool filtrate and shaken well. On completion, the resulting organic solvent was separated and mixed with ammonia to form an ammoniacal layer that turns pink/red to showcase the existence of anthraquinone glycosides.

*Modified Borntrager's* test [20] was also performed, where the test sample was mixed with 5 ml of dilute HCl and 5 ml of 5% solution of ferric chloride under boiling condition initially for few minutes, followed by subsequently cooling and filtration. Finally, this solution was shaken vigorously with benzene and the separated benzene layer was treated with an equal volume of a diluted solution of ammonia to form pink color as an indication of anthraquinone glycosides.

*Keller-Killiani, Legal's,* and *Baljet's* tests were performed to check the presence of cardiac glycosides. The test sample was prepared by dissolving 1 g of crude extract in 15 mL of ethanol under reflux for ~ 10–15 min followed by the addition of 5 mL lead acetate solution (10%). The cool filtrate of the previous solution was further extracted by gentle shaking in 3:2 ratios with 1 mL dichloromethane. On completion, the lower part was separated and the solvent was evaporated to get the dry powder, further dry powder was again dissolved in 1 mL dichloromethane on 3:2 ratios [18].

In the *Keller-Killiani* test [20] 2 mL of the test sample, glacial acetic acid, one drop of 5% ferric chloride, and concentrated sulfuric acid were mixed, resulting in reddish-brown color at the junction of two liquid layer and blue-green at the upper layer indicating the presence of cardiac glycosides [21].

For *Legal's* test [20], the test sample was treated with pyridine (1 ml) and sodium nitroprusside solution (1 ml), resulting in the formation of pink to red color if cardiac glycoside presence.

Whereas, the sample was treated with picric acid/sodium picrate in *Baljet's* test [20]. Turing of the orange color solution also indicates the presence of cardiac glycoside.

For the presence of *saponin* glycosides, a *Froth formation* test [20] was performed thrice. The test sample was prepared by dissolving 1 g of crude extract in 5 mL of ethanol (70%) under moderate heating for ~ 8–10 min [18]. This sample was further treated with water in a test tube followed by vigorous shaking so that the stable froth (form) is formed.

Tests for *tannins* were investigated by *ferric chloride* and *gelatin* tests. Directly solvent extracted solutions (`0.02 g/mL) were used as a test sample and performed thrice for each test [19].

In the *ferric chloric* test [20], the test sample was treated with a ferric chloride solution, resulting in either blue color which indicated the presence of hydrolyzable tannins; while the resulting green color indicated the presence of condensed tannins.

On the *gelatin* test [20], a test sample along with a 1% gelatin solution containing 10% sodium chloride was mixed, resulting in the formation of a precipitate that indicated the presence of tannins.

The presence of *protein and amino acids* was evaluated using the *Biuret* test, *Ninhydrine* test reagents, *hydrolysis* test, and *heat* test. Directly solvent extracted solutions (`0.02 g/mL) were used as a test sample and performed thrice for each test [21].

A *Biuret* test [20] was performed by adding a few drops of 2% copper solution to the test sample followed by 1 ml of ethanol and potassium hydroxide leads to the formation of pink color to indicate the presence of protein.

In the *Ninhydrin* test [20], the test sample was mixed with an alcoholic solution of ninhydrin and boiled, emerging the appearance of red to violet color, demonstrated the presence of amino acid.

In the *Heat* test [20], the test solution was boiled in a water bath and coagulation of solution indicates the presence of protein.

Finally, the *Hydrolysis* test [20] was performed by hydrolyzing the test solution with 1 N hydrochloric acid, followed by confirmation with ninhydrine test reagents.

*Steroid* test was performed using *Salkowski's* test [20], where concentrate extract (~ 2 mg) of different solvent was added into 5 ml of chloroform, an equal volume of concentrated $H_2SO_4$ were mixed and performed in triplicate to confirm. The existence of steroids was proved by the formation of the upper layer of chloroform displaying a play of colors first from bluish-red to gradually violet and lower acid layer showing yellow color with green fluorescence [21].

Test for *flavonoids* was performed using *Shinoda* and *Zinc hydrochloride* tests in a triplicate manner. The sample was prepared by dissolving 300 mg of crude extract from different solvents in 3 mL of methanol under ~ 60 °C using a water bath [18].

In the *Shinoda* test [20], the test solution was prepared by mixing the test sample with few magnesium turnings followed by the addition of concentrated hydrochloric acid (dropwise). Emerging pink scarlet, crimson red, and sometimes green to the blue color indicated the presence of flavonoids.

