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Neuropharmacological Activities of Toddalia Asiatica Root Extract in Swiss Albino Rats

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KEYWORDS

***Toddalia asiatica*, *antidepression activity*, *behavioral tests*, *neuropharmacological studies*, *swiss albino rats*.**

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ABSTRACT

The present study was focused to investigate the neuropharmacological (antidepressant) activities of *Toddalia asiatica* root extract. Antidepressant activities of methanolic extract of *Toddalia asiatica* root was evaluated by behavioural tests like Locomotor Activity Test, Forced Swimming Test and Tail Suspension Test in male Swiss albino rats. We also examined the neuropharmacological activities by estimating the levels of stress parameters, such as plasma nitrites, plasma corticosterone and malondialdehyde, catalase, glutathione peroxidase and superoxide dismutase in the rat brain homogenates. Methanolic extract of *Toddalia asiatica* root showed the significant neuropharmacological activity in Swiss albino rats. In both FST and TST, they significantly reduced the duration of immobility and demonstrated dose-dependent activity ($p < 0.001$). At 400 mg kg-1, it showed far better activity than imipramine in FST ($p < 0.001$). Our studies indicate that *Toddalia asiatica* root extract showed significant neuropharmacological activities which may be related to activation of serotonergic, noradrenergic and dopaminergic pathways

INTRODUCTION

India is frequently known to as the "botanical garden of the world" due to its abundant natural resources. The country is home to over 6,000 plant species used in traditional, folklore, and herbal medicine. Of these, the Indian medicinal system has officially recognized 1,500 plants, with around 500 being commonly utilized. *Toddalia asiatica* has been part of folk medicine in both India and China since the 18th century. Belonging to the Rutaceae family, this plant is a staple of Chinese traditional medicine, with its entire structure, especially the roots, used for medicinal purposes and historically distributed by local communities. [1]

In recent years, both domestic and international researchers have conducted extensive studies, revealing that the primary chemical constituents of *Toddalia asiatica* are coumarins and alkaloids. The plant's root bark exhibits "antimalarial, antipyretic, and carminative properties, while its leaves and stems, possess analgesic qualities. Possessing a wide range of clinical purposes, *T.asiatica* is particularly efficient in treating rheumatism, discomfort, bleeding from injuries and sprains. Given its substantial therapeutic importance makeup and neuropharmacological actions of the plant. [2]

Medicinal plants have garnered significant attention from biotechnology researchers, as many pharmaceutical companies rely on them for producing essential compounds. Plants have long served as traditional sources of various chemicals used in pharmaceuticals, fragrances, food colorants, and flavorings, especially in countries like India. [3]

Therefore, the purpose of this research work is to assess the neuropsychiatric effects of *Toddalia asiatica* methanolic root extract" in Swiss albino rats. The study aims to offer insights into the possible therapeutic utility of the *Toddalia asiatica* methanolic root extract compound as a depression treatment option by assessing both behavioral and biochemical attributes.

MATERIALS AND METHODS

Collection of Sample and other Ingredients

The fresh *Toddalia asiatica* root were collected from Thenkasi district in the month of July, 2022 and authenticated (Reg No: XCH-40454) by Dr.S.Mutheeswaran, Scientist, Xavier Research Foundation, St.Xavier's College, Playamkottai, Tamil Nadu, India.

Extraction of *Toddalia asiatica* Root

T.asiatica root powder was extracted three times by reflux technique for two hours each time in a constant temperature waterbath at 65^0C after being separately steeped in methanol for 24 hours at a solid-liquid ratio of 1:50 (g:ml). Pass the mixture through lipophilic microporous filters with a diameter of 0.22µm for additional examination. [4]

Phytochemical Analysis

To conduct the initial phytochemical analysis, a gram of freshly prepared *T.asiatica* root was made upto in 100 ml with the corresponding mother solvent, resulting in a 1% w/v concentration stock solution. This stock solution was then subjected to standard phytochemical procedures. [5] Quantitative phytochemical analysis of protein by Lowry's method, phenols [6], tannins [7], alkaloids, flavonoids, carbohydrates (anthrone method) were estimated.

FTIR Spectrum Analysis

Using a mortar and pestle, combine the *Toddalia asiatica* root extract with KBr salt then squeeze into a thin pellet. These samples analyzed using FTIR spectroscopy, specifically on a Shimadzu FTIR Spectrometer 8000 series, with scans conducted over the range of 4,000–400 cm^-1 [8].

Antidepression Activity

Experimental animals

Swiss male albino rats 6 per group were acclimatized to the experimental room under conditions of 23±2°C temperature as well as a 12-hour light-dark cycle. Female rats had not been included in the study due to reports indicating that estrogen, a female sex hormone, may have antidepressant effects. Water and food were available to the animals at all times. Before and after the drugs were administered, they fasted for two hours. The Institutional Animals Ethics Committee (IAEC) met on March 23, 2023, and approved the experimental procedure. Guidelines for animal care procedures were provided by the Government of India's Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (Ref: SBCP/2022-23/ CCSEA/IAEC/I(2)/F16/368).

