Research Article

Exploration of Antidepressant Efficacy of Kantakari Flower (Solanum Xanthocarpum) Extract and Its Comparative Analysis With Fluoxetine Hydrochloride on Behavioural and Neurochemical Alterations

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Abstract

Major depressive disorder affects an estimated 280–330 million people worldwide and remains a leading contributor to years lived with disability. Beyond the classical monoaminergic deficit, converging evidence implicates hypothalamic–pituitary–adrenal (HPA) axis hyperactivity, neuroinflammation and oxidative stress in the pathogenesis of stress-related depression. Currently available antidepressants act slowly, fail in a substantial minority of patients and carry a considerable adverse-effect burden, which sustains interest in multi-target, plant-derived candidates. Solanum xanthocarpum Schrad. & Wendl. (Kantakari), a Solanaceous medicinal plant used extensively in Ayurveda with documented antioxidant and anti-inflammatory activity; its flowers, however, remain pharmacologically unexplored. To evaluate the antidepressant potential of a hydroalcoholic Kantakari flower extract in a cold water immersion (CWI) model of stress-induced depression in rats and to benchmark it against fluoxetine. Twenty-five Wistar rats (150–250 g) were randomised into five groups (n = 5): normal control; CWI (disease) control; CWI + fluoxetine (10 mg/kg); CWI + Kantakari flower extract (200 mg/kg); and CWI + Kantakari flower extract (400 mg/kg). Animals were immersed in cold water for 10–15 min daily for 21 days, with oral treatment given concurrently. Locomotor activity (actophotometer) was recorded on days 8, 10, 12, 14, 18 and 21, and immobility time (forced swim test) on days 10, 14, 18 and 21. Brain superoxide dismutase (SOD) and reduced glutathione (GSH) were quantified, and cortical and hippocampal sections were examined after haematoxylin–eosin staining. Data were analysed by one-way ANOVA with Tukey's post hoc test. CWI produced a progressive fall in locomotor activity (93.6 ± 5.3 vs. 290.4 ± 2.3 in normal controls on day 21; $p < 0.05$) and a rise in immobility time (207.0 ± 6.7 s vs. 63.4 ± 7.2 s; $p < 0.05$), together with depletion of brain SOD and GSH and marked neuronal degeneration. Both Kantakari flower extract doses significantly reversed these changes relative to disease controls ($p < 0.05$). Kantakari flower extract 400 mg/kg restored locomotor activity to 333.0 ± 8.5 and Reduced immobility to 75.2 ± 6.3 s by day 21, normalized antioxidant indices and largely preserved neuronal architecture, matching fluoxetine. Kantakari flower extract exerts dose-dependent antidepressant-like activity in CWI-stressed rats, plausibly through reinforcement of endogenous antioxidant defenses and consequent neuroprotection.

Keywords: Solanum xanthocarpum, Kantakari, Depression, Cold water immersion stress,Oxidative stress, Superoxide dismutase.

Introduction

Depression is among the most burdensome non-communicable conditions of the present era. Global Burden of Disease estimates place the number of people living with a depressive disorder at roughly 280–330 million, equivalent to 4–5% of the world population, and depressive disorders consistently rank among the top contributors to years lived with disability across almost every region and age band [1–4]. The COVID-19 pandemic added a further 27% to global prevalence in a single year, an increment that has not fully receded [5]. Prevalence is approximately twofold higher in women than in men, peaks in adolescence and early adulthood, and is amplified by unemployment, financial insecurity and social fragmentation [6–8]. The economic consequences are equally striking: in the United States alone, the annual societal cost attributable to adults with major depressive disorder (MDD) has been estimated at well above USD 300 billion [8]. Clinically, MDD is defined by at least two weeks of persistently depressed mood or anhedonia accompanied by neurovegetative, cognitive and psychomotor disturbance, and it carries a substantial excess mortality driven largely by suicide [9].



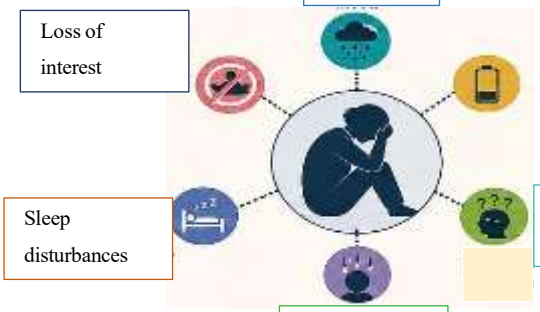
INTRODUCTION OF DEPRESSION

A persistent, recurrent & disabling psychiatric disorder that significantly affects behavior, mood, cognitive function and overall quality of life



WHAT IS DEPRESSION?

Depression is a mood disorder characterized by:
Prolonged sadness
Loss of interest
Emotional numbness
Reduced motivation
Impairment in personal, social & occupational functioning



CLINICAL SIGNIFICANCE

Major Depressive Disorder (MDD) represents the most severe form & contributes heavily to the global disease burden.



Among the leading causes of disability

Table no.1: Global Epidemiology and Burden of Depression

Aspect	Summary	References
Global prevalence	lion people (4-5% of the global population)	[10]
Disease burden	e of Years Lived with Disability (YLDs) worldwide	[11]
High-risk groups	Women, adolescents & young adults	[12]
Economic burden	exceeds USD 300 billion in the United States	[13]
Major risk factors	ent, social isolation and financial stress	[14]
Clinical features	ressed mood or anhedonia for more than 2 weeks	[15]
Mortality	Increased risk of suicide	[16]

Oxidative stress sits at the convergence of these pathways and is increasingly regarded as a mechanistic hub rather than an epiphenomenon. The brain is intrinsically vulnerable to oxidative injury: it consumes a

disproportionate share of body oxygen, is enriched in peroxidisable polyunsaturated fatty acids and possesses comparatively modest antioxidant reserves. Chronic stress accelerates mitochondrial reactive oxygen species (ROS) leakage while simultaneously depleting superoxide dismutase (SOD) and reduced glutathione (GSH), mitochondrial membrane depolarisation [17–19]. Clinical and preclinical data converge on the same signature, and reduced antioxidant capacity has been reported to track with symptom severity in patients with MDD [20,21]. Because oxidative injury both amplifies neuroinflammation and suppresses brain-derived neurotrophic factor (BDNF) signalling, agents capable of restoring redox homeostasis are attractive candidates for antidepressant development [22,23].

Pharmacological management nevertheless remains dominated by selective serotonin reuptake inhibitors (SSRIs). Network meta-analytic evidence confirms that these agents are effective at the group level, yet their limitations are well documented: a therapeutic latency of two to four weeks, remission in fewer than half of patients after a first adequate trial, and a side-effect profile encompassing nausea, insomnia, weight change, sexual dysfunction and discontinuation symptoms [24–26]. Fluoxetine, the prototypical SSRI, blocks the serotonin transporter and, over weeks, promotes downstream neuroplastic adaptations including enhanced BDNF–TrkB signalling; it remains the reference comparator in preclinical antidepressant screening [27,28]. The gap between statistical efficacy and clinical satisfaction has revived interest in phytotherapeutics, which characteristically act on several pathogenic nodes simultaneously and are generally well tolerated [29–31].