Now, in the *zinc hydrochloride* test [20], the mixture was prepared by mixing the test sample with zinc dust and concentrated hydrochloric acid. The formation of red color after a few minutes indicated the presence of flavonoids.

*Carbohydrate* test was performed using *Molisch's* test [20], where a direct crude extract of different solvent systems was mixed with 2–3 ml of the alcoholic alpha-naphthol solution followed by few drops of concentrated $H_2SO_4$ was added from the side of the test tube in triplicate manner. The appearance of a purple to violet color ring at the junction demonstrated the presence of carbohydrates [19].

### Preparation of the buffer and salt solution

*Hank's Balanced Salt Solution (HBSS) 1X:* prepared by following the standard method of the previous researchers [22] and composition was stated in Table 1.

Phosphate Buffer 0.1 M (pH 7.0) was prepared by following the standards as described by Indian Pharmacopoeia [23].

Table 2. Preparation of antimicrobial solutions [24]: Antimicrobial solutions were added to prevent any type of microbial growth (Bacteria & Fungi) which can interrupt the study and result also. As the goat liver slice/homogenates are very much susceptible to microbial contamination.

### Collection of the liver

Fresh goat (*Capra Capra*) liver samples were collected from a local slaughterhouse, washed in normal saline, and immersed into the cold sterile Hank's Balanced Salt Solution (HBSS) and maintained at 4 °C till further use as illustrated by previous researchers [25, 26].

### Preparation of liver slices for the development of experimental models

The liver samples, as collected, were cut into very thin slices with sterile student lancet (~ 1 g each approx.) and dipped into 2.5 mL of cold sterile HBSS solution containing 1 mL of Penicillin, Gentamycin, Fluconazole for the prevention of any type of microbial contamination. The whole process was done under the laminar airflow bench to maintain the sterility and aseptic condition for the experiments, according to the method as proclaimed by the previous researchers [25, 26].

### Preparation for biological models

Three models were developed to induce hepatotoxicity. Carbon tetrachloride ($CCl_4$) with the dose of 2 mL/kg was used according to Meera et al. 2009 [25], followed by Acetaminophen having a dose of 7 g/kg as prescribed by Sasidharan et al. 2010 [27], finally, 1.4 g/kg dose of Erythromycin as proclaimed by Okwa et al. 2013 [28].

**Table 1** Composition of reagents used for the preparation of buffer solution

| Reagents | mg/L |
|---|---|
| Sodium chloride | 8000 |
| Potassium chloride | 400 |
| Potassium phosphate, Monobasic | 60 |
| Glucose | 1000 |
| Sodium phosphate, Dibasic anhydride | 48 |
| Magnesium sulfate anhydride | 98 |
| Calcium chloride anhydride | 140 |
| Sodium bi-carbonate | 350 |

**Table 2** Preparation of antimicrobial solution

| Antibiotics | Concentration | Spectrum | Stability at 37 °C (days) |
|---|---|---|---|
| Penicillin | 100 Unit / mL | Gram (+ve) bacteria | 3 |
| Gentamycin | 50 μg / mL | Gram (+ve)& gram (−ve) bacteria | 5 |
| Fluconazole | 2 μg / mL | Yeasts and molds | 3 |

The reason for the selectivity of this biological method is because toxicity can be raised from different sources and can damage the liver reversibly or irreversibly, but the most common ways, such as free radical generation (mimic by $CCl_4$ model) were challenged here to evaluate the efficacy. All the test tubes or vials containing liver samples were sterile and divided into 6 groups (each group in triplicate) to perform the experimental study, as shown below in Table 3. Table 3 elaborated on the experimental study (design) or method.

### Homogenization and centrifugation

After the incubation period, the test tubes or vials containing liver samples were homogenized at 8000 rpm (Remi, Homogenizer) with 10mLof 0.1 M Phosphate Buffer Solution (PBS) (pH 7.0) at 4–8 °C. After homogenization, the liver homogenates were centrifuged at 10000 rpm (Remi Centrifuge) and the supernatants were collected and kept in a refrigerator for further investigations and the procedure followed for homogenization and centrifugation is according to the previous researchers [25, 26].

### Protein estimation by Lowry method

Protein estimation was carried out in triplicate by following the Lowry method [29] using Bovine Serum Albumin (BSA) as standard. Briefly, 5 mL of Lowry reagent was added to 1 mL of sample or standard solution and kept for 10 min at room temperature. Later on, 0.5 mL of 1 N Folin-Ciocalteu Phenol reagent was added to it (sample or standard solution) with immediate vortexing. The color was allowed to develop and optical density at a wavelength of 750 nm was recorded after 30 min using UV-VIS Spectrophotometer (UV 1800 from Shimadzu).