Acute Toxicity Studies

The methanol extract of *Toddalia asiatica* root underwent acute oral toxicity testing following the revised OECD (2002) guidelines No. 423. Animals were monitored for behavioral changes hourly for four hours and daily for fourteen days. Administration of the extract at doses up to 2000 mg/kg orally did not result in any toxicity in rats. Therefore, "doses ranging from 100 mg/kg to 400 mg/kg of the extract were selected for subsequent studies.

Experimental Design

Rats were put into five groups (n = 5). The animals had been administered medication 60 minutes before the research "began.

Group I: Normal control- rats received normal saline 2ml/kg orally

Group II: Positive control- rats received imipramine 30mg/kg orally

Group III: rats received META 100mg/kg orally

Group IV: rats received META 200mg/kg orally

Group V: rats received META 400mg/kg orally

Procedure" for ARS

To induce acute restraint stress (ARS), rats were individually placed in plastic rodent restraint devices for 12 hours. This method effectively restricted their physical movements without causing pain. Animals were denied water and food during this time of stress exposure. After 12 hours of restraint, the rats were released from the devices, and 40 minutes after release, they underwent behavioral tests followed by biochemical measurements. In contrast, rats in the normal control group were housed in standard animal cages within the experimental room.

Behavioral tests

Depression-like behaviors in rats were evaluated using tests like the Locomotor Activity Test and TST, conducted 40 minutes after subjecting them to restraint stress. Each rat also underwent a 10-minute pretest for the forced swim test (FST) simultaneously. Relevant samples were given out the next day, 23.5 hours later, and the primary test was carried out 30 minutes later. Rats placed under restraint were compared to a control group, and following the FST on the 90th day, "oxidative stress markers like catalase (CAT), malondialdehyde (MDA), superoxide dismutase (SOD), and lipid peroxidation (LPO) had been assessed. [9]

Tail Suspension Test

After subjecting the animals to restraint stress, each rat was individually suspended by the end of its tail utilizing micropore adhesive tape, positioned approximately 1cm from the end, in a suspension box with the head 50 cm above the floor. This occurred 40 minutes after the restraint stress procedure. For six minutes in total, each rat was suspended, and the last four minutes of that time were used to measure the immobility period. While hanging passively and without movement, the rat was said to be immobile. Antidepressant medication usually shortens the period of time without movement during this test.

Forced Swimming Test

On the fourteenth day of the experiment, each rat was given ten minutes to swim on their own to get used to the environment. The relevant samples were then given out 23.5 hours later, and on the fifteenth day, the primary test was run 30 minutes later. Rats were used in the experiment, and they were kept in a cylindrical tank 40 cm in diameter and 60 cm in height with 30 cm of fresh water kept at 25°C ± 1°C. To maintain consistency and avoid any impact from previously used water on the following behaviors, the water was replaced after every rat. Each rat exhibited vigorous movement in the initial 2-minute period of the test. An observer manually recorded the length of immobility for the next 4 minutes of the 6-minute testing period. Rats had been considered motionless when they floated upright as well as moved very slightly to keep their heads above water. Rats were dried with a cotton towel after their swim and put back in their own cages. When compared to a control group, an increase in immobility time suggests depressive-like effects, while a decrease in immobility duration supports antidepressant-like benefits.

Locomotor Activity Test

To evaluate the locomotor activity of albino rats, thirty rats in all were split up into five groups of six rats each (n = 6). Methanol extract was given to the experimental groups at doses of 25mg/kg, 50mg/kg, and 100mg/kg respectively, while imipramine hydrochloride served as the standard treatment. Control rats were administered physiological saline. Thirty minutes before the experimental trials began, the treatments for the control and experimental animals were administered.

The albino rats were placed in an open field apparatus consisting of a 45 cm diameter arena divided into 16 equal areas. This occurred 15 hours after the final treatment. The rats were positioned in the arena's center, and their behavioral responses, including rearing frequencies, locomotion, and defecations, were observed for a period of 5 minutes.

Biochemical estimations: Collection of blood samples

On the 90th day, both unstressed and stressed rats underwent testing for antidepressant activity. On the 91st day, one hour after administering the drug, blood samples (1.0–1.5mL) had been collected from the rat's retro-orbital plexus. Following the separation of the plasma, the levels of corticosterone and nitrite were assessed using a refrigerated centrifuge (Remi, Mumbai, India) set at 350 g for ten minutes. [10]

Estimation of plasma nitrite levels

The Green et al. (1982) approach was utilized to determine the levels of plasma nitrite. One part of "a 5% w/v aqueous solution of m-phosphoric acid was mixed with one part of 0.1% w/v N-(1-Naphthyl) ethylene diamine dihydrochloride, and the combination was refrigerated at 0°C for 60 minutes. Following a 0.5 mL/0.5 mL mixture of this solution and plasma, the mixture was left to stand at room temperature in the dark for 10 minutes. The absorbance at 546 nm was then measured using a UV-visible spectrophotometer.

