Exploring the Pharmacological Potential of *Ipomoea aquatica forsk* for Neuropathic Conditions: Extraction, Pharmacognostic Evaluation, and Toxicity Assessment in Animal Models

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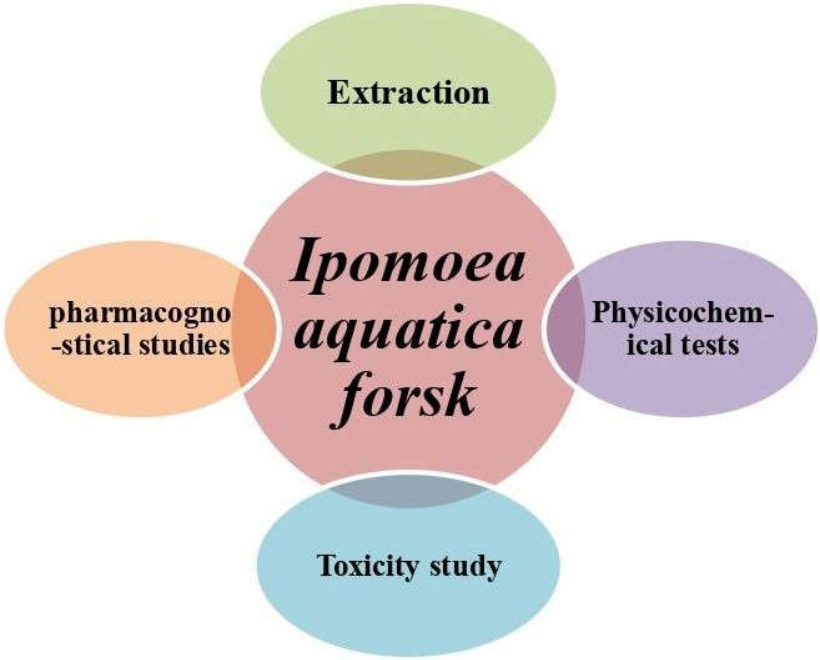
Abstract:

The present study embarked on a comprehensive investigation of *Ipomoea aquatica forsk*, a plant with promising therapeutic potential. We aimed to extract *Ipomoea aquatica forsk*, conduct a thorough pharmacognostic analysis to identify its physicochemical properties and perform a range of physicochemical tests to determine its composition and characteristics. Furthermore, an animal toxicity study was conducted to elucidate the underlying mechanisms of action of the hydroalcoholic extract of *Ipomoea aquatica forsk*, with a particular focus on its safety profile. The results of the toxicity study were reassuring, revealing no significant signs of hepatotoxicity, and nephrotoxicity in the specimens tested. These findings suggest that the plant could potentially serve as an effective and safe treatment option for the clinical management of various health conditions, without the risk of toxicity. Moreover, the study's results imply that the hydroalcoholic extract of *Ipomoea aquatica forsk* may hold significant promise as a therapeutic agent for the management of neurological problems.

Keywords: Parkinson's disease, *Ipomoea aquatica forsk,* and neurological problems.

Graphical Abstract:



Introduction:

Neurological disorders, including Alzheimer's, Parkinson's, multiple sclerosis, epilepsy, and headache disorders, account for 3% of the global disease burden, with exponential growth in the next decade [1]. Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized primarily by motor impairments, including tremors, rigidity, and bradykinesia. Additionally, cognitive deficits and neuropsychiatric symptoms often accompany the motor symptoms, contributing to the overall burden of the disease. The deposition of alpha-synuclein aggregates, known as Lewy bodies, in dopaminergic neurons of the substantia nigra is a pathological hallmark of Parkinson's disease. PD is defined as a progressive loss of muscle control, which increases the risk of dying young. It is more prevalent in the sixth decade of life, with men being more susceptible. Symptoms include tremors, rigidity, bradykinesia, and compromised balance. Non-motor disturbances include cognitive deficits, anxiety, stress, and other symptoms [2-4]. PD is the second most prevalent neurological disorder globally, impacting 1.5 million Americans [5-10]. The brain's significant oxygen consumption, accounting for 20% of the body's basal oxygen utilization, makes it vulnerable to oxidative stress. The interplay between oxidative stress, dopamine metabolism, and iron accumulation is crucial in PD neuropathogenesis [16-39]. Natural products have been extensively studied for their potential protective effects against neurological problems using animal models. Studies have shown that ceftriaxone, quercetin, acacia catechu leaf extract, safflower flavonoid extract, isolecanoric acid, carnosic acid, ocimum sanctum leaf extract, and hesperidin, have shown antioxidant and memory-enhancing activities. These studies highlight the therapeutic potential of natural products in mitigating neurodegenerative processes associated with neurological problems [40-48].

Ipomoea aquatica forsk, commonly known as water spinach, is a vascular plant characterized by its glabrous, semi-aquatic trailing vine structure and milky sap. It thrives in water environments, either submerged or floating, and is indigenous to tropical and subtropical regions, where it can be found growing wild or cultivated.