Enzyme activity investigation can be easily studied by preparing goat liver homogenate protein. To assess the enzyme activity and the effect of samples on it, standardizations of the assay conditions are necessary. As a part of standardization, the changes in activity with the variation of protein concentration were investigated. The specific activity means the amount of product formed by the enzyme per unit time (min) per unit amount of protein (mg) under given conditions. The determination of protein concentration in terms of absorbance of the liver homogenate was measured.

The protein concentration employed for each assay was resolved and estimated in comparison with that of standard protein (BSA) concentration. Such determination of the protein content is essential to estimate the specific activity of the enzyme present in such preparation.

### Preparation of standard curve

*Preparation of Reagents:* 5% (w/v) solution of potassium di-chromate ($K_2Cr_2O_7$) was prepared by using water and

**Table 3** Experimental Design/ Biological Models

| Sr. No | Model | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | Group 6 |
|---|---|---|---|---|---|---|---|
| | | | | Co-administration | | | |
| | *Biological Experimental Model* | *Normal Control* | *Inducer Control (Only)* | *Standard Treatment* | *Extract Treatment Dose 1* | *Extract Treatment Dose 2* | *Extract Treatment Dose 3* |
| 1. | Carbon Tetrachloride ($CCl_4$) induced hepatotoxicity. | Only HBSS | $CCl_4$ 2 mL/kg (in 0.5% Tween 80 Solution) | $CCl_4$ 2 mL/kg (in 0.5% Tween 80 Solution) | $CCl_4$ 2 mL/kg (in 0.5% Tween 80 Solution) | $CCl_4$ 2 mL/kg (in 0.5% Tween 80 Solution) | $CCl_4$ 2 mL/kg (in 0.5% Tween 80 Solution) |
| | | | | Silymarin (500 mg/kg) | Ethanol fruit extract, 100 mg/kg | Ethanol fruit extract, 250 mg/kg | Ethanol fruit extract, 500 mg/kg |
| 2. | Acetaminophen induced hepatotoxicity | Only HBSS | Acetaminophen (7 g/kg) | Acetaminophen (7 g/kg) | Acetaminophen (7 g/kg) | Acetaminophen (7 g/kg) | Acetaminophen (7 g/kg) |
| | | | | Silymarin (500 mg/kg) | Ethanol fruit extract, 100 mg/kg | Ethanol fruit extract, 250 mg/kg | Ethanol fruit extract, 500 mg/kg |
| 3. | Erythromycin induced hepatotoxicity | Only HBSS | Erythromycin (1.4 g/kg) | Erythromycin (1.4 g/kg) | Erythromycin (1.4 g/kg) | Erythromycin (1.4 g/kg) | Erythromycin (1.4 g/kg) |
| | | | | Silymarin (500 mg/kg) | Ethanol fruit extract, 100 mg/kg | Ethanol fruit extract, 250 mg/kg | Ethanol fruit extract, 500 mg/kg |

Note: After processing, all the test tubes or vials were kept in a BOD incubator at 37 °C overnight to mimic the body temperature

glacial acetic acid as a solvent which was mixed in the ratio 1:3. 0.2 M of hydrogen peroxide ($H_2O_2$) was prepared from 30% (v/v) $H_2O_2$, which is equivalent to 8.8 M. Phosphate Buffer 0.1 M (pH 7.0) was also prepared. It has been done in triplicate.

### Colorimetric determination of $H_2O_2$

Six different test tubes or vials containing an increasing amount of $H_2O_2$ (18–120 μmol) were taken according to the previous researcher [30]. Then the volume of each test tube was made up to 10 mL with 0.1 M phosphate buffer (pH 7.0). After 1 min, 1 mL from each test tube was withdrawn and added to another test tube containing 2 mL of 5% $K_2Cr_2O_7$ reagent, instantaneously with the addition of $H_2O_2$ lead to generating an unstable blue precipitate of perchromic acid. On subsequent heating for 10 min in a boiling water bath, the color of the solution turns into stable green owing to the formation of chromic acetate. At cooling at room temperature, 3 mL from each test tube was relocated to a clean cuvette and the absorbance was measured at a wavelength of 570 nm in the UV-Vis spectrophotometer using phosphate buffer 0.1 M as blank. Using the results obtained, a graphical representation was plotted, against absorbance at a wavelength of 570 nm (Y-axis) as a function of micromoles of the $H_2O_2$ (X-axis). It has been done in triplicate.