Estimation of plasma corticosterone levels

The method outlined by Bartos and Pesez in 1979 had been used to assess plasma corticosterone levels. Initially, 1.0 mL of the sample was mixed" with 0.50 mL of a 0.10% w/v solution of p-nitroso-N, N-dimethylaniline in ethanol. The liquid was mixed with 0.50mL of 0.10M sodium hydroxide after it had cooled for five minutes in ice water. Cotton wool was used to seal the tubes, which were then left in the dark at 0°C for five hours.

Then, 2.0mL of sodium carbonate/bicarbonate buffer (pH-9.8), 5.0mL of phenol in ethanol (0.10% solution), and 0.50mL "of potassium ferricyanide (1.0 w/v aqueous solution) had been added to the mixture. Subsequently, the tubes were incubated in a water bath at 20 ± 2°C for 10 minutes. Lastly, the absorbance of the solution at 650 nm was measured using a UV-visible spectrophotometer.

Biochemical estimations in brain homogenate

On the ninetieth day, after blood samples had been taken, the mice were beheaded and their brains removed. After the isolated brain samples were rinsed in a cold buffer (pH 7.4) containing 0.02 M ethylenediamine tetraacetic acid (EDTA), 0.1 M Tris", and 0.25 M sucrose, they were weighed. Using a Teflon glass homogenizer, the brain samples had been homogenized in nine "liters of cold 0.25M sucrose–0.1 M Tris–0.02 M EDTA buffer (pH-7.4). Next, the homogenate was placed in a refrigerated centrifuge and

centrifuged twice at 350g for ten minutes at 4°C. The obtained pellet was disposed of and the supernatant was collected.

The recovered supernatant was then centrifuged" for 20 minutes at 4°C at 8064 g. Assays for superoxide dismutase, reduced glutathione, malondialdehyde, as well as catalase activity were conducted using the supernatant that was produced from this step.

Determination of Malondialdehyde Level

The spectrophotometric assay method using TBA (thiobarbituric acid) was utilized to detect the level of malondialdehyde (MDA), which is utilized as an indication of lipid peroxidation. Ohkawa et al. first developed this approach in 1979. In this experiment, TBA reacts with specific lipid peroxidation products at a high temperature and acidic environment to form a compound that is pink in color.

250µl of liver homogenate, "500µl of distilled water, 500µl of 15% trichloroacetic acid (TCA), and 500µl of 0.37% TBA were employed in the experiment. The TCA and TBA solutions were prepared in 0.25 M HCl. For ten minutes, the mixture was boiled in a bath of boiling water. The samples undergo a 10-minute, 12,000×g centrifugation after cooling. The absorbance of the resulting supernatant was determined with a spectrophotometer set at 535 nm.

Determination of Superoxide Dismutase Activity

Superoxide dismutase (SOD) activity had been measured employing a colorimetric technique" that was reported by Misra and Fridovich in 1972. When oxygen is present and epinephrine is oxidized at an alkaline pH, superoxide radical anions (O_2) are produced indirectly. Then, spectrophotometric measurements were made at 485 nm to determine the pink-colored oxidation product of epinephrine, known as adrenochrome.

Estimation of reduced glutathione

The technique reported by Jollow et al. in 1974 was used to measure reduced glutathione levels. First, 1.0 mL of 10% v/v diluted post-mitochondrial supernatant was combined with a corresponding volume of 4% w/v sulfosalicylic "acid, and the mixture was allowed to incubate for at least one hour at 4°C. The proteins were then precipitated by centrifuging the mixture at 81g for 15 minutes at 4°C.

The test was carried out by adding 0.2mL of 5,5-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent, 0.1mM, pH-8.0) and 0.1mL of the supernatant to a total volume of 3.0mL. The phosphate buffer was added at a concentration of 0.1M", pH 7.4. Immediately after, a spectrophotometer was used to measure the yellow color that had emerged at "412nm. Using a molar extinction value of 1.36 × 10^4 M^-1 cm^-1, glutathione (GSH) concentrations had been calculated and expressed in micromoles per milligram of protein.

Estimation of catalase activity

Catalase activity had" been determined following the approach outlined by Saint-Denis et al, 1988. The reaction mixture for this experiment "included 1.0mL of hydrogen peroxide (0.019M), 3.0 mL of total volume, 1.95mL of phosphate buffer (0.05M, pH-7.0), and 0.05mL of post-mitochondrial supernatant (diluted 10% v/v). Using a spectrophotometer set at 240 nm, changes in absorbance were observed.