The primary objective of the current research was to extract *Ipomoea aquatica forsk*, a plant species known for its potential medicinal properties. Through the process of pharmacognostic studies, we aimed to identify and analyze the various chemical compounds present in the plant material. Physicochemical tests were conducted to assess the physical and chemical

characteristics of the extracted compounds. Furthermore, an animal toxicity study was carried out to investigate the effects of the hydroalcoholic extract of *Ipomoea aquatic forsk* on Swiss albino rats. This study was crucial in understanding the potential risks and benefits associated with the use of this plant extract in various medical applications. By elucidating the underlying mechanisms of action of the extract, the researchers aimed to provide valuable insights into its pharmacological properties and potential therapeutic uses. Overall, the comprehensive approach taken in this study sought to deepen our understanding of *Ipomoea aquatica forsk* and its potential benefits for human health. The findings of this research could potentially contribute to the development of new drugs or treatments derived from natural sources, ultimately benefiting patient care and improving overall health outcomes.

Materials and Methods:

Ipomoea aquatica forsk, and ethanol.

Collection and authentication of the plant:

The entire plant specimens were gathered from the pond area in Cooch Behar, located in North Bengal. The validity and classification of these plants were verified by renowned expert Dr. A. P. Das, who serves as the Professor and Head of Taxonomy and Environmental Botany, as well as the Herbarium- in charge at The North Bengal University in Darjeeling. Additionally, an official herbarium was successfully recorded and stored under the Department of Botany with a designated accession number 09696 on May 20th, 2013 at The North Bengal University in Darjeeling.

Method of extraction:

The aerial components of *Ipomoea aquatica forsk* were harvested and subjected to thorough washing with running water. Subsequently, they were shade-dried under ambient room conditions. Upon drying, 1 kg of the resultant material was processed into coarse powder and sieved through a 60-mesh sieve to ensure uniformity. The air-dried powdered drug was then subjected to maceration with ethanol (90% v/v) in a glass percolator and left to infuse at room temperature for approximately 24 hours. Following this period, the extract was meticulously filtered, and the filtrate was concentrated by a rotary vacuum pump, yielding a solid mass. The weight of the extract obtained from this process amounted to 19.6%.

Pharmacognostical Studies:

Morphological studies:

A thorough examination of the morphological attributes of fresh leaves and stems was conducted as part of the study. Key macroscopic characteristics observed for the fresh leaves encompassed size and shape, coloration, surface features, venation patterns, presence or absence of petioles, apex morphology, margin delineations, base structure, lamina properties, texture, odor, and taste profiles. Similarly, the study encompassed the analysis of various stem characteristics such as nodes, internodes, diverse buds, and surface attributes including color and odor. This comprehensive assessment aimed to elucidate the distinctive morphological features of the plant material under investigation [49, 50].

Microscopic Studies:

The outer epidermal membranous layers from both the upper and lower surfaces of freshly collected leaves were carefully cleared using chloral hydrate solution, followed by mounting with glycerin. This allowed for thorough scrutiny under a high-powered compound microscope, allowing for detailed observation and analysis of the presence, types, and distribution patterns of stomata and epidermal cells. The quantification of stomatal number, stomatal index, vein-islet number, and vein-let termination number was performed using precise measurement tools such as a camera Lucida and a stage micrometer. Additionally, transverse sections of the leaf and stem underwent clearance, mounting, staining, and subsequent microscopic observation. These detailed analyses aimed to provide insights into the anatomical characteristics of the plant material, facilitating a comprehensive understanding of its morphological structure [51].

Powder microscopy

The examination under the microscope revealed detailed information about the particle size, shape, and structural features of the powdered plant material. This analysis is crucial for identifying the authenticity, purity, and quality of the botanical product. Additionally, microscopic observation allowed for the detection of any contaminants or adulterants present in the powder, ensuring the safety and efficacy of the plant material for various applications. The comprehensive evaluation of the physical and chemical properties of the powdered substance is essential for further research, development, and utilization in industries such as pharmaceuticals, cosmetics, and food processing [52].

Fluorescence analysis

The plant material's fluorescence was studied in different solvents under visible, short UV, and long UV light. For this analysis, alcohols, mineral acids in various concentrations, halogens, and

other chemical and organic reagents were used. This thorough examination aimed to reveal the fluorescence traits of the plant material in different conditions and solvents, offering valuable insights into its chemical makeup and possible pharmacological properties [53, 54].

Physicochemical analysis

The World Health Organization (WHO) guidelines were followed to determine the physicochemical characteristics of powdered leaves. Several ash values, including total, acid-insoluble, water-soluble, sulphated ash, and extractive values, such as alcohol, water-soluble, methanol, and chloroform soluble, were included in these parameters. Additionally, loss on drying, swelling index, and foaming index were included. This comprehensive analysis helps understand the quality and purity of the leaves, assessing their suitability for phytochemical applications [55-57].

Physicochemical Tests:

Confirmatory tests

A series of tests was conducted to evaluate various phytochemical constituents present in the plant material. These included examinations for carbohydrates, proteins, tannins, saponins, flavonoids, terpenoids, and alkaloids. Here's a breakdown of the tests conducted:

Molisch's Test: To find out if there were any carbohydrates present, Molisch's reagent (α-naphthol in ethanol) was applied to the analyte, and then a few drops of concentrated H2SO4.

Biuret Test: The formation of a purple or purplish-red ring between H_2SO_4 and the analyte plus Molisch's reagent indicated the presence of carbohydrates.

$FeCl_3$ Test: The plant extract has been mixed with distilled water, filtered, and exposed to a ferric chloride reagent for the FeCl3 test. The presence of tannins was confirmed by the formation of a blue-black, green, or blue-green precipitate.

Foam Test: Distilled water was mixed with the plant extract in a test tube, vigorously stirred, and boiled with olive oil. The presence of froth indicated the presence of saponins.