### Measurement of catalase activity by colorimetric assay

The catalase activity was measured according to the method of Sinha et al. 1972 [30], Hadwan 2018 [31], and Verma et al. 2008 [32] with slight alteration for appropriate measurement of the samples. A definite amount of liver homogenate (20 μL) was mixed with 6 mL of 0.1 M PBS (pH 7.0) and incubated with 4 mL of 0.2 M $H_2O_2$ (800 μmoles) at 37 °C accurately for 1 min. After 1 min, 1 ml was taken from each test tube and added to another containing 2 mL of 5% $K_2Cr_2O_7$ reagent to make up volume up to 3 mL. Each test tube or vial was incubated for 10 min in a boiling water bath and then cooled to room temperature. Afterward, tubes were centrifuged at 5000 rpm for 15 min and the supernatant was used to quantify the amount of $H_2O_2$ to regulate catalase activity by UV-Vis Spectrophotometer at $\lambda_{max}$570 nm (using phosphate buffer 0.1 M as blank). Its unit expressed in *μ mole* of $H_2O_2$ consumed/min/mg protein. It has been done in triplicate.

$$2H_2O_2 \xrightarrow{Catalase} O_2 + 2H_2O$$

Generally, 130 μmole of $H_2O_2$ is liable for the absorbance 1.0 (from the standard curve). Therefore, the specific activity is indicated as 130 / (X × Y) μmole/min/mg of protein, where X is the amount of protein transferred per assay and Y is the time in a minute. The specific activity of the Catalase = Factor (f) × Optical Density (O.D) of the sample.

## Results

### Extractive yield value (w/w)

On completion of extraction with different solvent, the highest extraction yield was found to be in Direct Ethanol, whereas the lowest in Indirect Water. Detailed extraction yields were shown in Table 4.

**Phytochemical examination Results:**

Phytochemical examination results of different extraction showed the following response as when performed with the standard method of analysis. From Table 5, it can be inferred that in the case of alkaloids, all the reagents used turned out to be positive for direct ethanol extract, ethyl acetate fraction, and indirect ethanol fraction only. In the case of other tests, like glycoside, Tannin, protein, and amino acid, steroid, flavonoids, and carbohydrates, certain tests turned out to be positive whether as rest as negative.

### Results of protein concentration of the liver homogenate

The enzymatic activity bears a linear relationship with the amount of protein used during the assay. The protein concentration of the liver homogenate preparation was found to be 1.6 mg/mL as measured at $\lambda_{max}$ of 750 nm.

### Result of standard curve for $H_2O_2$

The standard curve showed a linear relationship between the optical density and the amount of $H_2O_2$ used. Further detailed analysis can be inferred that the presence *μ mole* of $H_2O_2$ increases monotonically as the absorbance (nm) is increased. The regression result from the graphical representation is obtained to be 0.9887.

### Effect of ethanol fruit extract of *F. benghalensis* on the antioxidant enzyme (catalase) level in $CCl_4$-induced liver damage

As demonstrated in Table 6, as compared to the control, the $CCl_4$-treated group exhibited a lower level of catalase activity in the liver. Conversely, the groups which received more ethanol extract (100–500 mg/kg) showed a dose-dependent increase in the reduced level of catalase. The ethanol extract of fruits of the plant showed promising activity in terms of the restoration of reduced enzyme level as compared to the standard drug, Silymarin, which also restored the altered level of catalase very nearby.

**Table 4** Extraction yield of various solvents

| Single Solvent System | | Multiple Solvent System (Gradient/Successive) | | | | |
|---|---|---|---|---|---|---|
| **Ethanol** | **Water** | **Pet. Ether** | **Chloroform** | **Ethyl Acetate** | **Ethanol** | **Water** |
| 2.96% | 1.75% | 0.44% | 0.34% | 0.84% | 0.25% | 0.22% |

### Effect of ethanol fruit extract of *F. benghalensis* on the antioxidant enzyme (catalase) level in acetaminophen-induced liver damage

As presented in Table 7, as compared to the control, similarly, the Acetaminophen-treated group exhibited a lower level of catalase activity in liver homogenate. Conversely, the groups which received more ethanol extract (100–500 mg/kg) showed a dose-dependent increase in catalase level. The ethanol extract of fruits of the plant showed moderate activity in terms of the restoration of reduced enzyme level as compared to the standard drug, Silymarin, which also restored the altered level of catalase.

### Effect of ethanol fruit extract of *F. benghalensis* on the antioxidant enzyme (catalase) level in erythromycin -induced liver damage

As exhibited in Table 8, similar to those previous models here also the catalase level in control reduced significantly in the Erythromycin-treated group in liver homogenate. Conversely, the groups which received more ethanol extract (100–500 mg/kg) showed a dose-dependent increase to restore the catalase level. The ethanol extract of fruits of the plant showed moderate activity in terms of the restoration of catalase as compared to the standard drug, Silymarin, which also restored the altered level of catalase.