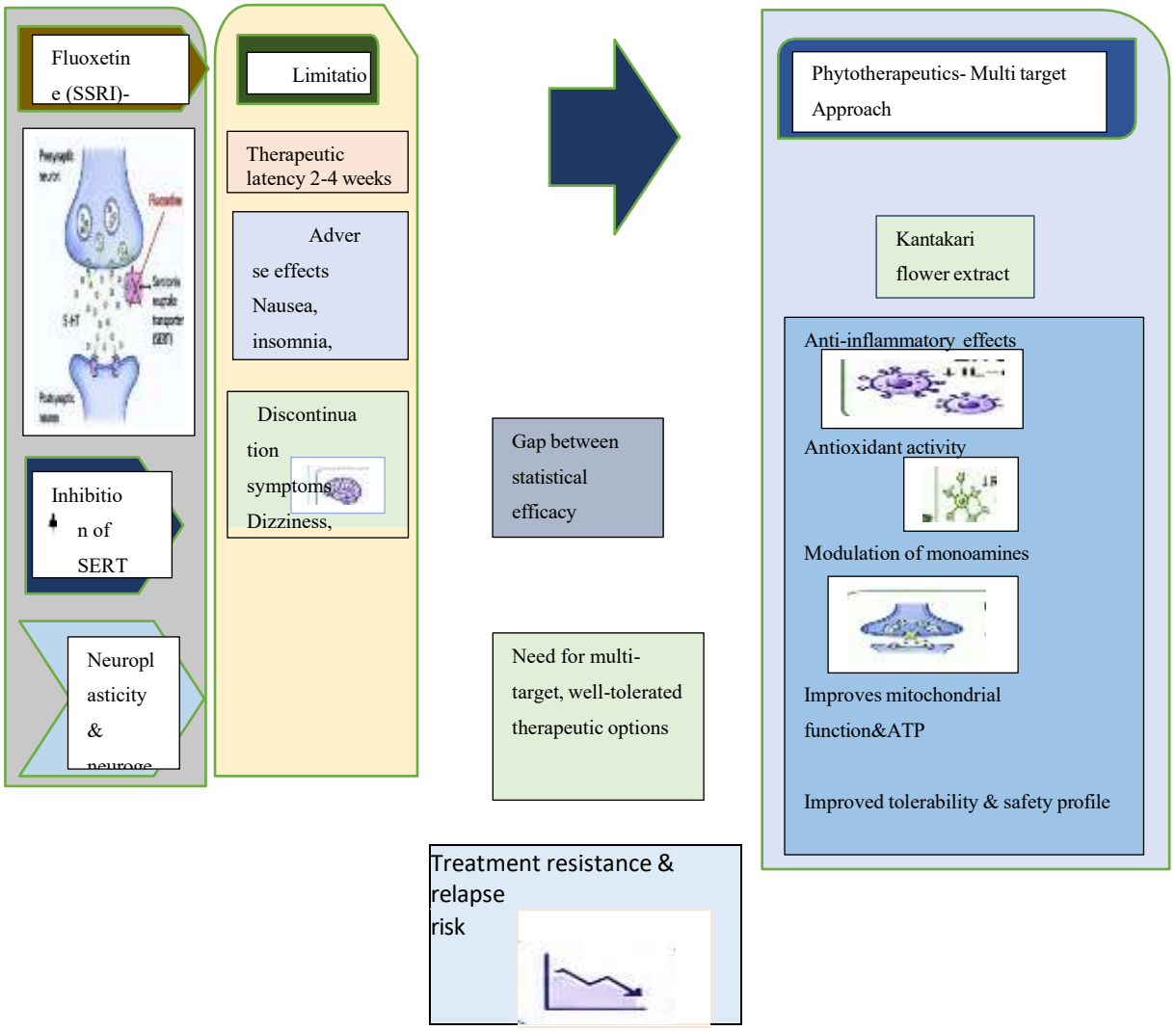


Figure no.2: Pharmacological Management of Depression: From SSRI to Multi-Target Phytotherapeutics

Solanum xanthocarpum Schrad. & Wendl. (syn. S. virginianum L.; Solanaceae), known in Ayurveda as Kantakari and colloquially as yellow-berried nightshade, is a prickly perennial undershrub distributed throughout the

Indian subcontinent and much of tropical Asia. It is a constituent of the classical Dashamoola formulation and has been used for centuries in respiratory, febrile and inflammatory conditions [32–34]. Phytochemical work on the genus has characterised steroidal glycoalkaloids (notably solasodine and solanine), steroidal saponins, flavonoids, phenolic acids, tannins and terpenoids [35–37]. Pharmacologically, extracts of the species have demonstrated free-radical scavenging, anti-inflammatory, antimicrobial, immunomodulatory, hepatoprotective, analgesic, antipyretic and bronchodilator activity [32,33,38–43]. Glycoalkaloids and flavonoids from Solanum species scavenge ROS, inhibit lipid peroxidation and stabilize cellular membranes, providing a plausible route to neuroprotection [38,44].

Two gaps in this literature motivated the present work. First, essentially all published pharmacological evaluation of S. xanthocarpum concerns the fruit, root, leaf or undifferentiated aerial parts; the flower, despite being the organ in which flavonoid and phenolic accumulation is typically highest in angiosperms, has not been examined for neuropharmacological activity. Second, herbal antidepressant screening has relied overwhelmingly on chronic unpredictable mild stress, the forced swim test used as an inducer, or the tail suspension test. The cold water immersion (CWI) paradigm has been used far less frequently, even though it is inexpensive, highly reproducible and reproduces the triad of HPA axis activation, oxidative brain injury and behavioural despair that characterises stress-related depression in humans [45–48]. Where herbal extracts have been tested, behavioural and biochemical readouts have often been reported in isolation, single doses have been used, and direct comparison with a standard antidepressant has frequently been omitted.

The present study was therefore designed to address these gaps in a single, internally consistent protocol. We hypothesised that a Kantakari flower extract, by virtue of its flavonoid content, would attenuate CWI-induced depressive-like behaviour in rats in a dose-related manner, and that this behavioural benefit would be accompanied by restoration of brain SOD and GSH and by preservation of cortical and hippocampal neuronal architecture. Accordingly, the objectives were to establish depressive-like behaviour by repeated cold water immersion; to determine the effect of Kantakari flower extract at 200 and 400 mg/kg on locomotor and on immobility time in the forced swim test; to quantify brain SOD and GSH as indices of endogenous antioxidant capacity; to assess neuronal integrity histopathologically; and to compare all outcomes directly with fluoxetine (10 mg/kg).

1. Materials and Methods

1.1. Experimental animals

Healthy adult Wistar rats weighing 150–250 g were obtained from a registered breeding facility and housed in the animal house of the University School of Pharmaceutical Sciences. Animals were maintained in polypropylene cages under conventional conditions: ambient room temperature, relative humidity 60–70%, a 12 h light/dark cycle and adequate ventilation. A standard pellet diet and tap water were available ad libitum except during the brief periods of behavioral testing. All animals were acclimatized to the housing environment and to routine handling for seven days before the start of the experimental protocol in order to minimize handling-related stress artefacts.