Sodium Hydroxide Test: Incremental addition of sodium hydroxide to the extract followed by acid addition resulted in the disappearance of a yellow color.

Lead Acetate Test: A yellow-colored precipitate formed when a few drops of 10% lead acetate were added to the extract, indicating the presence of flavonoids.

Shinoda Test: After adding magnesium turning pieces and concentrated hydrochloric acid drop by drop, the test solution's color changed to pink, scarlet, crimson red, or infrequently green to blue.

Salkowski Test: The presence of terpenoids was indicated by a reddish-brown coloration created at the interface by a mixture of extract, chloroform, and strong sulfuric acid.

Dragendorff's Test: An orange-red precipitate was formed when Dragendorff's reagent was added to the extract, signifying the presence of alkaloids.

Animal Toxicity Study:

Acute (14 days) oral toxicity study of ethanolic extract of Ipomoea Aquatica Forsk in rats bearing the Study No: TOXA (R) EEIA 0322 at the Facility of TAAB Biostudy Services, 69 Ibrahimpur Road, 1st Floor, Flat No. 1A, Kolkata – 700032. Animal House: 46C, Bidhan Pally, 1st Floor, Kolkata – 700 032.

Test item information

The test item is the ethanolic extract of *Ipomoea aquatica forsk*, a brownish liquid with storage conditions below 25℃.

Reference item (control item)

The reference item is Sodium Chloride Solution, 0.9%, a liquid, clear, and colorless solution, coded SCSTBS, and stored at room temperature between 15 to 30°C.

Justification for selection of control item

The reference item is any item used to provide a basis for comparison with the test item. In this experiment 0.9% saline solution is used because, normal saline is an isotonic solution, meaning that it has the same salt concentration as the body. Isotonic solutions are the same as the body, so the cells can maintain their normal appearance and it is non-corrosive.

Test system

The study involved Swiss Albino Rats of both males and females aged 6-8 weeks, with 6 rats per dose level. The rats were housed in polycarbonate cages with husk bedding, maintained at 20-24°C, relative humidity at 30-70%, and a 12-hour dark/light cycle. A normal Laboratory diet comprised of whole grain cereals was given to rats. The rats were given different doses for male rats (0 mg/Kg, 1000mg/kg, and 2000 mg/kg body weight) and female rats (0 mg/Kg, 1000mg/kg, and 2000 mg/kg body weight). These parameters ensure standardized conditions for the

experimental animals, allowing for accurate assessment of the effects of the administered doses on both male and female rats within the specified age range.

Acute Oral Toxicity Study (LD 50 Calculations, Limit Test Method):

Procedure

Suspensions of the ethanolic extract of *Ipomoea aquatica forsk* at different doses were prepared. A simple method for acute toxicity study is the limit test method. 2000 mg/kg body weight dose of test sample suspension was administered orally to six rats and observed for 24 hours. All the animals treated with a 2000 mg/kg body weight dose survived throughout the dosing periods. So there is no need for further dose preparation and up and down toxicity study.

Methods of randomization, numbering, and grouping of animals

The study involved 24 rats divided into two groups, each receiving doses of 0 mg/kg and 2000 mg/kg body weight. The rats underwent a 7-day acclimation period before dosing, with female rats being nulliparous. Rats were housed in cages with six individuals per cage, segregated by sex, and each animal was marked with picric acid for identification.

Dose preparation

In the acute toxicity study, rats were administered the ethanolic extract of Ipomoea aquatica Forsk at dose levels of 0 mg/kg and 2000 mg/kg. The administration was conducted at a dose volume of 1 ml per 100 g of body weight. The control group of animals received only the vehicle without the extract.

Observations

Throughout the study, the following parameters were carefully monitored:

Symptoms: Daily observation of all animals was conducted to detect any clinical signs. The onset, intensity, and duration of these symptoms, if present, were meticulously recorded.

Mortality: Animals were observed twice daily to monitor mortality rates throughout the study.

Body Weight: The weight of each mouse was measured on day 0 and at weekly intervals thereafter. Mean body weights for each group were calculated to assess any changes throughout the study.

Food Consumption

Weekly food consumption data were collected for each group, comprising six rats per group. The quantity of food consumed by each group was recorded, and then the average food consumption per rat was calculated separately for the control and dose groups. This allowed for the assessment

of any potential differences in food consumption between the control and treated groups throughout the study.

Necropsy:

On day 29 of the study, all animals were humanely euthanized using diethyl ether anesthesia. Subsequently, a necropsy was performed on each animal, and the weights of specific organs including the liver, kidneys, and heart were recorded. These organ weights were documented both as absolute values and as relative values, calculated as a percentage of the body weight. This comprehensive assessment provided insight into any potential effects of the administered substance on organ morphology and physiology.

Histopathology:

Tissue samples from the heart, kidneys, liver, lungs, and stomach of both control animals and those treated at the highest dose level of 2000 mg/kg were meticulously preserved in 10% formalin solution. This preservation method ensures the tissues maintain their structural integrity for subsequent histopathological examination. By examining these tissues under a microscope, we can gain valuable insights into any potential histological changes or pathological findings induced by the administered substance.