## Discussion

There are lots of root causes for the hepatic disease or else developed hepatic necrosis. Amongst them, long term exposure to conventional treatment/use is the most eventual cause behind as e.g. 'Paracetamol (Acetaminophen) for any normal infection/disease associated with pain, fever and inflammation, which is readily & most widely available in the market as an over the counter (OTC) to be used as an antipyretic. Similarly, to be a choice of an anti-infective agent, 'Macrolide Group' stands apart from other antibiotics due to its broad-spectrum & quick activity, so drugs like, Azithromycin, Erythromycin is used quite often.

**Table 5** Phytochemical examination results

| Solvent System | | Single solvent system | | Multiple solvent systems (Gradient Extracts) | | | | |
|---|---|---|---|---|---|---|---|---|
| Chemical for test | Reagent | E | W | PE | CF | EA | GE | GW |
| Alkaloids | Mayer's reagent | + | – | – | – | + | + | – |
| | Dragendorff reagent | + | – | – | – | + | + | – |
| | Hager reagent | + | – | – | – | + | + | – |
| | Wagner reagent | + | – | – | – | + | + | – |
| Glycosides | Borntrager's | – | + | – | – | – | – | + |
| | Mod. Borntrager's | + | + | + | + | – | + | + |
| | Killer-Killiani | – | – | – | – | – | – | – |
| | Legal's | – | – | – | – | – | – | – |
| | Baljet | – | – | – | – | – | – | – |
| | Forth formation | – | – | – | – | – | – | – |
| Tannin | $Fecl_3$ test | + | – | – | – | + | + | – |
| | Gelatine test | – | – | – | – | – | – | – |
| Protein and amino acid | Biuret test | – | + | – | – | – | – | – |
| | Ninhydrine test | – | + | – | – | – | – | – |
| | Hydrolysis test | – | – | – | – | – | – | – |
| | Heat test | – | – | – | – | – | – | – |
| Steroid | Salkowski | + | + | – | + | + | + | + |
| Flavonoids | Shinoda test | + | + | – | – | – | + | + |
| | Zn-HCl test | + | + | – | – | – | + | + |
| Carbohydrate | Molisch's test | + | + | – | – | – | + | + |

*E* Ethanol extract; *W* Aqueous extract; *PE* Petroleum ether fraction; *CF* Chloroform fraction; *EA* Ethyl acetate fraction; *GE* Gradient ethanol fraction; *GW* Gradient aqueous fraction
'+' indicates present; '-' indicates absent

**Table 6** Effect of ethanol fruit extract of *F. benghalensis* on the activity of the antioxidant enzyme (Catalase) in goat liver after Carbon Tetrachloride ($CCl_4$) administration

| Groups | Specific Activity |
|---|---|
| Initial $H_2O_2$ | – |
| Normal control | 1198.44 |
| $CCl_4$ Control (2 mL/kg) | 455 |
| $CCl_4$ + Silymarin (500 mg/kg) | 865.31 |
| $CCl_4$+ Extract (100 mg/kg) | 698.75 |
| $CCl_4$ + Extract (250 mg / kg) | 706.88 |
| $CCl_4$ + Extract (500 mg / kg) | 857.18 |

[a]All the values of Optical Density (OD) and Specific Activity are in triplicate and taken as the average of three

**Table 8** Effect of ethanol fruit extract of *F. benghalensis* on the activity of the antioxidant enzyme (Catalase) in goat liver after Erythromycin administration

| Groups | Specific Activity |
|---|---|
| Initial $H_2O_2$ | – |
| Normal control | 1182.18 |
| Erythromycin Control (1.4 g/kg) | 288.44 |
| Erythromycin+ Silymarin (500 mg/kg) | 719.06 |
| Erythromycin+ Extract (100 mg/kg) | 300.62 |
| Erythromycin + Extract (250 mg/kg) | 446.88 |
| Erythromycin + Extract (500 mg/kg) | 528.13 |

[a]All the values of Optical Density (OD) and Specific Activity are in triplicate and taken as the average of three

Excessive stress, exposure to toxins, different kinds of chemicals, etc. can easily generate free radicals within the body from different sources. Even a metabolite or product from the metabolism process (mostly performed in the liver), can also be responsible for such kind of problematic free radicals, which can radically attack some of the major organs to developed serious issues/ damages.