The extinction coefficient of hydrogen peroxide (43.6 M^-1 cm^-1) had" been employed to evaluate the catalase activity. The results were revealed as micromoles of hydrogen peroxide broken down in a minute per milligram of protein.

Statistical "analysis

All results are presented as Mean ± S.E.M. Using SPSS software 2.0, a one-way analysis of variance (ANOVA) was carried out on the data with a significance threshold of $p<0.05$.

RESULTS AND DISCUSSION

Phytochemical screening plays a crucial role in isolating various constituents from plants to evaluate their medicinal uses and biological activities. The presence of particular phytoconstituents in plants that have precise physiological effects on living systems is thought to confer medicinal value upon them. To find the secondary metabolites in leaf extracts and evaluate their possible medicinal uses, phytochemical screening is a must. Regarding *Toddalia asiatica*, the plant's therapeutic qualities are ascribed to the existence of particular secondary metabolites that have different physiological effects on human beings. The phytochemicals found in the *Toddalia asiatica* root extracts were documented in Table 1, while Table 2 provides information on the quantification of phytochemicals present in it. The presence of bioactive elements such as "flavanoids, tannins, phenols, terpenoids, carbohydrates, saponins, alkaloids, cardiac glycosides, proteins, sterols, and tri terpenoids" accounts for *Toddalia asiatica* root medicinal effectiveness.

Table 1: Phytochemical Screening of *Toddalia asiatica* root extracts

S.No	Phytochemicals	Methanol	Ethanol	Aqueous	Ethyl Acetate
1.	Tannins	Present	Present	Present	present
2.	Saponins	Present	Absent	Absent	Absent
3.	Flavonoids	Present	Present	Present	Present
4.	Steroids	Present	Present	Absent	Absent
5.	Glycosides	Present	Present	Absent	Present
6.	Alkaloids	Present	Present	Present	Present
7.	Phenol	Present	Present	Present	Present
8.	Terpenoids	Present	Present	Absent	Absent
9.	Carbohydrates	Present	Present	Present	Present

Table 2: Quantitative Phytochemical Analysis of *Toddalia asiatica* root extracts (mg/g)

Phytochemicals	Aqueous extract	Ethanol extract	Ethyl acetate extract	Methanol extract
Flavonoids	1.5±0.046	2.3±0.059	1.9±0.018	2.3±0.087
Alkaloids	1.4±0.052	2.1±0.112	1.7±0.058	2.0±0.024

Tannins	0.3±0.028	0.5±0.063	0.4±0.028	0.5±0.035
Phenols	0.2±0.016	0.43±0.241	0.3±0.006	0.39±0.346
Carbohydrates	0.38±0.017	0.45±0.058	0.35±0.017	0.45±0.0.058

FTIR analysis is employed to find out functional groups in a sample by analyzing the peak values within the infrared radiation range. In the case of *Toddalia asiatica* root extracts, FTIR spectroscopy was utilized to examine the samples and separate the functional groups of the elements based on their peak ratios. The results of methanol extract of *Toddalia asiatica* Root FTIR analysis were shown in figure 4 and the peak values also given in table 3.

Table 3: FTIR interpretation of compounds of methanol extract of *Toddalia asiatica* Root

S.No	Peak	Functional Group	Phytocompound Identified
1.	473.49	S-S stretch	Aryl disulphides
2.	519.78	C-I, C-Cl	Halogen compound (Chloro compound, Iodo compound) alkyl halides
3.	599.82	C-I, C-Cl	Halogen compound (Chloro compound, Iodo compound) alkyl halides
4.	649	C-Br stretch	Aliphatic Bromo compounds
5.	676.97	C-Br stretch	Aliphatic Bromo compounds
6.	754.12	C-Cl stretch	Aliphatic chloro compound
7.	824.51	=C-H, bonding	Alkane
8.	920.95	=C-H, bonding	Alkane
9.	1012.56	PO3 stretch	Phosphate ion
10.	1059.81	PO3 stretch	Phosphate ion
11.	1135.99	O-H Bond, alcoholic group	Phenol or tertiary alcohol
12.	1204.46	O-H Bond, alcoholic group	Phenol or tertiary alcohol
13.	1338.51	O-H Bond, alcoholic group	Phenol or tertiary alcohol
14.	1400.22	O-H Bond, alcoholic group	Phenol or tertiary alcohol
15.	1457.12	C=C-C, Aromatic ring stretch	Aromatic compound
16.	1481.23	C=C-C, Aromatic ring stretch	Aromatic compound
17.	1511.12	C=C-C, Aromatic ring stretch	Aromatic compound
18.	1609.49	C=O Stretching	Ketone compound
19.	1731.96	C=O Stretch	Aldehyde compound
20.	2332.74	Multiple bonding, carbon-carbon triple bond	Nitrile compound, terminal alkynes
21.	2828.41	C-H stretch	Methoxy methyl ether
22.	2882.42	O-H stretch, carboxylic group, acidic, H- bonded	Carboxylic acids, hydroxyl compound