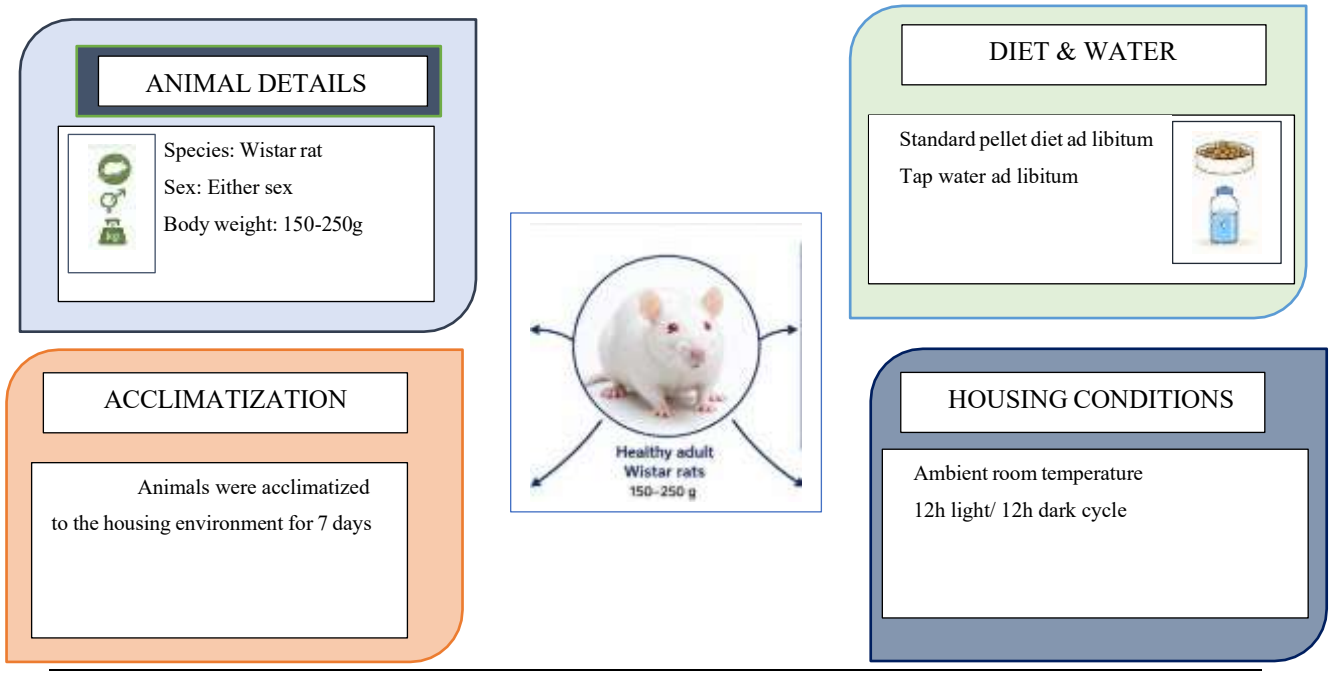


Figure no.3 : Experimental animals

1.2. Ethical approval

The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of the University School of Pharmaceutical Sciences, Rayat-Bahra University (approval number USPS/IAEC/CCSEA/2024/Protocol No. 118). All procedures involving animals were carried out in accordance with the guidelines of the Committee for Control and Supervision of Experiments on Animals (CCSEA), Government of India, and the study is reported in line with the ARRIVE recommendations. Animal numbers were kept to the minimum required to obtain interpretable data, and every effort was made to limit distress.

1.3. Drugs, chemicals and reagents

Fluoxetine hydrochloride was procured from Sun Pharmaceutical Industries Ltd., India. Carboxymethyl cellulose (0.5% w/v) was used as the suspending vehicle, and normal saline (0.9% w/v NaCl; Otsuka Pharmaceutical India Pvt. Ltd.) was used where an isotonic diluent was required.

1.4. Plant material

A standardised dry extract of Solanum xanthocarpum flowers was procured from AVAM Pvt. Ltd., India, a commercial supplier of herbal extracts. The identity of the raw material was confirmed against pharmacopoeial descriptors for the species, and a voucher specimen was retained in the departmental herbarium for future reference. The extract was supplied as a free-flowing dry powder and was stored in an amber, airtight container, protected from light and moisture, until use. Dosing suspensions were prepared immediately before administration by triturating the required quantity of extract in 0.5% w/v carboxymethyl cellulose to give a uniform suspension delivered at a constant volume of 1 mL/100 g body weight.

1.5. Experimental design and grouping

Twenty-five rats were allocated at random to five groups of five animals each (n = 5). Group I (normal group) received the vehicle only and was not exposed to cold water. Group II (disease Induced group) was exposed to cold water immersion without pharmacological treatment. Group III received fluoxetine 10 mg/kg with cold water immersion and served as the standard comparator. Groups IV and V received Kantakari flower extract at 200 and 400 mg/kg respectively, with cold water immersion. All treatments were administered by oral gavage once daily.

The complete protocol spanned 28 days. Days 0–7 were reserved for acclimatisation. From day 8 to day 21, animals in Groups II–V were subjected to daily cold water immersion, with drug or extract administered concurrently during the same period. Behavioural assessment was interleaved with the stress–treatment phase. Terminal sample collection and biochemical estimation were performed on days 23–24, and histopathological processing and analysis on days 24–28.

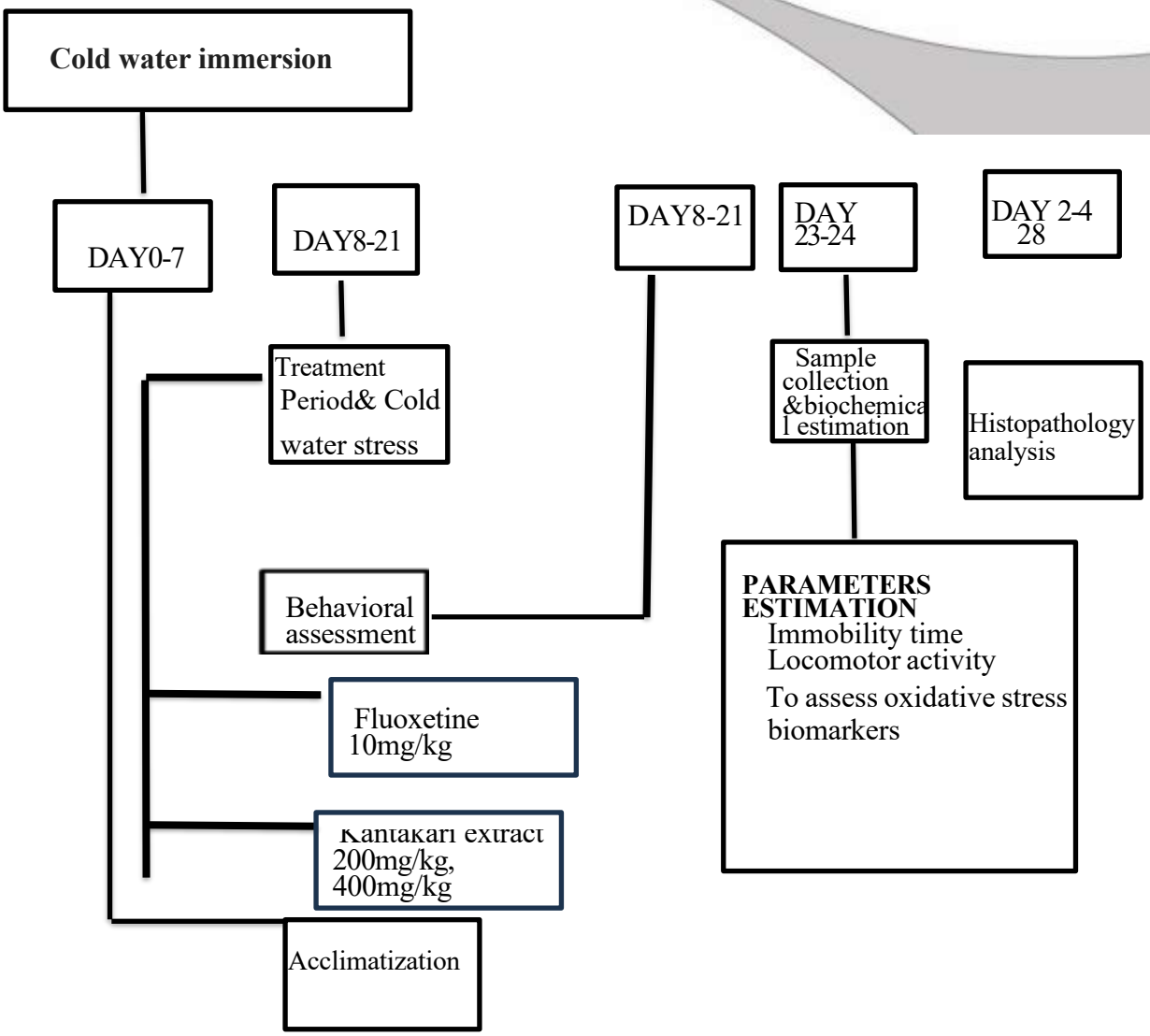


Figure no. 4: Experimental Design

Table no. 2. Experimental groups, interventions and dosing schedule.

Group	Intervention	Dose (mg/kg)	Route	n
I	Normal group (vehicle only, no stress)	Vehicle	p.o.	5
II	Induced group (cold water immersion)	—	—	5
III	Standard drug (fluoxetine)	10	p.o.	5
IV	ter immersion + Kantakari flower extract	200	p.o.	5
V	ter immersion + Kantakari flower extract	400	p.o.	5

p.o., per oral. Treatments were given once daily from day 8 to day 21 in parallel with the cold water immersion schedule.