RESULTS

Pharmacognostical Evaluation:

Morphological characters:

Ipomoea aquatic forsk belongs to the family Convolvulaceae and typically exhibits long, hollow, and tiny stems measuring 2 to 3 meters (7 to 10 ft.) in length, although they can grow even longer. The stems sprawl prostrate or float on water surfaces, and roots develop from the nodes, extending into the moist soil or mud below.The leaves of *Ipomoea aquatica forsk* are arranged alternately along the stem and are characterized by glabrous petioles ranging from 3 to 14 cm (1 to 6 in) in length. Leaf morphology varies, with shapes ranging from typically sagittate (arrowhead-shaped) to lanceolate, measuring 5 to 15 centimeters (2 to 6 in) in length and 2 to 8 centimeters (0.8 to 3 in) in width. The leaf margins may be entire, angular, or sub-lobed, while the leaf surface is typically glabrous, although occasionally pilose. *Ipomoea aquatica forsk* stems and leaves are green in color, tasteless, and sagittate in shape, with one main nerve, reticulated veins, and no stipules.

Ipomoea aquatica's leaf morphology is green, sagittate, reticular, lacking stipules, simple, with one main nerve and present petioles, measuring 5.0-15.0cm in length. The stems of *Ipomoea aquatica* have detailed morphological characteristics, including green color, no smell, taste, pubescent surface, and nodes with appendages like leaves, branches, and flowers. The stems of Ipomoea aquatica exhibit morphological characteristics such as hollow interiors, axillary buds, lenticels, leaf scars, terminal buds, and flower buds.

Microscopical characters:

Under microscopic examination, stomata were observed to be distributed on both the abaxial (lower) and adaxial (upper) surfaces of the leaves. Notably, both surfaces exhibited paracytic stomata, as depicted in Figure 1.

Under microscopic examination, unicellular covering trichomes were observed on both surfaces of the leaf, with a higher frequency on the upper surface, particularly on the midrib portion. The leaf's transverse section revealed a compact, rectangular epidermal layer with a cuticle on the upper and lower layers. The upper epidermis is enveloped by a cuticle, while the lamina contains a double layer of large, elongated, chlorophyll-containing palisade parenchyma. The midrib portion has a collenchymatous hypodermis and parenchyma cells. Starch granules were found to be present in the midrib portion, as depicted in Fig.2.

The transverse section of the stem appears round in shape, with a glabrous surface and a large hollow pith at the center. The cortex portion is notably thick and consists of several layers, including the exodermis, hypodermis (collenchyma), and endodermis (parenchyma). Large numbers of vascular bundles are arranged in a concentric ring with the pericycle. Within each vascular bundle, the xylem vessels appear prominent and large, followed by the phloem, which is capped by sclerenchyma fibers. Additionally, a few starch grains are observed both below and above the vascular bundle ring, as illustrated in Fig.3 and 4.

Stomatal density was found to be higher on the upper epidermis compared to the lower epidermis. The upper surface of the plant showed frequent vein islets and vein terminals, with a stomatal number of 305/square mm in the upper epidermis and 375/square mm in the lower epidermis. The upper surface of the plant showed frequent vein islets and vein terminals, with a stomatal index of 14.78, a stomatal index of 17.16, and a palisade ratio of 7.24. The upper surface of the skin showed frequent vein islets and terminals, with vein-islet no. in the upper epidermis at 40.66/square mm and vein termination no. at 25.33/square mm.

Fig.1: Paracytic stomata in the upper surface of Ipomoea aquatica; P: Paracytic stomata, E: Epidermal cells

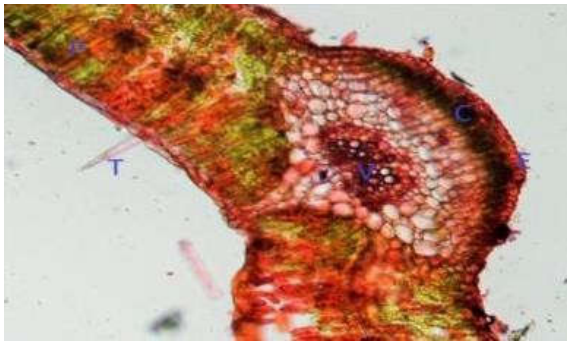


Fig.2: T.S. of Ipomoea aquatica leaf; T: Trichome, P: Palisade cells, V: Vascular bundle, C: Collenchyma, E: Epidermis

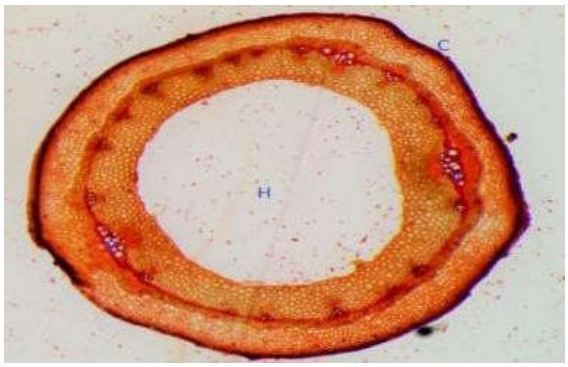


Fig.3: T.S. of Ipomoea aquatica stem; H: Hollow pith, C: Cortex, V: Vascular bundle

