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Efficacy of *Tabernaemontana divaricata* bark extract in mitigating oxidative stress in rodent

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Abstract
The present study aimed to evaluate the phytochemical constituents, total phenolic content, antioxidant activity, and hepatoprotective efficacy of *Tabernaemontana divaricata* bark extract against D-galactosamine-induced oxidative stress in rats. The bark of *T. divaricata* was extracted successively using petroleum ether, chloroform, methanol, and water. Phytochemical screening and estimation of total phenolic content were performed. The methanolic bark extract, which exhibited the highest phenolic content, was selected for in vivo evaluation. Hepatotoxicity was induced in Wistar rats using D-galactosamine (25 mg/kg), and the extract was administered orally at doses of 200 and 400 mg/kg for 21 days. Biochemical markers including SGOT, SGPT, ALP, ACP, bilirubin, catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and lipid peroxidation levels were assessed. Phytochemical analysis revealed the presence of alkaloids, flavonoids, phenols, tannins, and triterpenoids. The methanolic extract showed the highest total phenolic content (207.09 ± 4.705 GAE mg/g) among all extracts. D-galactosamine administration significantly elevated hepatic biochemical markers and lipid peroxidation while reducing endogenous antioxidant enzymes. Treatment with *T. divaricata* methanolic bark extract produced a dose-dependent reduction in SGOT, SGPT, ALP, ACP, and bilirubin levels and significantly restored CAT, SOD, and GPx activities. The higher dose (400 mg/kg) demonstrated pronounced protection comparable to vitamin E treatment. The findings suggest that the plant may serve as a promising natural therapeutic agent for managing oxidative stress-associated hepatic disorders.

Keywords: *Tabernaemontana divaricate*, oxidative stress, antioxidant activity, hepatoprotective activity, D-Galactosamine

Introduction
Oxidative stress is a physiological condition resulting from an imbalance between the generation of reactive oxygen species (ROS) and the antioxidant defense mechanisms of the body [1]. Excessive production of free radicals can damage cellular lipids, proteins, and nucleic acids, thereby contributing to the pathogenesis of several chronic diseases, including liver disorders, diabetes, cardiovascular diseases, neurodegenerative conditions, and cancer [2]. The liver, being the primary organ responsible for xenobiotic metabolism and detoxification, is particularly susceptible to oxidative damage induced by toxic chemicals and drugs. Consequently, the search for natural antioxidants capable of protecting hepatic tissues against oxidative stress has attracted considerable scientific attention [3].
Medicinal plants represent a rich source of bioactive phytochemicals with antioxidant, anti-inflammatory, and hepatoprotective properties [4]. Among these phytochemicals, phenolic compounds, flavonoids, alkaloids, and tannins have been extensively investigated for their ability to scavenge free radicals, inhibit lipid peroxidation, and enhance endogenous antioxidant defense systems. Traditional medicinal systems have long utilized plant-derived preparations for the management of liver ailments and other oxidative stress-related disorders, providing a valuable foundation for modern pharmacological investigations [5].
Tabernaemontana divaricata (L.) R.Br. ex Roem. & Schult., commonly known as crape jasmine, belongs to the family Apocynaceae [6]. The plant is widely distributed throughout tropical and subtropical regions of Asia and has been extensively employed in traditional medicine for the treatment of inflammation, pain, fever, skin diseases, wounds, and various systemic disorders [7]. Previous phytochemical studies have revealed the presence of indole alkaloids, flavonoids, phenolic compounds, triterpenoids, and other secondary metabolites in different parts of the plant. These constituents are known to possess significant pharmacological activities, including antioxidant, antimicrobial, anti-inflammatory, analgesic, and hepatoprotective effects [8, 10].
Although several biological activities of *T. divaricata* have been reported, scientific evidence regarding the antioxidant potential of its bark extract and its role in protecting against oxidative stress-induced hepatic damage remains limited. Since oxidative injury is one of the major mechanisms involved in hepatotoxicity, evaluating the antioxidant properties of *T. divaricata* bark may provide insight into its therapeutic significance. D-Galactosamine-induced hepatotoxicity is a well-established experimental model that closely resembles human viral hepatitis and is characterized by excessive oxidative stress, lipid peroxidation, and liver cell injury. Therefore, the present study was undertaken to evaluate the phytochemical profile, total phenolic content, and antioxidant efficacy of different extracts of *Tabernaemontana divaricata* bark.

Material and Methods
Collection and identification of plant material
The bark of *Tabernaemontana divaricata* were collected from the local surrounding of Gwalior, Madhya Pradesh in the month of January and authenticated at RB Science; Bhopal by voucher specimen**.** The authenticated plant bark was washed with distilled water and were dried under shade. The dried bark was powdered using a blender at low speed. The powdered bark was stored in closed container till use.