2.7. Cold water immersion protocol

Depressive-like behaviour was induced by repeated cold water immersion, a paradigm that reproducibly activates the HPA axis and generates oxidative brain injury [49,50]. Each animal was placed individually in an

acrylic tank containing cold water, the depth of which was adjusted so that the animal could keep its head above the surface throughout the session without being able to rest or escape. Immersion lasted 10–15 min and was repeated daily for 21 days. Immediately after each session animals were gently towel-dried, allowed to recover under ambient conditions and returned to their home cages. Normal control animals were handled identically but not immersed.

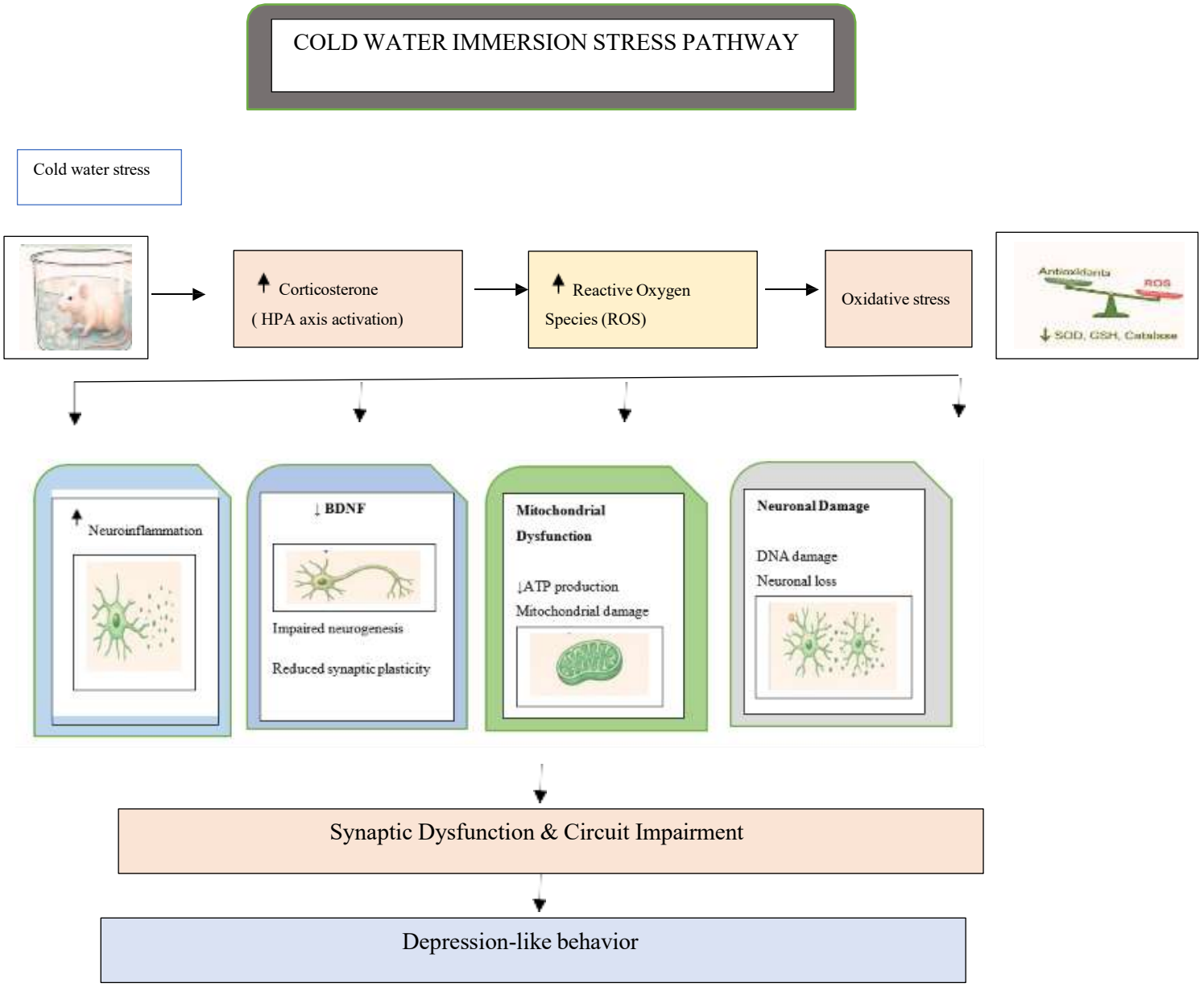


Figure no.5: Cold Water Immersion Stress Pathway

Table no. 3: Experimental Protocol for repeated Cold water immersion

Parameter	Protocol
Experimental model	Repeated cold water immersion
Purpose	of stress-associated depressive-like behavioral changes
Exposure duration	10-15 minutes/session

Animals	Individual rat per session
Exposure frequency	Once daily
Total duration	21 days
Post-immersion care	ere gently towel-dried immediately after each session
Recovery	Animals were allowed to recover under ambient conditions before returning to home cages
Study relevance	Establishment of a reproducible depressive-like phenotype for evaluating the antidepressant potential of Kantakari flower extract

2.8. Drug administration

Fluoxetine hydrochloride (10 mg/kg) and Kantakari flower extract (200 and 400 mg/kg) were suspended in 0.5% w/v carboxymethyl cellulose and administered once daily by oral gavage using a stainless-steel feeding needle. Dosing was performed at a fixed time each morning, approximately 60 min before the cold water immersion session, to ensure that behavioural testing occurred within a comparable pharmacokinetic window across groups. Normal and disease control animals received an equal volume of vehicle by the same route.