So, Carbon tetrachloride is such a class of chemicals, which are very much familiar to be associated with a quick free radical generator. As a result, $CCl_4$ can be associated with a metabolic imbalance in the liver leads to the formation of reactive oxygen species (ROS).

As per the existing literature of many scientific types of research which demonstrated that the ROS including oxygen free radicals are causative factors in the etiology of degenerative diseases, including some hepatopathies. Interestingly, in vitro and in vivo examinations so far published till today, where it has been clearly shown that several classical antioxidants can protect hepatocytes against lipid peroxidation or inflammation, thereby preventing the occurrence of hepatic necrosis as reported by Hsiao et al. 2001 [33].

**Table 7** Effect of ethanol fruit extract of *F. benghalensis* on the activity of the antioxidant enzyme (Catalase) in goat liver after Acetaminophen administration

| Groups | Specific Activity |
|---|---|
| Initial $H_2O_2$ | – |
| Normal control | 1076.56 |
| Acetaminophen Control (7 g/kg) | 316.88 |
| Acetaminophen + Silymarin (500 mg/kg) | 589.06 |
| Acetaminophen + Extract (100 mg/kg) | 320.93 |
| Acetaminophen + Extract (250 mg/kg) | 381.88 |
| Acetaminophen + Extract (500 mg/kg) | 455 |

[a]All the values of Optical Density (OD) and Specific Activity are in triplicate and taken as the average of three

Exocrine systems are bound to release antioxidant enzyme after getting induced on the generation of free radicals in cells, like Catalase (CAT) which protect cells from the oxidative stress of highly reactive free radicals as a consequent event of normal homeostasis mechanism. To authorize the above physiological event, the activity of CAT was checked in goat liver samples, which were cultured and treated with different concentrations of ethanol fruit extract of *F. benghalensis* (100–500 mg/kg).

The stem bark, roots, leaves, fruits, and other different parts of *F. benghalensis* were used in India for the treatment of numbers of diseases traditionally and the hunt for pharmacological activity is at full pace of research, as explained and elaborated in the supplementary material, as Table A1. The fruits were traditionally used in folk medicine for respiratory disorders and certain skin diseases. The phytoconstituents present in *F. benghalensis* (Aerial root) revealed significant hepatoprotective activity against isoniazid-rifampicin induced oxidative liver injury in rats, along with free radical scavenging effect, which may be attributed to their polyphenolic compounds including flavonoids and terpenoids, which are similar to the report as described by Parameswari et al. 2012 [34]. Along with bark have also demonstrated promising results in $CCl_4$ and Paracetamol induced model of hepatotoxicity, as explained and elaborated by Baheti, and Goyal 2011 [35]. Extracts from different parts of this plant were found to be safe up to 4 g/kg (*p.o.)* when investigated for acute toxicity study in rats similar to the observations observed by Manocha et al. 2011 [36] and Gupta et al. 2002 [37]. The different extracts and fractions of the fruit of the plant have revealed some potential phytochemicals like alkaloids, glycosides, flavonoids, carbohydrates, steroids, and tannins. Mainly flavonoids, steroids, and carbohydrates are present in

both the aqueous and ethanol extract. Alkaloids and tannins are found in both the ethanol extract and ethyl acetate fraction. Glycosides are also present in all the extracts and fractions except ethyl acetate fraction as shown in Table 5.

The hepatoprotection of the ethanol extract of *F. benghalensis* against $CCl_4$-induced, Acetaminophen-induced, and Erythromycin-induced hepatotoxicity in vitro could be attributed to its ability to restore the antioxidant enzyme catalase of the liver or to the free radical scavenging activity of the extract. The observed hepatoprotective activities of the fruit extract are shown in Figs. 1, 2, and 3 along with Tables 6, 7, and 8 respectively. From the detailed analysis of each figure in conjunction with a table, it can be explained that a significant result is observed in terms of restoration of catalase as a function of the dose depends on the manner, akin to the standard i.e. Silymarin.

## Conclusion

The results of the present investigations suggest that the traditionally or conventionally used potent medicinal plant amongst all the thousands of medicinal plants, *F. benghalensis* fruit extract could strongly prevent oxidative liver damage caused by the administration of conventional medicine, paracetamol, macrolide, and chemical carbon –tetrachloride may be due to the presence of the high amount of phenolic, flavonoids and tannin compounds in this plant extract (Tables 4 and 5) as these compounds are believed to have the ability for scavenging and stabilizing oxidation.