Preparation of Extract

Powdered bark (102 g) was evenly packed in the extractor of the soxhlet apparatus and extracted using solvents of increasing polarity (petroleum ether, chloroform, methanol, water) using hot continuous extraction process for about 6-12 h for each solvent. The extract was filtered while hot through Whatman filter paper to remove any impurity and concentration by evaporating the solvent using rotary vacuum evaporator. The oleo-resinous extract was collected and placed in desiccator to get rid of the excessive moisture**.** The dried/semidried extracts were stored in desiccator for further experimental procedures.

Qualitative Phytochemical Screening

All the four extracts were evaluated by phytochemical qualitative reactions for identifying the presence or absence of usual plant secondary metabolites. The screening was performed for triterpenes/steroids, alkaloids, glycosides, flavonoids, saponins, tannins, and phenolic acids. The color intensity or the precipitate formation was used as analytical responses to these tests [11].

Total Phenolic Content

The extraction of phenolic compounds was based on a modification of the method reported [12]. The extracts (0.1 g each) were macerated with methanol (5 mL) for 8 hours. The solution was filtered and the filtrate was used for determination of total phenolic content. The solutions were stored at 4°C in amber colored bottles and served as the stock solutions for subsequent analyses.

For determining the total phenolic content 200 µL of extract sample was mixed with 1.4 mL purified water and 100 µL of Folin-Ciocalteu reagent was added to it. After 2 min, 300 µL of 20% Na_2CO_3 aqueous solution was added and the mixture was allowed to stand for 2 h. The absorbance was measured at 765 nm with a UV-Vis spectrophotometer. Standard solutions of gallic acid (10-100 ppm) were treated similarly for constructing the calibration curve. The control solution contained 200 µL of methanol and suitable reagents, and it was prepared and incubated under the same conditions as the rest of the samples. Results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g of the dry sample.

Pharmacological Activity

Selection of animals

Male Wistar rats (150-200 gm), were used, and kept in quarantine for 10 days under standard husbandry conditions (27.3°C, Relative humidity 65 ±10%) for 12 h in dark and light cycle respectively and were given standard food and water *ad libitum*. All experiments were approved by the institutional ethical committee and were carried out according to the animal ethical committee guidelines.

Treatment protocol

The extract of *Tabernaemontana divaricata* was dissolved in 20 mL of sterile water and was administered orally at a dose of 200 mg/kg and 400 mg/kg. D-Galactosamine was diluted in sterile water and administered intraperitoneally (i.p) at a dose of 25 mg/kg. Vitamin E was diluted in sterile water and administered orally at a dose of 25 mg/kg.

The acclimatized animals were divided into 5 groups of each 6 animals as per the approved protocol. Each group served individual purpose.

- **Group 1:** Served as normal control and received normal diet and water.
- **Group 2:** Toxic control received 25 mg/kg of D-galactosamine i.p for 21 days.
- **Group 3:** Standard control received 25 mg/kg of vitamin E per oral for 21 days + 25 mg/kg of D-galactosamine i.p for 21 days
- **Group 4:** Received 200 mg/kg of *Tabernaemontana divaricata* methanolic bark extract (TDMBE) for 21 days + 25 mg/kg of D-galactosamine i.p for 21 days.
- **Group 5:** Received 400 mg/kg of *Tabernaemontana divaricata* methanolic bark extract (TDMBE) for 21 days + 25 mg/kg of D-galactosamine i.p for 21 days.

On day 22nd day (24 h after administration of the last dose) the animals from all the groups were sacrificed by cervical dislocation and the blood was collected from the carotid artery and allowed to clot for 45 min at room temperature; serum was separated by centrifugation at 2500 rpm for 15 min, used for the estimation of various biochemical parameters like serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (ALP), serum acid phosphatase (ACP) and serum bilirubin [13, 15].

The livers were dissected out immediately, washed with ice-cold saline and homogenized in phosphate buffer solution (PH 7.4) to prepare 10% homogenates. The homogenate was centrifuged at 1700 rpm for 10 min at 4°C using a refrigerated centrifuge and the level of lipid peroxidation was evaluated. The supernatant was used for the assay of Superoxide dismutase (SOD), catalase (CAT) and Glutathione peroxidase (GPx).

Statistical Analysis

The results are expressed as mean ± SEM of six independent experiments. Statistical significance between the groups was evaluated by one-way analysis of variance (ANOVA) followed by Dunett's test. A $P < 0.05$ value was considered as statistically significant.