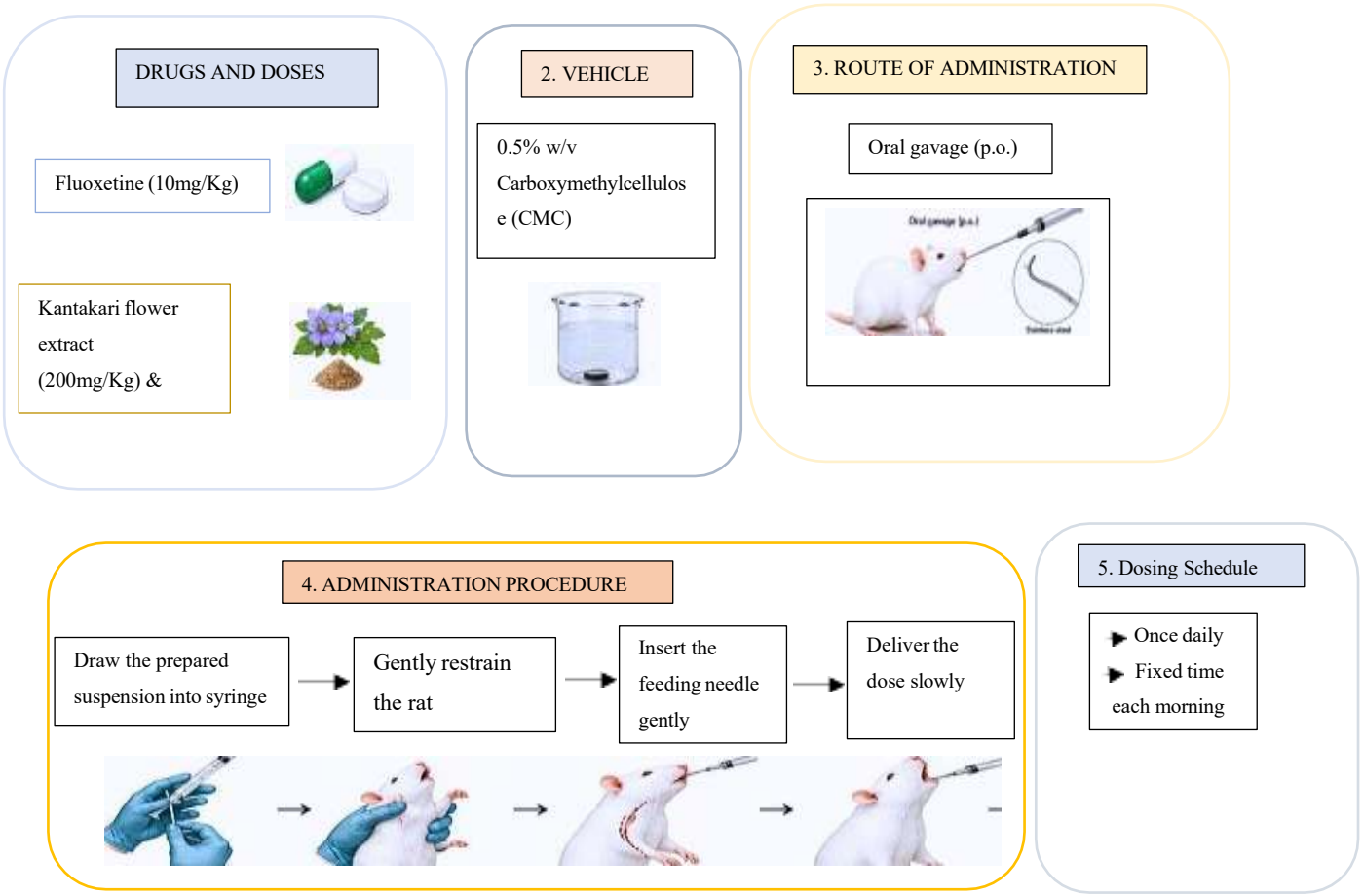


Figure no. 6 : Drug Administration

2.9. Behavioural assessment

2.9.1. Locomotor activity

Spontaneous locomotor activity was quantified using actophotometer. Each rat was placed individually in the activity chamber and allowed to move freely; interruptions of the photobeam array were counted automatically over a fixed observation period, and activity was expressed per unit time. Locomotor activity was recorded on days 8, 10, 12, 14, 18 and 21. This parameter serves two purposes: it is itself a sensitive index of stress-induced behavioral suppression, and it provides the control necessary to distinguish a genuine antidepressant-like effect from non-specific psychostimulant or sedative action of the test substance. Testing was conducted in a quiet room under uniform illumination.

2.9.2. Immobility time (forced swim test)

Behavioural despair was assessed by the forced swim test [51]. Each rat was placed individually in a transparent glass cylinder containing water maintained at 25 ± 2 °C to a depth sufficient to prevent the animal from touching the base with its hind paws or tail. The session lasted 6 min and immobility was scored during the final 5 min by an observer blinded to group allocation. An animal was scored as immobile when it floated passively, making only those movements necessary to keep its head above water. Immobility was recorded on days 10, 14,
18 and 21. Prolongation of immobility is interpreted as behavioural despair, whereas a reduction after treatment is taken as evidence of antidepressant-like activity [52,53].

2.10. Biochemical estimation

At the end of the protocol animals were euthanised humanely, the brains were rapidly excised, rinsed in ice-cold saline and homogenised in ice-cold 0.1 M phosphate buffer (pH 7.4). Homogenates were centrifuged in the cold and the supernatant was used for enzymatic assays. All estimations were carried out in duplicate on a UV–visible spectrophotometer.

2.10.1. Superoxide dismutase

SOD activity was determined by the method of Misra and Fridovich, which exploits the capacity of the enzyme to inhibit the auto-oxidation of epinephrine at alkaline pH. Briefly, 1.0 mL of tissue supernatant was mixed with 0.5 mL carbonate buffer (pH 10.2) and 0.1 mL EDTA (1×10^{-1} M). The reaction was initiated by adding 1.0 mL epinephrine (3×10^{-3} M), and the increase in absorbance due to adrenochrome formation was recorded at 480 nm at 30 s intervals for 3 min. Enzyme activity was calculated from the percentage inhibition of epinephrine auto-oxidation and expressed as units per gram of tissue.

2.10.2. Reduced glutathione

Brain GSH was estimated by Ellman's method [56]. Tissue homogenate prepared in 0.1 M phosphate buffer (pH 7.4) was deproteinised with an equal volume of 20% trichloroacetic acid containing 1 mM EDTA, allowed to stand for 5 min and centrifuged. To 200 µL of the clear supernatant, 1.8 mL of Ellman's reagent (0.1 mM 5,5′-dithiobis-2-nitrobenzoic acid prepared in 0.3 M phosphate buffer containing 1% sodium citrate) was added to a final volume of 2 mL. After completion of the reaction, absorbance of the yellow chromophore was read at 412 nm against a reagent blank, and concentrations were interpolated from a calibration curve constructed with a standard GSH preparation.

2.11. Histopathological examination

Following euthanasia, the hippocampus and cerebral cortex — regions central to affective regulation and particularly susceptible to glucocorticoid- and oxidant-mediated injury — were dissected and fixed in 10% neutral buffered formalin for 24–48 h. Tissues were dehydrated through an ascending ethanol series (70%, 80%, 90%, 95% and absolute), cleared in xylene and embedded in molten paraffin wax. Blocks were sectioned at 5 µm on a rotary microtome, mounted on clean glass slides and stained with haematoxylin and eosin. Sections were examined under a light microscope by an assessor blinded to treatment allocation, and were scored qualitatively for neuronal degeneration, pyknosis, cytoplasmic vacuolation, cellular disarray, inflammatory infiltration and neuronal loss. The neuroprotective effect of the extract was judged by direct comparison of treated groups with the normal and disease control sections.

2.12. Statistical analysis

Data are expressed as mean ± standard error of the mean (SEM) for n = 5 animals per group. Between-group comparisons were made by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test, using GraphPad Prism. A probability value below 0.05 was regarded as statistically significant; the

significance actually attained for the principal behavioural comparisons is reported at $p < 0.05$. Comparisons were structured a priori as follows: each stressed group against the normal control (to verify induction), each treated group against the disease control (to establish efficacy) and each extract dose against fluoxetine (to benchmark potency).