23.	2972.1	C-H stretch, aliphatic	Alkanes
24.	3389.66	O-H stretch Hydroxyl group, H-bonded	Poly hydroxyl compound

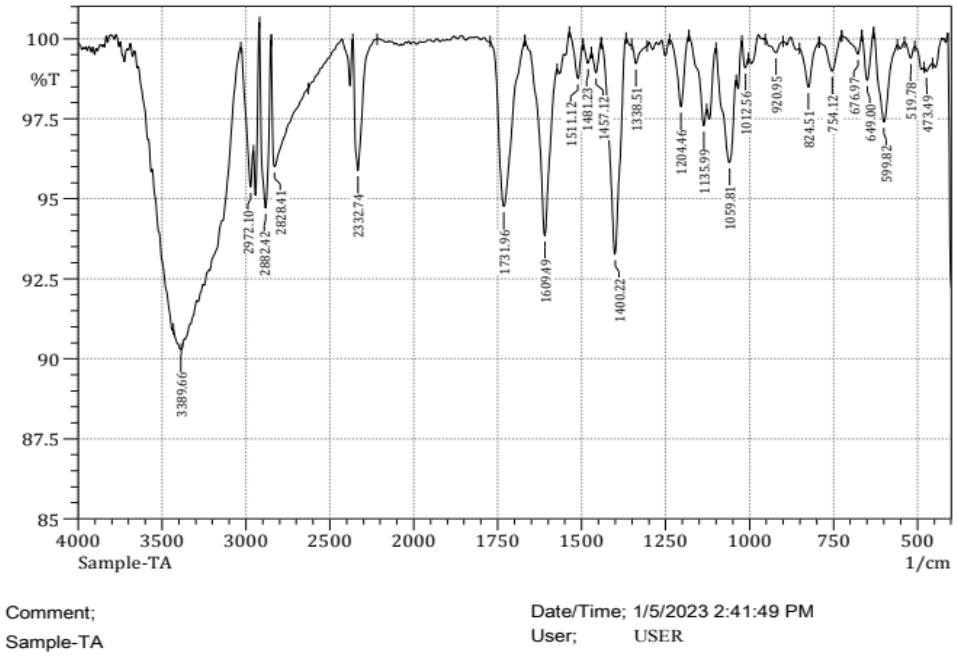

Fig 1: FTIR analysis of Methanolic extract of *Toddalia asiatica* Root

These results verify the existence of functional groups such as "H-bonded, O-H stretch, carboxylic group, C=N stretch, carbonyl compound, C=O stretch, =C-H bending and C-Cl bonds were present. FTIR analysis reveals the presence of various functional groups such as Aryl disulphides, phenols, halogen compounds, alkenes, phosphate ions, aromatic compounds, ketones and poly hydroxyl compounds. These FTIR bands correspond to "stretching and vibrational bands associated with compounds like flavonoids, terpenoids, phenols, amino acids, and alkaloids.

Acute Toxicity Studies

At a dose of 2000 mg/kg, a methanolic extract of *Toddalia asiatica* root in Swiss albino rats did not trigger any physiological alterations or mortality.

Antidepression Activities

Table 4, 5 and 6 showed the results of behavioral tests done in swiss albino rats after the oral administration of Toddalia asiatica root extract. They showed dose-dependent action ($p < 0.001$) and dramatically shortened the immobility period in both FST and TST. It demonstrated much more action than imipramine in FST at 400 mg kg-1 ($p < 0.001$). When compared to the control group, they demonstrated a considerable increase in swimming time ($p < 0.001$). Figure 2,3,4 and 5 showed the results of effect on the levels of Plasma Nitritre and Cortisterone, MDA activity, SOD level, "reduced glutathione and catalase activity in mice by oral administration of *Toddalia asiatcia* root methanol extract respectively which revealed that *Toddalia asiatcia* root extract significantly had the antidepressant – like activity.

Table 4: Effect of Methanolic Extract of *Toddalia asiatcia* root on mice behaviors in TST

Group No.	Drug and dose	Tail Suspension test Duration of Immobility (Sec)		
		30 days	60 days	90 days
1	Control	185 ± 6.12	174 ± 5.8	182 ± 5.16
2	Imipramine 30mg/kg	93.4 ± 3.71***	63.9 ± 3.65***	46 ± 2.98***
3	*Toddalia asiatica* 100 mg/kg p.o	151.6 ± 3.24*	128.5 ± 4.7*	106.3 ± 3.2**
4	*Toddalia asiatica* 200 mg/kg p.o	135.2 ± 2.43*	98.2 ± 3.5***	89.8 ± 3.59***
5	*Toddalia asiatica* 400 mg/kg p.o	94.6 ± 3.5**	67.9 ± 2.48***	47.6 ± 1.3***