Results and Discussion

Extraction and phytochemical screening

The bark was extracted using petroleum ether, chloroform, methanol and water as solvents and the weight of the extract obtained was observed and yield was calculated. The petroleum ether extract yielded were fewer extractable contents suggesting the absence of much lipid soluble components. The yields were obtained in the following order: methanol (11.8%)>water (10.1%)>chloroform (6.3%)>petroleum ether (1.7%). The extracts were dark brown to green and obtained as sticky or dry solids. The qualitative phytochemical screening revealed the presence of alkaloids, flavonoids, triterpenoids, phenols and tannins in various extracts (Table 1).

Table 1: Phytochemical screening of *Tabernaemontana divaricata* extracts

Chemical Tests	Petroleum Ether extract	Chloroform extract	Methanolic extract	Aqueous extract
Mayer's reagent	-	-	+	-
Hager's reagent	-	-	+	-
Wagner's reagent	-	-	+	-
Dragendorff's reagent	-	-	+	-

Froth test	-	+	-	+
Kedde's Test	-	-	-	-
Bontrager's Test	-	-	-	-
Keller-Kiliani	-	-	-	-
Ferric chloride	-	-	+	-
Gelatin Solution	+	+	+	+
Alkaline reagent test	-	+	+	+
Vanillin HCl test	-	-	+	+
Shinoda test	-	+	+	+
Alkaline reagent test	-	-	+	+
Zinc HCl reduction test	-	+	+	+
Millon's Test	-	-	-	-
Ninhydrin Test	-	-	-	-
Liberman-Burchard Test	-	+	+	-
Salkowski Test	+	-	+	-

+ indicates positive observation; - indicates negative observation

Total Phenolic content

The standard curve of gallic acid was constructed using the absorption data of the blue color obtained in the concentration range of 10 to 100 ppm (Figure 1). The total phenolic content of the extract of *Tabernaemontana divaricata* was calculated as GAE mg/g and was found to be 21.98 ± 1.064, 60.99 ± 2.044, 207.09 ± 4.705 and 149.28 ± 1.809 for petroleum ether, chloroform, methanol and aqueous extracts respectively.

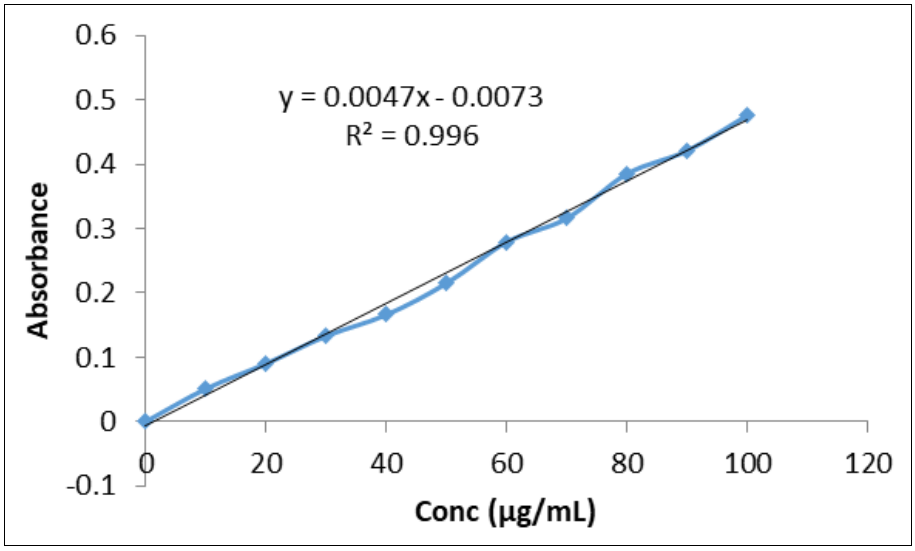


Fig 1: Calibration curve of gallic acid

Pharmacological Action

The methanolic extract was employed to assess the hepatoprotective activity since it had the highest phenolic content. The D-galactosamine induced hepatotoxicity model was used to assess the hepatoprotective efficacy. Rats given D-galactosamine in the current study showed significant liver damage, as evidenced by increased serum levels of liver-specific enzymes and significant changes in other biochemical parameters. The rats treated with D-galactosamine had considerably higher values of the biochemical parameters (Table 2).

The D-galactosamine-induced changes in SGOT, SGPT, ALP, ACP, total bilirubin, and direct bilirubin in blood were reduced by treatment with both dosages of Tabernaemontana divaricata bark extract. The test samples were found to provide protection against toxins, as demonstrated by a significant decrease in all biochemical indicators ($P<0.05$). Additionally, it showed that both separated compounds had lower levels of lowered biochemical parameters than typical drugs.