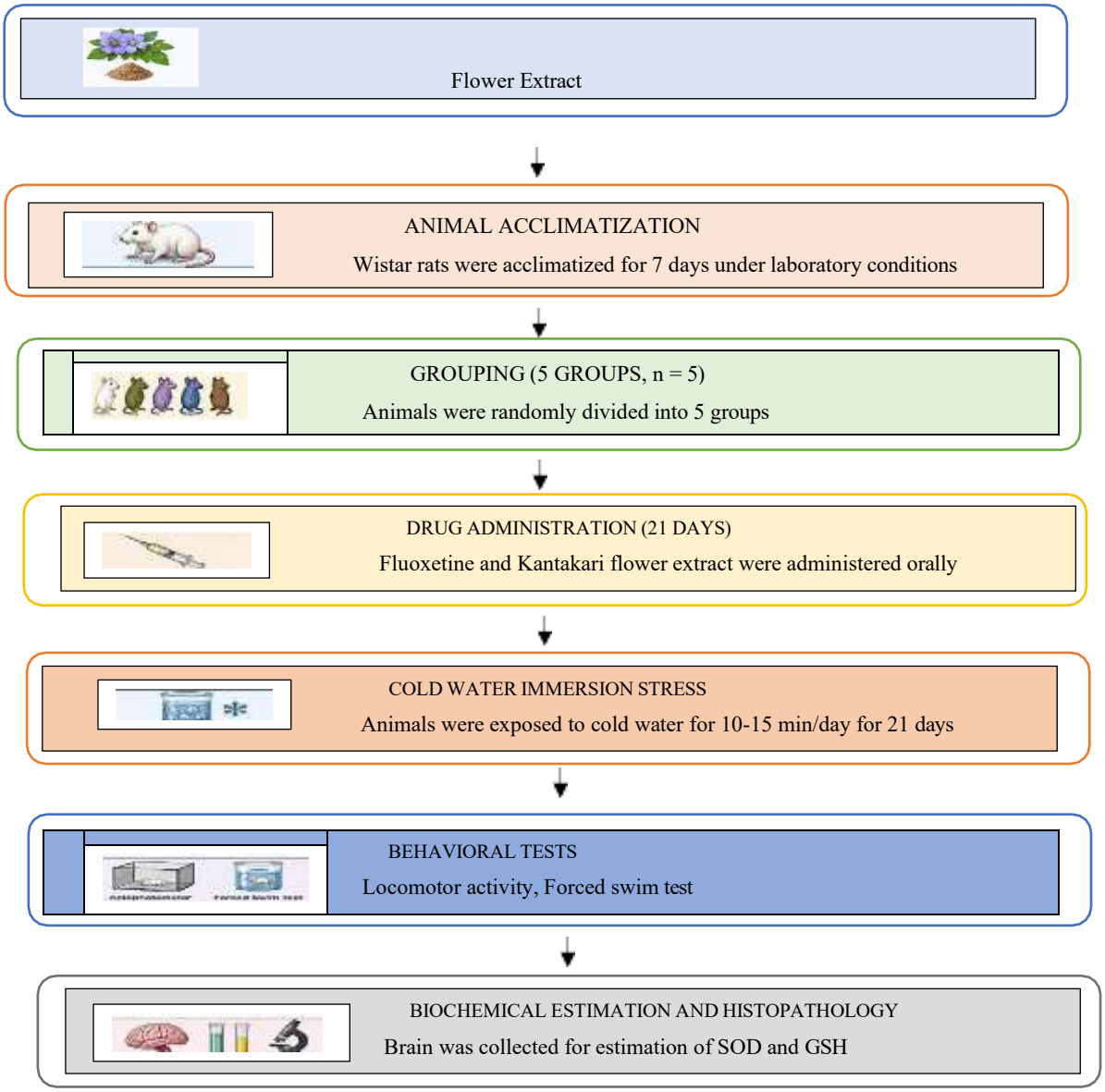


Figure no. 7 : Experimental workfow

2. Results

2.1. Phytochemical profile of the flower extract

Qualitative screening confirmed a broad secondary-metabolite profile in the Kantakari flower extract (Table 2). Alkaloids, flavonoids, glycosides, saponins, tannins, steroids, terpenoids and carbohydrates were all detected. The simultaneous presence of flavonoids, tannins and steroidal constituents is directly relevant to the pharmacological outcomes described below, since these classes account for most of the reported free-radical scavenging and membrane-stabilising activity of the genus.

Table no. 4. Preliminary qualitative phytochemical screening of Kantakari (Solanum xanthocarpum) flower extract

S. No.	Phytoconstituent	Test applied	Result
1	Alkaloids	Dragendorff's test	Present (+)
2	Flavonoids	Shinoda test	Present (+)
3	Saponins	Foam test	Present (+)
4	Steroids	Salkowski test	Present (+)
5	Anthraquinones	Borntrager's test	Absent (-)
6	Cardiac glycosides	Keller-Killianitest	Absent (-)

(+) denotes a positive reaction and (-) denotes a negative reaction

2.2. Effect on locomotor activity

Repeated cold water immersion caused a marked and progressive suppression of spontaneous locomotor activity. Disease control animals were significantly less active than normal controls at every time point ($p < 0.05$), and the deficit deepened as the stress schedule continued, falling from 169.4 ± 8.3 on day 8 to 93.6 ± 5.3 on day 21, against 290.4 ± 2.3 in unstressed animals.This progressive decline confirms that the model produced a cumulative rather than transient behavioural impairment.

Fluoxetine (10 mg/kg) significantly reversed this suppression relative to disease controls from day 8 onward ($p < 0.05$), reaching 285.8 ± 9.7 by day 21 — essentially the level of unstressed animals. Kantakari flower extract at 200 mg/kg also produced a significant improvement over disease controls at every time point ($p < 0.05$), rising from 191.0 ± 9.8 on day 8 to 268.4 ± 9.7 on day 21; values at this dose nevertheless remained significantly below those of the fluoxetine group ($p < 0.05$), indicating incomplete recovery at the lower dose.

The higher dose behaved differently. Kantakari flower extract at 400 mg/kg not only exceeded the disease control at all time points ($p < 0.05$) but produced values significantly different from the normal control as well ($p < 0.05$), reaching 333.0 ± 8.5 on day 21 against 290.4 ± 2.3 in normal animals. The extract at this dose therefore restored locomotion beyond the unstressed baseline rather than merely to it, and the consistent separation between the 200 and 400 mg/kg arms across all six assessment days establishes a clear dose–response relationship.

Table no. 5: Effect of Kantakari Flower Extract on Locomotor Activity In Cold Water Immersion-Stressed Rats

Group	Day 8	Day 10	Day 12	Day 14	Day 18	Day 21
Normal group	283.8 ± 3.0	251.96 ± 3.5	290.4 ± 0.7	288.4 ± 2.4	294.4 ± 1.4	290.4 ± 2.3
Disease Induced group	169.4 ± 8.3^{a}	150.6 ± 7.2^{a}	148.2 ± 2.0^{a}	122.2 ± 7.0^{a}	103.6 ± 5.5^{a}	93.6 ± 5.3^{a}
Standard drug (10 mg/kg)	216.8 ± 12.6^{b}	244.0 ± 10.1^{b}	247.8 ± 10.8	260.8 ± 11.0^{b}	281.8 ± 9.5^{b}	285.8 ± 9.7^{b}
Test drug-I Kantakari (200 mg/kg)	191.0 ± 9.8^{bc}	201.4 ± 11.8^{bc}	224.4 ± 8.7^{bc}	237.4 ± 7.11^{bc}	260.8 ± 6.5^{bc}	268.4 ± 9.7^{bc}
Test drug-II Kantakari (400 mg/kg)	271.0 ± 8.8^{ab}	282.0 ± 4.2^{ab}	292.8 ± 7.3^{ab}	302.8 ± 4.1^{ab}	324.6 ± 9.5^{ab}	333.0 ± 8.5^{ab}

- $^{a}P < 0.05$ compared with the Normal group
- $^{b}P < 0.05$ compared with the Disease group
- $^{bc}P < 0.05$ compared with the Standard Drug (Fluoxetine, 10 mg/kg) group
- $^{ab}P < 0.05$ compared with Test Group I (Kantakari flower extract, 200 mg/kg) group.