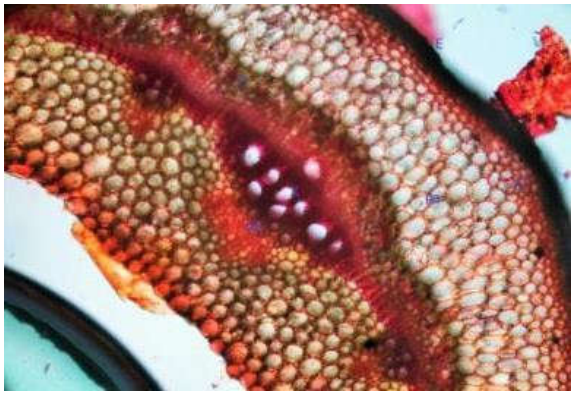


Fig.4: T.S. of the stem of Ipomoea aquatica; E: Exodermis, Pa: Parenchyma, C: Collenchyma, X: Xylem vessel, P: Pericycle, Ph: Phloem

Powder microscopy:

The herb's fine powder has a fibrous texture and a greyish-green tint. It has no flavor and no smell. When the powder of the herb is stained with phloroglucinol in distinct solutions of sulfuric acid and iodine under a microscope, it exhibits many properties. These include, among other things, the existence of paracytic stomata, oil cells, phloem fibers, long unicellular uniseriate covering trichomes, and epidermal cells with thin, somewhat sinuous walls. These features are depicted in Fig.5-12.

Fig. 5: Powder microscopy: Epidermal cells

Fig. 6: Powder microscopy: stomata

Fig. 7: Powder microscopy: Phloem fibre

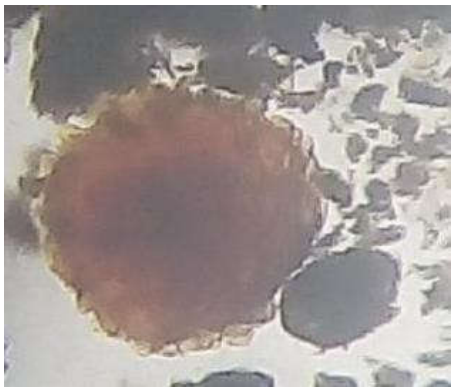


Fig. 8: Powder microscopy: Laticiferous cells

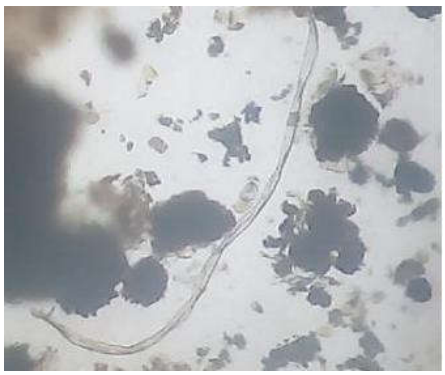


Fig. 9: Powder microscopy: Annular vessels

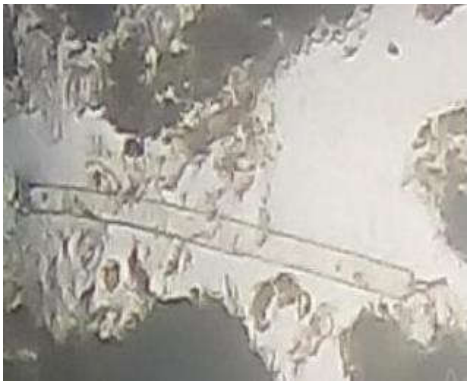


Fig. 10: Powder microscopy: volatile oil cell

Fig. 11: Powder microscopy: Unicellular trichome

Fig. 12: Powder microscopy: calcium oxalate

Analysis of Fluorescence:

Using visible, short UV, and long UV light in a variety of solvents, such as alcohols, mineral acids at varying concentrations, halogens, and other distinct chemical and organic reagents, the fluorescence properties of the powdered plant material(PPM) were studied. The observations are summarized in Table 1.

Powdered drug	Visible light	UV light (short)	UV light (long)
PPM	Green	Greenish brown	Greenish brown
PPM + 5%$FeCl_3$	Deep green	Green	Black Brown
PPM + 1 N HCl	Pale green	Green	Brown
PPM + 1 N HNO_3	Reddish brown	Green	Brown
PPM + 10% $K_2Cr_2O_7$	Reddish	Green	Brown black
PPM + 1M NaOH	Green	Reddish green	Red
PPM + $AgNO_3$	Green	Greenish brown	Light brown
PPM + Ammonia	Citrine green	Greenish black	Brown
PPM + 1 N H_2SO_4	Green	Green	Brown black
PPM + Br_2 water	Reddish green	Brown	Light brown
PPM + 5% H_2O_2	Pale green	Green	Brown
PPM + CCl_4	Green	Green	Greenish brown
PPM + Methanol	Green	Brown	Dark brown
PPM + CH_3COOH	Green	Reddish brown	Dark brown
PPM + Xylene	Greyish green	Grey	Orange
PPM + 5% KOH	Cascade green	Reddish brown	Dark brown
PPM + I_2	Reddish green	Brown	Dark brown

Table 1: Fluorescence Study

Physicochemical analysis:

The physicochemical parameters of powdered leaves were evaluated according to WHO guidelines. The study evaluated various ash values, extractive values, loss on drying, swelling index, and foaming index of a product, resulting in a loss on drying of 4.30, total ash of 9.90, water-soluble ash value of 1.75, acid insoluble ash value of 6.50, extractive value of 42.31, chloroform soluble extractive value at 5.98, ethanolic at 30.07, methanolic at 35.15, swelling index of 25.63 and foaming index of 16.42. Confirmatory tests of various physiochemical tests are given in Table 2.