Each group has n=4, with the mean ± SEM. Group I and Group II were contrasted. Group II was compared with the remaining groups. There were significant changes to the mean values *** = P< 0.001 highly significant; ** = P< 0.01 moderately significant; * = P< 0.05 significant

Table 5: Effect of Methanolic Extract of *Toddalia asiatcia* root on mice behaviors in Forced Swim Test

Group No.	Drug and dose	Duration of Immobility (Sec)		
		30 days	60 days	90 days
1	Control	171.20 ± 3.21	173.41 ± 3.53	172.87 ± 2.91
2	Imipramine 30mg/kg	32.10 ± 0.75***	28.45 ± 0.72***	27.52 ± 0.65***
3	*Toddalia asiatica* 100 mg/kg p.o	158.4 ± 2.7	132 ± 1.7	105.8 ± 2.5*

4	*Toddalia asiatica* 200 mg/kg p.o	141.5 ± 1.2	126.3 ± 2.3*	97.2 ± 1.2***
5	*Toddalia asiatica* 400 mg/kg p.o	124.3 ± 2.3*	97.2 ± 1.24**	72.8 ± 2.1***

The values in each group (n = 4) are mean ± SEM; Group I was compared to Group II. The remaining groups were compared using Group II. There were significant "changes to the mean values *** = P< 0.001 highly significant; ** = P< 0.01 moderately significant; * = P< 0.05 significant

Table 6: Effect of Methanolic Extract of *Toddalia asiatcia* root on mice behaviors in Locomotor Activity

Group No.	Drug and dose	Locomotor Activity in Minutes			
		Before Treatment	30 days	60 days	90 days
1	Control	51 ± 0.12	51 ± 0.12	53 ± 0.23	53 ± 0.35
2	Imipramine 30mg/kg	54 ± 0.20	98 ± 0.39***	114±0.45***	121±0.28***
3	*Toddalia asiatica* 100 mg/kg p.o	58 ± 0.24	65 ± 0.31	84 ± 0.2	98 ± 0.15**
4	*Toddalia asiatica* 200 mg/kg p.o	62 ± 0.17	72 ± 0.41	94 ± 0.25**	111±0.16***
5	*Toddalia asiatica* 400 mg/kg p.o	68 ± 0.28	86 ± 0.12*	117 ± 0.17**	136±0.25***

Each group has n=4, with the mean ± SEM. Every group was contrasted with group I. There were significant "changes to the mean values *** = P< 0.001 highly significant; ** = P< 0.01 moderately significant; * = P< 0.05" significant

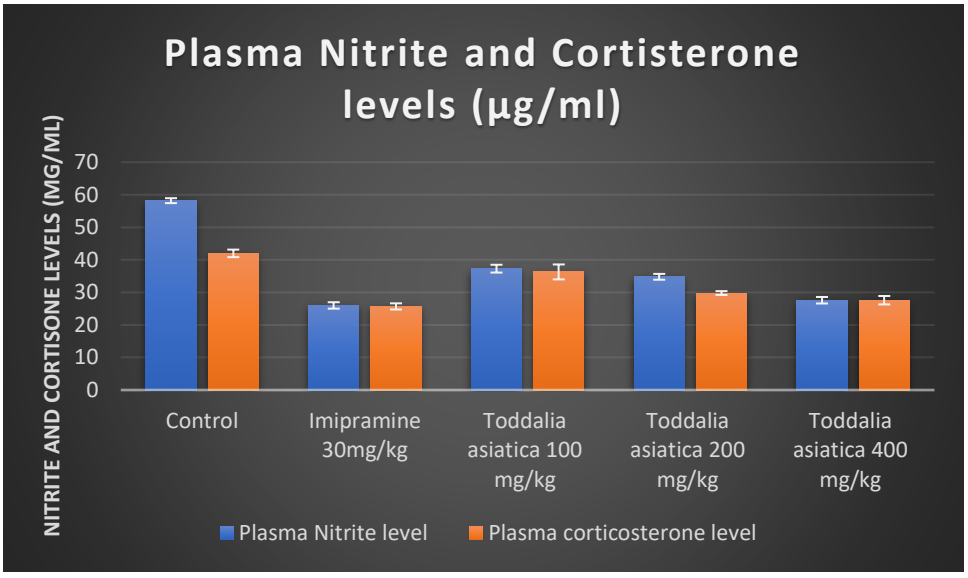

Fig 2: Effect on the levels of Plasma Nitritre and Cortisterone in mice by oral administration of *Toddalia asiatcia* root methanol extract