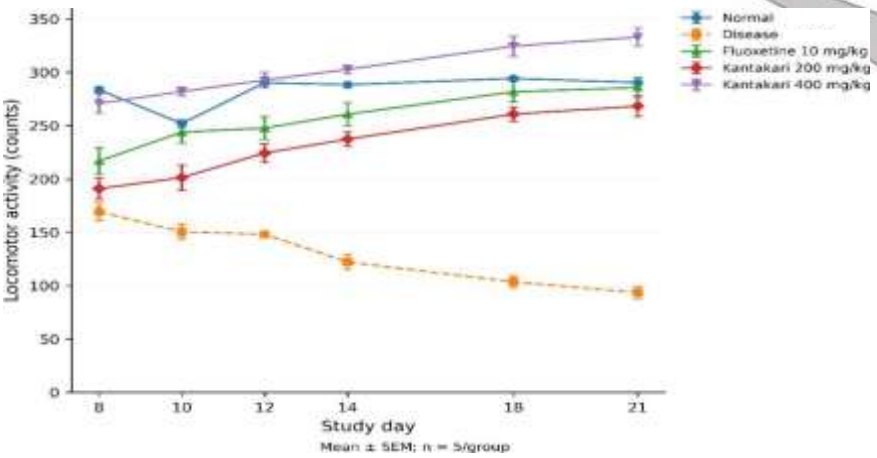


Figure no. 8: Locomotor activity over Days 8-21

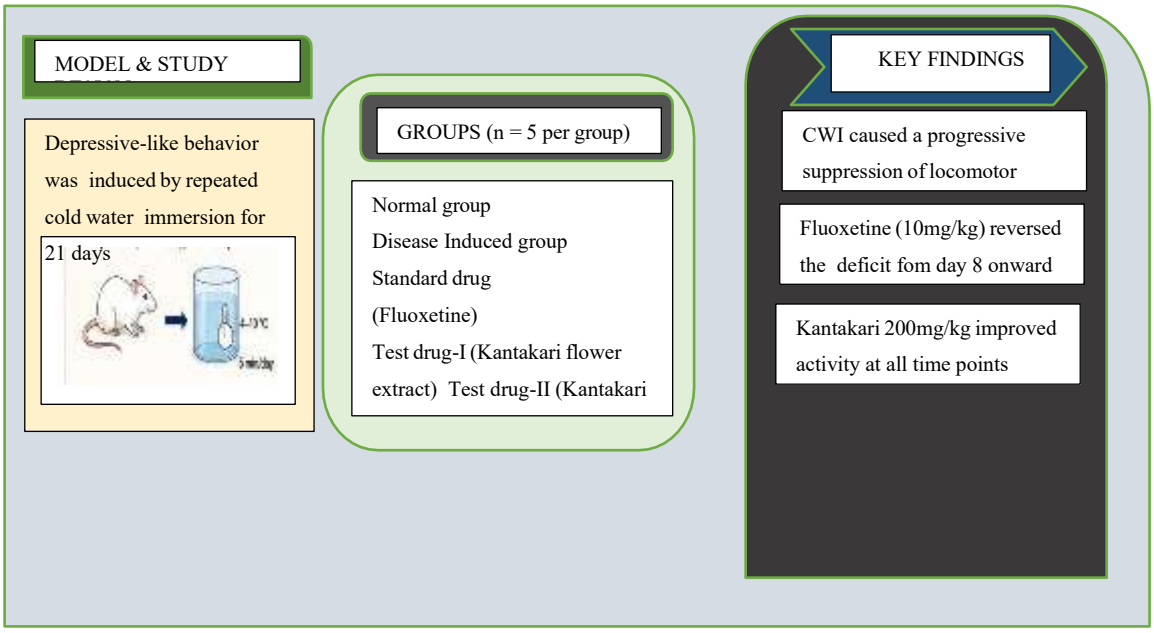


Figure no. 9: Kantakari Flower Extract Restores Locomotor Activity

2.3. Effect on immobility time in the forced swim test

Cold water immersion produced a pronounced increase in immobility. Disease control animals were immobile for 138.4 ± 13.1 s on day 10, and this lengthened steadily to 207.0 ± 6.7 s by day 21, more than three times the value recorded in normal controls (63.4 ± 7.2 s; $p < 0.05$). Immobility in unstressed animals fluctuated within a narrow band (55.2–68.2 s) across the four assessment days, confirming stability of the assay in the absence of stress.

Both active treatments reversed this effect, and the time course is informative. On day 10, after only a short exposure to treatment, immobility in the fluoxetine group (140.2 ± 7.3 s) and both extract groups (160.4 ± 10.5 s and 140.8 ± 8.6 s) remained close to the disease control value, consistent with the well-recognised latency of antidepressant action. Separation emerged from day 14 onward. By day 21 fluoxetine had reduced immobility to 56.6 ± 7.0 s, Kantakari flower extract 200 mg/kg to 84.6 ± 8.5 s and Kantakari flower extract 400 mg/kg to 75.2 ± 6.3 s, all significantly lower than the disease control ($p < 0.05$).

The higher extract dose produced a consistently greater reduction in immobility than the lower dose at every post-induction time point, mirroring the dose-related pattern seen for locomotor activity. Values in the 200 mg/kg group differed significantly from fluoxetine throughout, whereas the 400 mg/kg group approached the standard drug by day 21. Because locomotor activity was preserved or enhanced rather than reduced in the treated groups, the fall in immobility cannot be attributed to sedation and is therefore interpretable as a

genuine antidepressant-like effect.

Table no. 6: Effect of Kantakari flower extract on immobility time (s) in the forced swim test.

Group	Day 10	Day 14	Day 18	Day 21
Normal group	61.6 ± 6.0	68.2 ± 4.6	55.2 ± 7.8	63.4 ± 7.2
Disease Induced group	138.4 ± 13.1[a]	175.2 ± 6.5[a]	194.2 ± 59.0[a]	207.0 ± 6.7[a]
Standard drug (10 mg/kg)	140.2 ± 7.3[b]	99.2 ± 6.0[b]	81.2 ± 6.8[b]	56.6 ± 7.0[b]
Test drug-I Kantakari (200 mg/kg)	160.4 ± 10.5[bc]	120.8 ± 9.2[bc]	105.4 ± 7.1[bc]	84.6 ± 8.5[bc]
Test drug-II Kantakari (400 mg/kg)	140.8 ± 8.6[ab]	107.8 ± 8.5[ab]	85.6 ± 7.5[ab]	75.2 ± 6.3[ab]

Values are expressed as Mean ± SEM (n = 5). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test.

- [a]P < 0.05 compared with the Normal Control group
- [b]P < 0.05 compared with the Disease Control group
- [bc]P < 0.05 compared with the Standard Drug (Fluoxetine, 10 mg/kg) group
- [ab]P < 0.05 compared with Test Group I (Kantakari flower extract, 200 mg/kg) group.

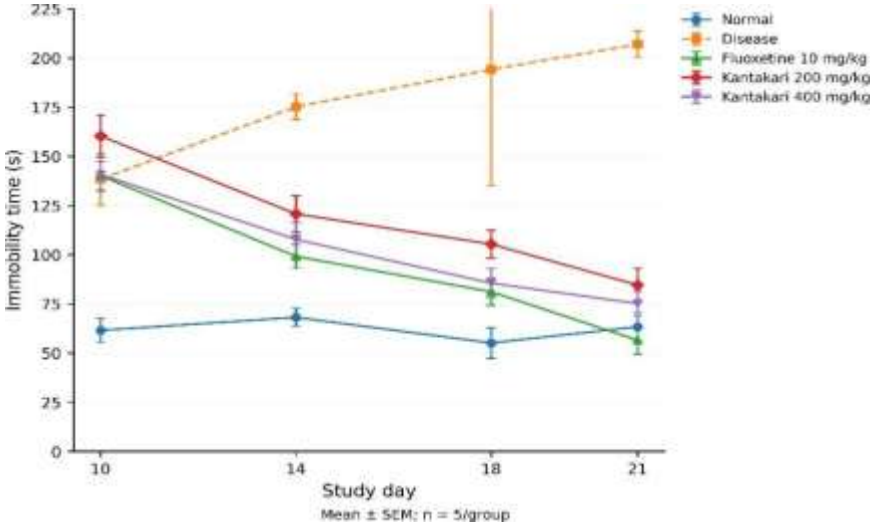


Figure no. 10: Forced swim test immobility over days 10-21

2.4. Effect on brain superoxide dismutase activity

Brain SOD activity was significantly reduced in disease control animals compared with normal controls ($p < 0.05$), indicating consumption of the first-line enzymatic defence against superoxide radicals under sustained cold stress. Fluoxetine treatment significantly restored SOD activity relative to the disease control ($p < 0.05$). Kantakari flower extract at 200 mg/kg likewise produced a significant increase over the disease control ($p < 0.05$), although activity in this group remained below that of the fluoxetine-treated animals. The 400 mg/kg dose generated a larger increment than the lower dose and approached normal control values, demonstrating a dose-dependent antioxidant effect that parallels the behavioural findings.

2.5. Effect on brain reduced glutathione

The same pattern was observed for the non-enzymatic arm of the antioxidant system. Cold water immersion significantly depleted brain GSH compared with normal controls, consistent with sustained oxidative consumption of thiol reserves. Fluoxetine significantly restored GSH concentrations relative to disease controls, as did Kantakari flower extract at 200 mg/kg. The 400 mg/kg dose produced a greater elevation than

200 mg/kg, returning GSH close to the concentration measured in unstressed animals. The concordance between the SOD and GSH results indicates that the extract acts on both enzymatic and non-enzymatic components of the cellular redox buffer rather than on a single target.