Sl. No.	Test	Observation	Inference
1.	Molisch's Test	Appearance of purple or violet	Presence of carbohydrates

		ring	
2.	Biuret Test	Formation of violet coloration	Presence of proteins
3.	$FeCl_3$ test	Appearance of greenish-black color precipitate	Presence of tannins
4.	Foam test	Development of stable foam	Presence of saponins
5.	Lead acetate test	Development of yellow color precipitate	Presence of flavonoids – quercetin
6.	Sodium Hydroxide test	The color of the substance was initially yellow, but it disappeared after the addition of acid.	Presence of flavonoids – quercetin
7.	Shinoda test	After a few minutes, a pink scarlet, crimson red, or occasionally green to blue color appeared.	Presence of flavonoids – quercetin
8.	Salkowski test	A reddish-brown coloration of the interface was formed	Presence of terpenoids
9.	Dragendorff's Test	Orange-red precipitate	Presence of alkaloids

Table 2: Confirmatory tests

Acute Oral Toxicity Study:

Clinical signs (Tables 3, 4, and 5)

Male

Throughout the 14-day dosage period, none of the animals in Group I (0 mg/kg) displayed any signs of intoxication (animals' nos. 1 to 6). Comparably, during the same dose interval, animals in Group II (2000 mg/kg) showed no evidence of intoxication (animals' nos. 13 to 18).

Female

Throughout the 14-day treatment period, none of the animals in Group I (0 mg/kg) displayed any signs of intoxication (animal nos. 7 to 12). Comparably, during the same 14-day treatment interval, animals in Group II (2000 mg/kg) showed no evidence of intoxication (animals nos. 19 to 24).

Group No.	Dose (mg/kg)	Sex	No. of Animals in each group	Animal Numbers
I	Control	Male	6	1 – 6
		Female	6	7 – 12
II	2000	Male	6	13 – 18
		Female	6	19 – 24

Table No. 3: Assignment of animals to different groups.

Sex	Group no.	Dose (mg/kg)	Observed signs	Total no. of animals	Animal no.	Period of signs in days From – To	Mortality
Male	I	Control	NIL	6	1 – 6	1 – 14	0/6
	II	2000	NIL	6	13 – 18	1 – 14	0/6
Female	I	Control	NIL	6	7 – 12	1 – 14	0/6
	II	2000	NIL	6	19 – 24	1 – 14	0/6

Table No. 4: Impact of treatment on clinical indicators of intoxication

Mortality

Male and Female: Mortality rates are shown in Table No.5

Group No.	Dose (mg/kg) Body weight		Mortality			
	Male	Female	Males		Females	
			Absolute	Relative %	Absolute	Relative %
I	Control	Control	0/6	0	0/6	0
II	2000	2000	0/6	0	0/6	0

Table No. 5: Mortality rate

Body Weight (Table 6 and Fig. 13, 14)

In male animals, those from the control groups maintained normal body weight. However, in the high-dose treated animals, the rate of weight gain decreased compared to the control group during the 14-day dosing period.

Similarly, in female animals, those from the control groups also exhibited normal body weight. However, among the high-dose treated animals, the rate of weight gain decreased compared to the control group over the same 14-day dosing period.

Sex	**Group no.**	**Dose (mg/kg)**	**Weight (g)**			
			Statistics	**0**	**7**	**14**
Male	I	Control	Mean	124.92	125.25	126.08
			± SD	3.28	2.66	2.83
	II	2000	Mean	124.48	124.80	125.52
			± SD	3.71	3.93	3.49
Female	I	Control	Mean	124.42	124.20	124.18
			± SD	3.62	3.86	3.30
	II	2000	Mean	124.80	125.60	126.25
			± SD	3.02	2.62	2.13

Table No. 6: Mean body weight

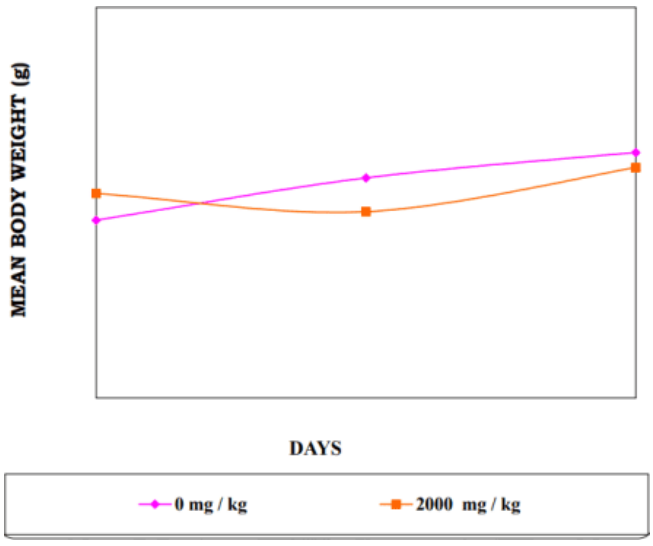


Fig. 13: Male (rat) group mean body weight (g)