Table 2: Effect of *Tabernaemontana divaricata* methanolic extract on SGOT, SGPT, ALP, ACP, total bilirubin and direct bilirubin in D-Galactosamine induced liver toxicity in rats

Group	Treatment	SGOT	SGPT	ALT	ACP	Bilirubin (mg/100 mL of blood)	
						Direct (mg/dl)	Total (mg/dl)
I	Normal Control	70.6 ± 4.6	123.0 ± 7.6	160.5 ± 1.9	138.1 ± 4.2	0.28 ± 0.1	0.56 ± 0.4
II	D-Galactosamine	207.9 ± 2.4	333.6 ± 4.2	320.5 ± 4.3	376.7 ± 9.0	2.13 ± 0.4	3.26 ± 0.2
III	Vitamin E	69.7 ± 3.5	129.7 ± 2.2	158.4 ± 4.6	133.1 ± 6.7	0.27 ± 0.1	0.60 ± 0.1
IV	TDMBE (200 mg/kg)	114.6 ± 6.2	171.1 ± 5.1	221.0 ± 2.1	208.2 ± 6.1	1.26 ± 1.1	2.03 ± 0.9
V	TDMBE (400 mg/kg)	82.4 ± 7.3	145.3 ± 4.5	177.1 ± 4.6	161.4 ± 7.5	0.31 ± 0.3	0.78 ± 0.4

Values are expressed as mean ± SEM, n = 6 in each group.

Antioxidant Activity

When compared to animals treated with D-galactosamine, the administration of TDME counteracted the free radical activity caused by D-galactosamine, which is similar to that of vitamin E. SOD, GPx, and CAT enzyme levels are statistically significantly raised, while lipid peroxidation is lowered ($P < 0.05$). These findings demonstrate the Tabernaemontana divaricata bark extract's potent antioxidant activity (Table 3).

The liver releases a wide range of enzymes and engages in a number of metabolic processes. Numerous toxicants, chemicals, and medications can harm the liver. D-galactosamine acts as a toxicant in our model. Elevated enzyme levels are linked to D-galactosamine-related hepatotoxicity, which may be caused by the hepatic microsomes' production of trichloromethyl free radical during metabolism, which initiates lipid peroxidation. SOD, CAT, and GPx activities are reduced by hepatocellular necrosis, and their return to basal levels is a strong sign of tissue healing and stabilization of the plasma membrane. Such a result is consistent with the idea that hepatocyte regeneration and healing of the hepatic parenchyma are seen, and that enzyme activity are returned to normal. An enzyme defence system against oxidative damage is made up of SOD, CAT, and GPx. Such enzyme activities are reduced under D-galactosamine settings, but they significantly increase under extract-treated circumstances. This could be a biochemical technique to lower lipid peroxidation [16].

Table 3: Antioxidant activity of *Tabernaemontana divaricata* extract in D-Galactosamine induced liver damage in rats

Group	Treatment	Catalase (mg liver protein)$^{-1}$	Superoxide Dismutase (mg liver protein)$^{-1}$	Glutathione Peroxidase (mg liver protein)$^{-1}$	TBA (mg liver protein)$^{-1}$
I	Normal Control	242.3 ± 2.21	80.7 ± 1.09	1.34 ± 0.04	1.66 ± 0.05
II	D-Galactosamine	113.8 ± 1.40	26.3 ± 1.03	0.29 ± 0.01	4.05 ± 0.03
III	Vitamin E	243.0 ± 2.34	81.6 ± 1.41	1.32 ± 0.02	1.54 ± 0.01
IV	TDMBE (200 mg/kg)	179.6 ± 1.76	54.6 ± 1.21	0.80 ± 0.07	2.74 ± 0.07
V	TDMBE (400 mg/kg)	226.0 ± 1.15	69.4 ± 1.05	1.22 ± 0.06	1.90 ± 0.05

Values are expressed as mean ± SEM, n = 6 in each group.

Conclusion

The present investigation highlights the significant antioxidant potential of the methanolic bark extract of *Tabernaemontana divaricata*, which was found to play a pivotal role in mitigating oxidative stress. The in vivo findings strongly support the inference that the hepatoprotective activity of the extract is primarily attributable to its free radical scavenging and antioxidant properties. By reducing oxidative damage, the extract not only preserved hepatic function but also demonstrated broader implications for systemic protection against conditions where oxidative stress is a central pathogenic factor. These results underscore the therapeutic promise of *T. divaricata* bark extract as a natural hepatoprotective agent.

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