Hence, *F. benghalensis* may be strongly considered as an easily available medicinal plant for the development of new and good efficacious drug(s) in near future with the least possible side effect, adverse drug reaction, and at very little cost. However, further comprehensive pharmacological investigations; detailed screening, and identification of molecules are needed for the advancement in both ex-vivo and in vivo model with the ultimate target to elucidate its mechanism of this hepatoprotective effect. Further studies are required to isolate and characterize the active principles, which are responsible for the hepatoprotective efficacy of this valuable medicinal plant, but this is the first step in advancement towards new technology.

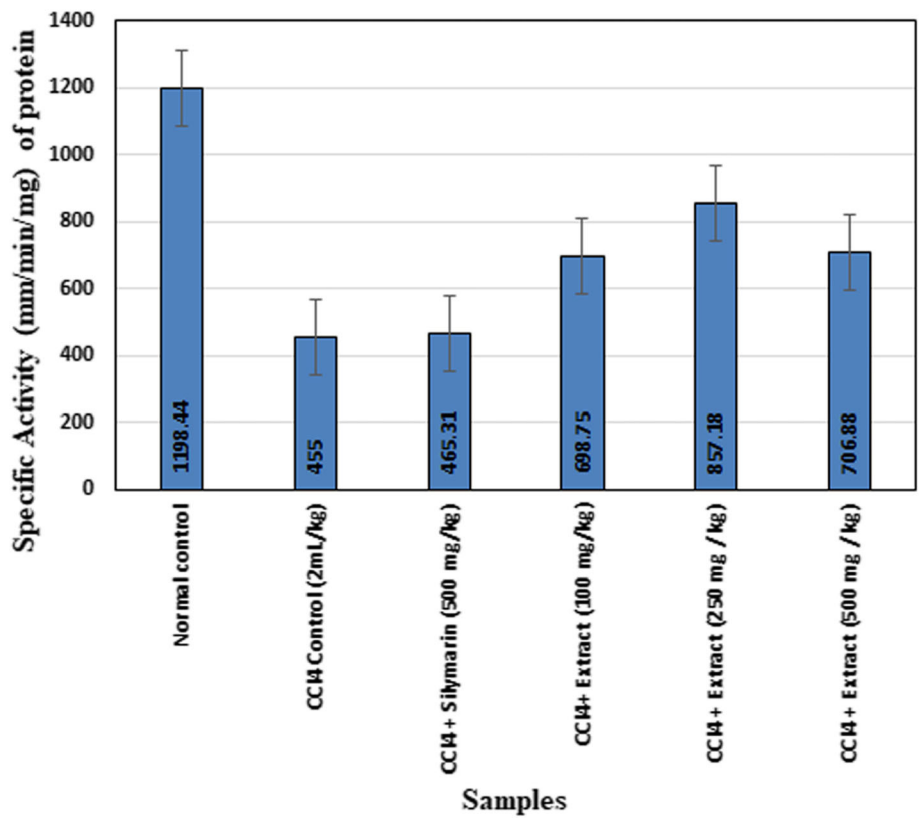


**Fig. 1** Antioxidant enzyme (catalase) level in $CCl_4$ -induced liver damage

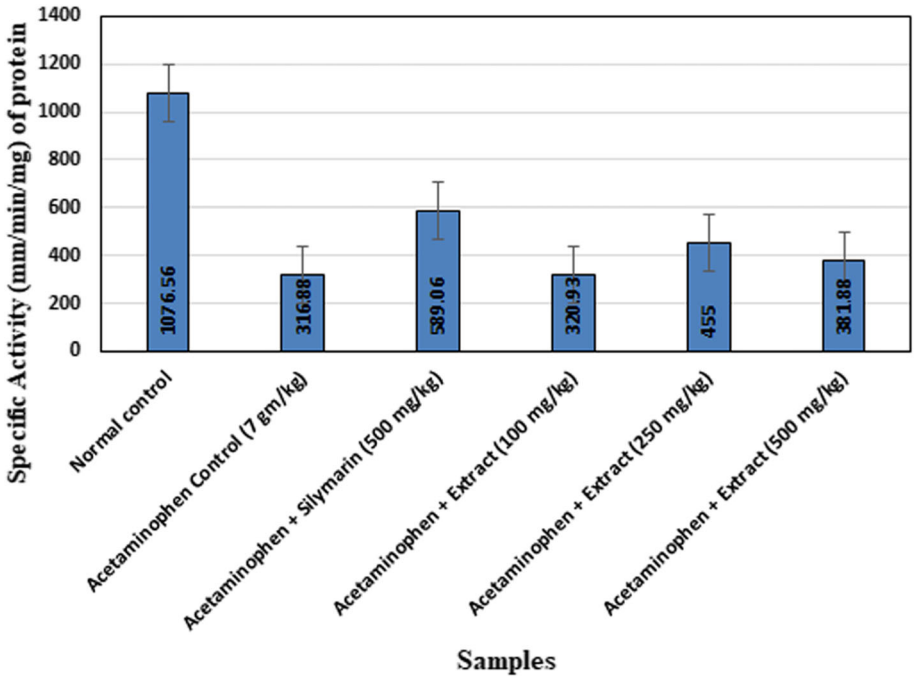


**Fig. 2** The antioxidant enzyme (catalase) level in Acetaminophen-induced liver damage

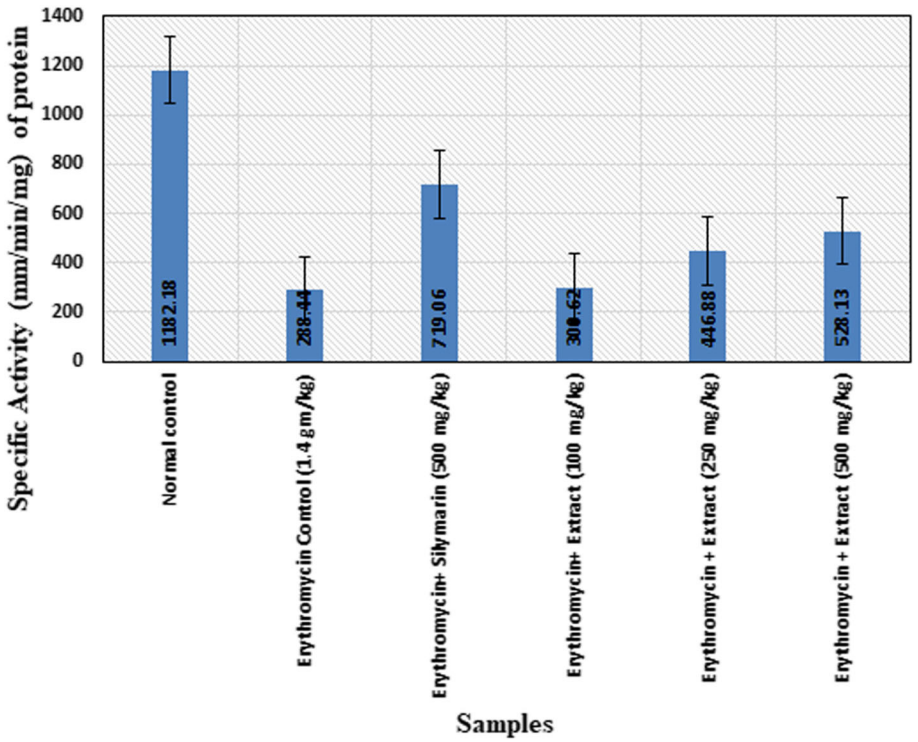


**Fig. 3** The antioxidant enzyme (catalase) level in Erythromycin-induced liver damage

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40816-020-00230-8.

**Additional file 1.**


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### Authors' contributions

*Shibam Karmakar*- Conceptualization, Data curation, Formal analysis, Investigation, Validation, writing the draft manuscript, *Somnath Paul*- Data curation Formal analysis, Investigation, Software utilization, *Nripendra Madhab Biswas*- Data curation Formal analysis, *Jasmina Khanam* - Writing, review and editing, *Sudip Kumar Kar*- Conceptualization, Validation, Visualization, *Hemanta Mukherjee*- Validation, Writing, review, and editing – original and final manuscript. *Sourav Poddar*- Conceptualization, Data curation, Formal analysis, Investigation, Software utilization, Validation, Visualization, and Writing, review, and editing – original and final manuscript. The author(s) read and approved the final manuscript.


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### Availability of data and materials

Soft copy of the identification of plant information sheet is attached herewith this manuscript in the supplementary section. Apart from all data generated and analyzed are available with this manuscript.

### Ethics approval and consent to participate

This article does not involve any human participation, so it is not applicable. However, the experimental procedures performed on the plant extracts were approved by the Government of India, Ministry of Environmental & Forests, Botanical Survey of India, provided in the supplementary section. All the experimental procedures were performed according to the guidelines of the Bengal School of Technology, Chinsurah, Hooghly, West Bengal, India. The article also follows the guidelines laid by the National Institute of Technology, Tiruchirappalli, Tamil Nadu, India.

### Consent for publication

Not applicable. However, the authors declare that there is no known competing financial interest or personal relationships that could have appeared to influence the work reported in this manuscript.

### Competing interests

The authors declare that they have no competing interests.


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## Publisher's Note