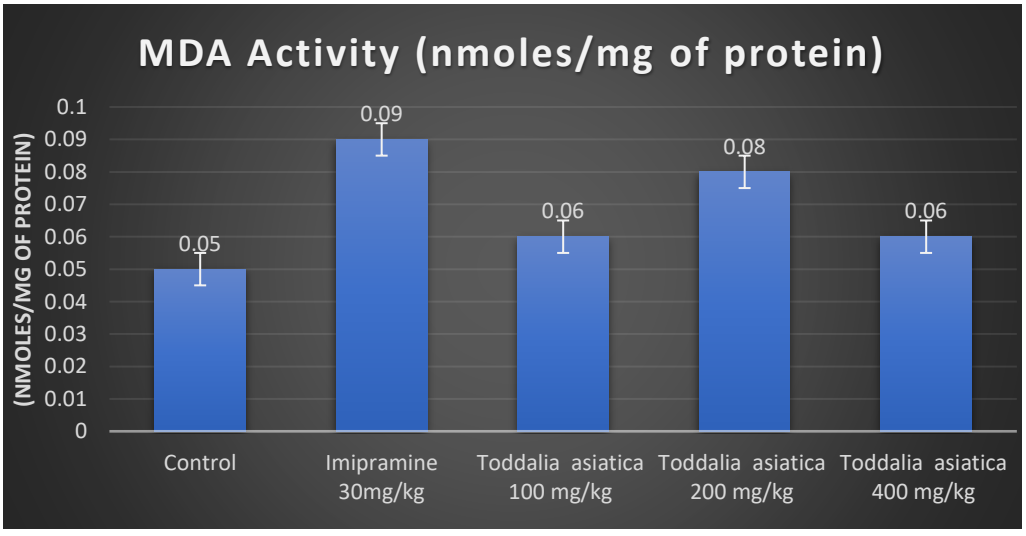

Fig 3: Effect on the MDA acitvity in mice by oral administration of *Toddalia asiatcia* root methanol extract

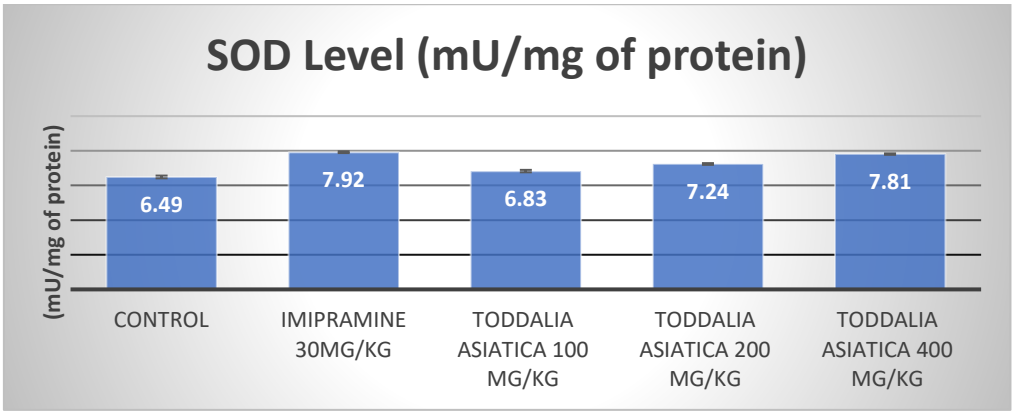

Fig 4: Effect on the levels of SOD in mice by oral administration of *Toddalia asiatcia* root methanol extract

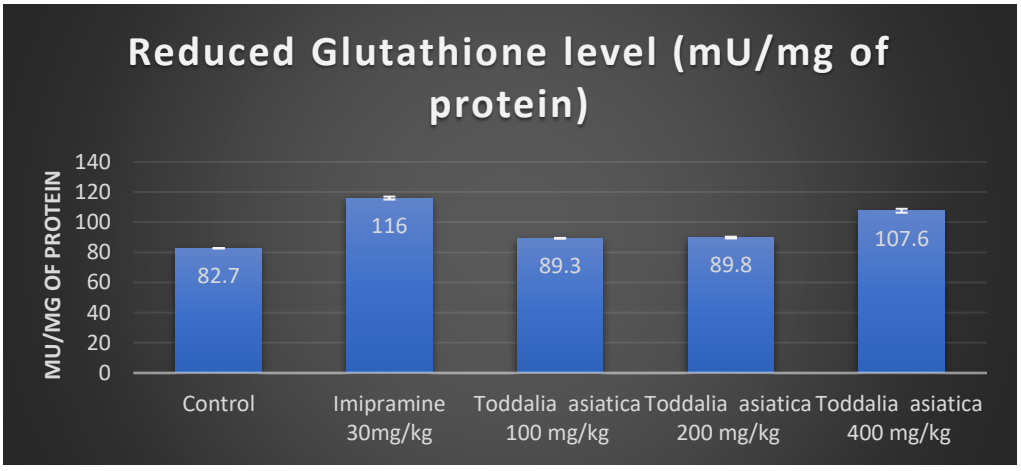

Fig 5: Effect on the levels of reduced glutathione in mice by oral administration of *Toddalia asiatcia* root methanol extract