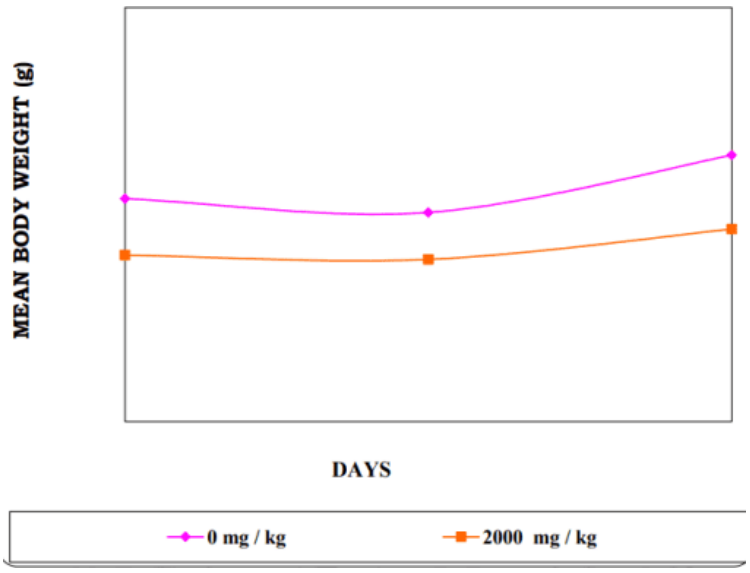


Fig. 14: Female (rat) group mean body weight (g)

Organ Weights (Table 7, 8)

Both male and female animals, irrespective of the treatment groups, displayed normal organ weights.

Sex	Group no.	Dose (mg/kg)	Statistics	Body weight (g)	Liver	Kidneys	Heart
Male	I	Control	Mean	126.28	4.80	0.79	0.48
			± SD	3.46	0.40	0.04	0.04
	II	2000	Mean	125.90	4.92	0.78	0.48
			± SD	3.77	0.90	0.08	0.06
Female	I	Control	Mean	126.43	4.66	0.77	0.46
			± SD	2.94	0.56	0.07	0.05
	II	2000	Mean	124.65	4.82	0.77	0.49
			± SD	1.32	0.77	0.07	0.06

Table 7: Group means – absolute values (g)

Sex	Group no.	Dose (mg/kg)	Statistics	Body weight (g)	Liver	Kidneys	Heart
Male	I	Control	Mean	126.28	3.78	0.62	0.38
			± SD	3.46	0.37	0.04	0.04
	II	2000	Mean	125.90	3.91	0.62	0.38
			± SD	3.77	0.75	0.06	0.05
Female	I	Control	Mean	126.43	3.68	0.61	0.36
			± SD	2.94	0.43	0.06	0.03
	II	2000	Mean	124.65	3.86	0.62	0.40
			± SD	1.32	0.61	0.06	0.05

Table 8: Group mean – relative values (%)

NECROPSY (Table 9)

Table 9 provides an overview of the necropsy results for both male and female animals. There were no anomalies found during the gross pathological examination that could be linked to the therapy.

Sex	Site and lesion observed	Group	
		I	II
Male	No Abnormality Detected	1 – 6	13 – 18
Female	No Abnormality Detected	7 – 12	19 – 24

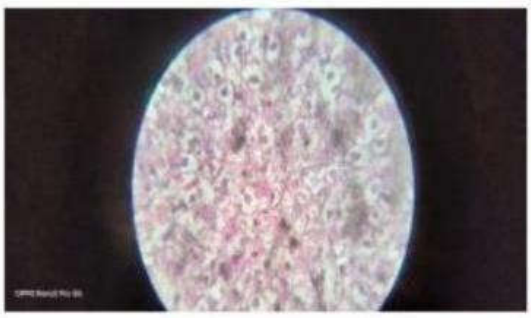
Table 9: Summary of Gross Pathology Findings

HISTOPATHOLOGY (Table 10)

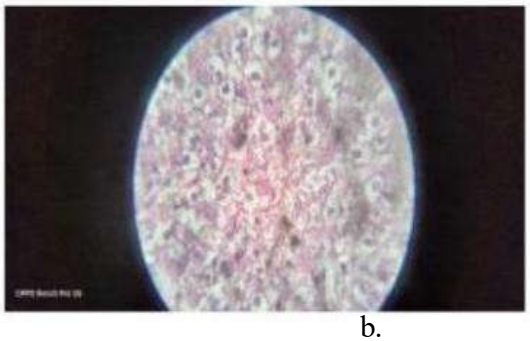
Table No. 10 provides an overview of the histological observations. Figure 15 (a-j) provides the histological observations for individual animals. Upon histopathological inspection, no abnormalities linked to the treatment were found in the animals from the high-dose group.

Fates		**Groups**			
		Male		**Female**	
		0 mg/kg (# SEV) (n = 6)	2000 mg/kg (# SEV) (n = 6)	0 mg/kg (# SEV) (n = 6)	2000 mg/kg (# SEV) (n = 6)
Heart	# Ex	5	5	5	5
Kidneys	# Ex	5	5	5	5
Liver	# Ex	5	5	5	5
Lungs	# Ex	5	5	5	5
Stomach	# Ex	5	5	5	5

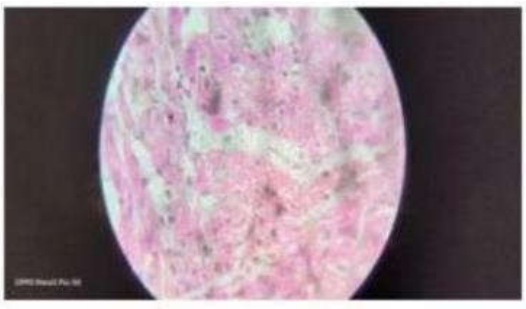
Table 10: Summary of histopathology findings



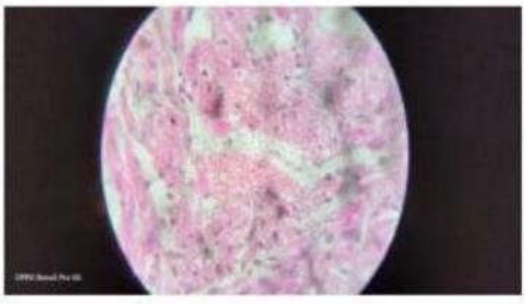
a.



b.



c.



d.