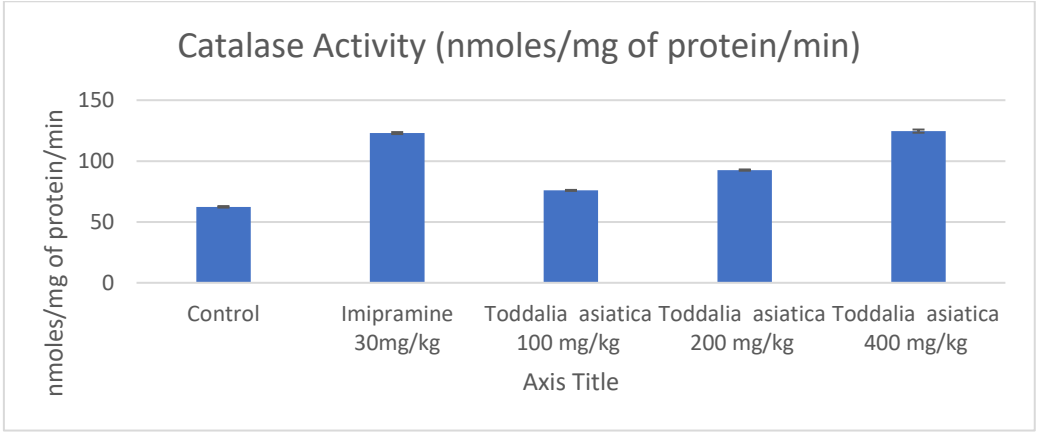

Fig 6: Effect on catalase activity in mice by oral administration of *Toddalia asiatcia* root methanol extract

When under stress, "the hypothalamic-pituitary-adrenal (HPA) axis increases the amount of glucocorticoids in the blood, such as cortisol in primates and corticosterone in rodents. Depression has been connected to increased blood glucocorticoid levels resulting from over-activation of the HPA axis. Neurogenesis, excitability, neuronal survival, and memory formation are all impacted by cortisol. By affecting these brain activities, high cortisol levels may exacerbate depressive symptoms [11]. According to studies, long-term antidepressant treatment in rats reduces HPA axis activity indicating that a key component of treating human depression may be getting the HPA axis back to normal. Major depression has been linked to reactive oxygen species (ROS). The pathophysiology of depression may be aided by processes that result in ROS overproduction, lipid peroxidation, and decreased antioxidant enzyme activity. These processes include immune-inflammatory activation, enhanced monoamine breakdown, and lipid abnormalities [12].

In this study, the methanol extract of *Toddalia asiatica* root was administered daily for 90 days, revealing substantial antidepressant-like effects in Swiss albino rats. Acute Restraint Stress (ARS) is widely accepted as the most reliable animal model for studying depressive behaviors akin to those observed in humans [13]. Tests like the Forced Swim Test (FST), Tail Suspension Test (TST), and locomotor activity were used to see whether the *Toddalia asiatica* root extract altered the rats' depressive-like behavior. When compared to control rats, ARS enhanced the total number of immobility phases in both TST and FST, revealing that depression-like behavior was effectively induced. Stressed rats' immobility times were considerably lowered in both TST and FST when they received oral treatments with imipramine (30 mg/kg) and various concentrations of methanol extract (100, 200, and 400 mg/kg). These treatments also showed considerable antidepressant-like effects. Rats' locomotor activity was also significantly boosted by imipramine and the methanol extract, indicating that the drugs had stronger central nervous system stimulant effects.

From this research work, we found that administering the methanol extract of *Toddalia asiatica* root to rats exposed to various stressors for 90 days led to elevated levels of malondialdehyde in the brain and nitrite in the plasma. Concurrently, there was decreased in plasma corticosterone levels, as well as in brain levels of reduced glutathione and catalase activity. Takomthong et al. (2020) discovered that seven of the nine coumarins isolated from *Toddalia asiatica* function as multifunctional agents capable of inhibiting the development of Alzheimer's disease (AD). Among these, phellopterin exhibited a notable protective effect against neuronal cell damage caused by H_2O_2 and Aβ1-42 toxicity.[14] These results align with earlier research, which demonstrated that acute restraint stress (ARS) negatively impacted the brain's antioxidant defenses, leading to elevated "lipid peroxidation and nitrite levels, alongside reduced glutathione levels and catalase activity [15].

CONCLUSION

In summary, the methanol extract of *Toddalia asiatica* root demonstrated significant antidepressant-like effects in Swiss albino rats, potentially by lowering plasma corticosterone levels, and increasing nitrite levels. It may be able to boost the overall response to antidepressant treatment through the integration of several drugs that have similar but diverse effects. Furthermore, this strategy might enable a more potent antidepressant effect without requiring larger amounts of a specific medication, which could result in more undesirable side effects. [16] These results suggest that further exploration of *Toddalia asiatica* root extract for treating depression in humans.

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