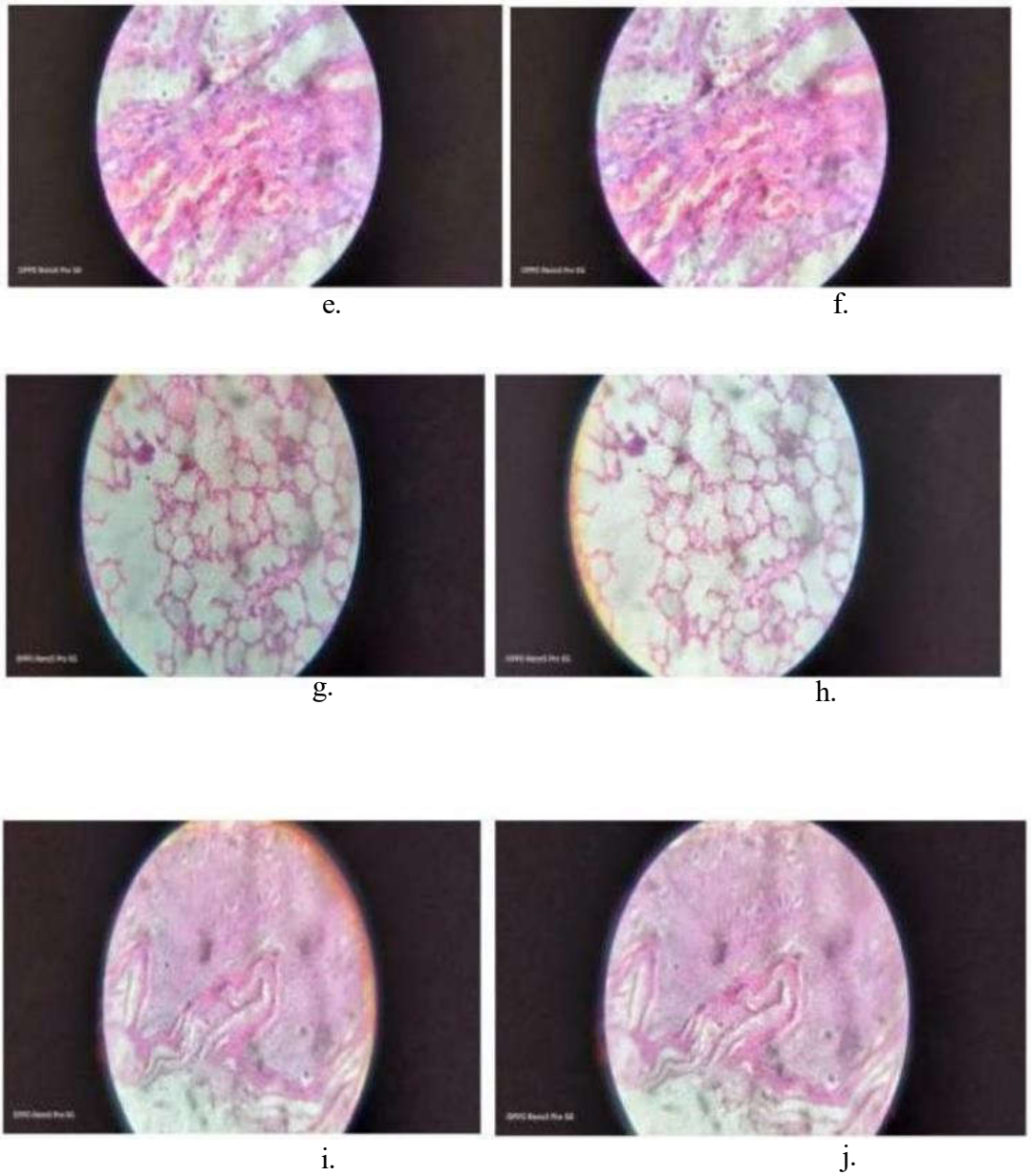


Fig 15: Photographs of Histopathological Slides of Organ Tissue [a. Control liver, b. Experimental liver, c. Control heart, d. Experimental heart, e. Control kidney, f. Experimental kidney, g. Control lungs, h. Experimental lungs, i. Control stomach, j. Experimental stomach]

Conclusion:

The present investigation deals with the acute (14 days) oral toxicity study of ethanolic extract of *Ipomoea aquatica forsk* on Swiss albino rats at the rate of 0 mg/Kg, 2000 mg/kg body weight. In

the case of the acute toxicity study oral LD50 was found to be more than 2000 mg/kg. The animals of both sexes from the control groups showed normal body weight gain throughout 14-day periods and administered dose groups also exhibited normal body weight gain throughout 14 days after the single administration of the drug at the rate of 2000 mg/kg. There was no abnormality or lesion found from the histopathological examination of the organs viz. heart, kidneys, liver, lungs, or stomach. The oral acute toxicity study in mice did not show significant features of hepatotoxicity and nephrotoxicity in the specimen. The findings from the current study suggest that the plant *Ipomoea aquatic forsk* may potentially serve as an effective treatment option for its clinical management against neurological problems.

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