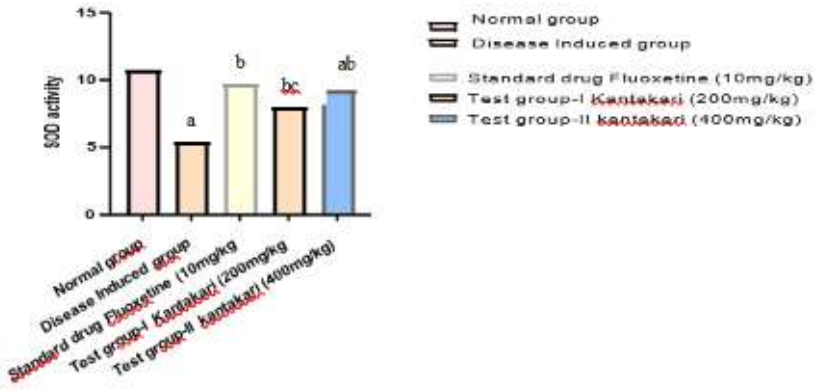


Figure no. 11 : Effect of Kantakari Flower Extract on Superoxide Dismutase (SOD) Activity. Values are expressed as Mean ± SEM (n = 5). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test.

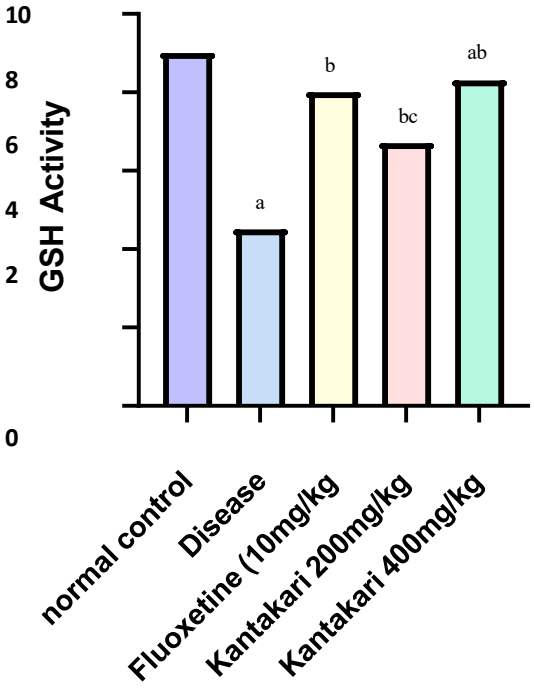


Figure no. 12: Effect of Kantakari Flower Extract on Glutathione (GSH) Activity.Values are expressed as Mean ± SEM (n = 5). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test.

2.6. Histopathological findings

Haematoxylin–eosin-stained sections of cerebral cortex and hippocampus from normal group animals showed intact cytoarchitecture, with regularly arranged neurons possessing well-defined nuclei and prominent nucleoli, and no evidence of degeneration or inflammatory infiltration. Sections from cold water immersion-stressed animals showed marked neuronal injury: shrunken and darkly stained degenerating neurons,

cytoplasmic vacuolation, loss of the orderly laminar arrangement and reduced neuronal density. These structural changes provide the morphological correlate of the behavioural and biochemical deficits recorded in the same animals.

Fluoxetine-treated animals showed substantially preserved architecture with only occasional degenerating neurons. Sections from animals given Kantakari flower extract at 200 mg/kg showed partial protection, with a reduction in vacuolation and in the number of pyknotic neurons compared with the disease control, but incomplete restoration of laminar organisation. At 400 mg/kg the extract produced near-normal cortical and hippocampal morphology, with well-preserved neuronal density and minimal degenerative change, comparable to that observed with fluoxetine. The gradation of histological protection across the two extract doses reinforces the dose-dependence evident in the behavioural and biochemical data.

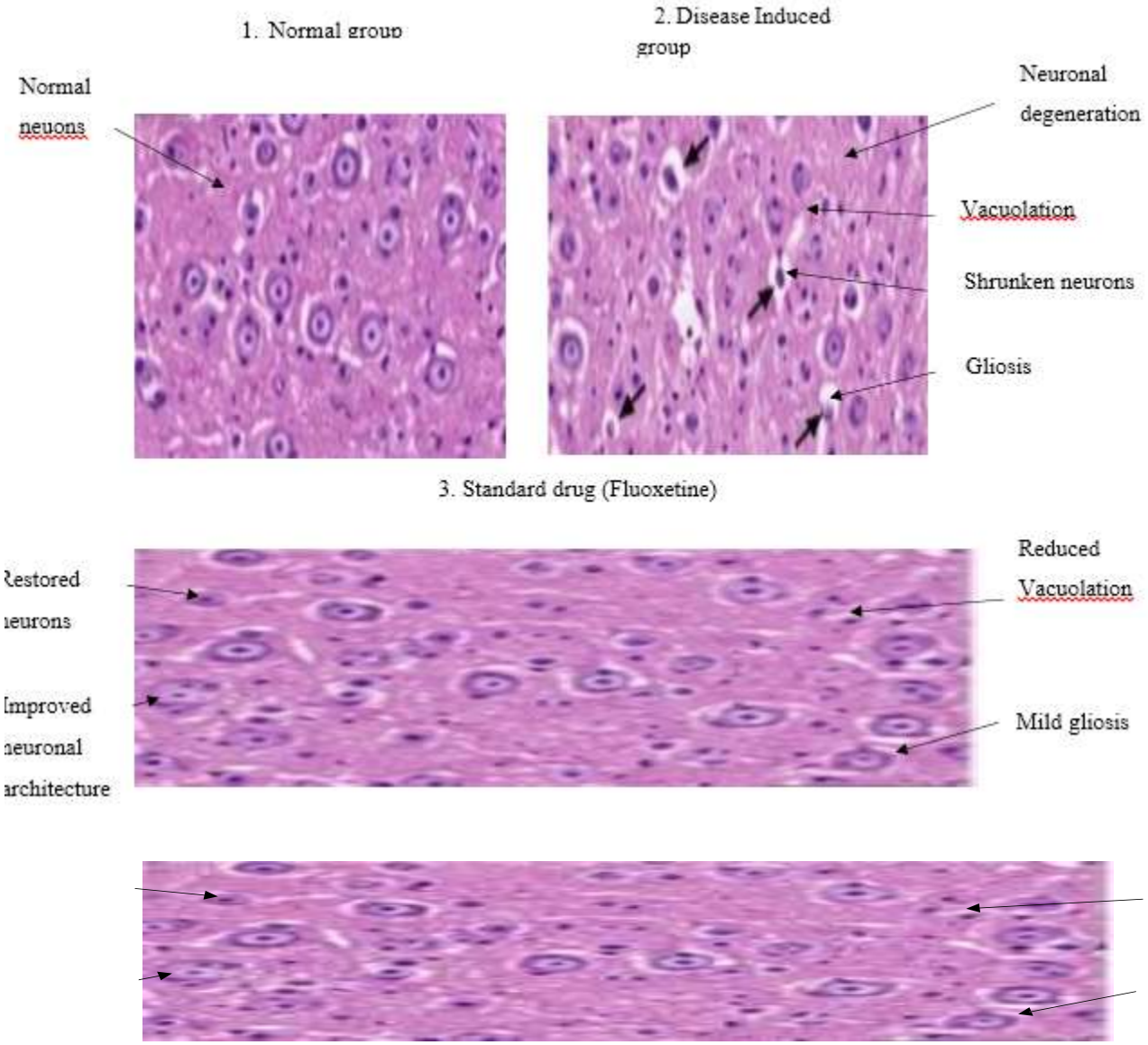


Figure no.13 Representative H&E-stained sections of rat brain following chronic cold water immersion stress. (1) Normal group showing normal neurons. (2) Disease control showing neuronal degeneration, vacuolation and gliosis. (3) Standard drug (Fluoxetine) showing restored neurons, improve architecture and reduced vacuolation with mild gliosis. (4) Kantakari flower extract (200mg/kg) showing partially restored neurons, reduced neuronal degeneration and few vacuoles. (5) Kantakari flower extract (400mg/kg) showing numerous intact neurons and minimal gliosis.

3. Discussion

The central finding of this study is that extract of Solanum xanthocarpum flowers produces dose-dependent antidepressant-like activity in rats subjected to repeated cold water immersion, and that this behavioural benefit is accompanied by restoration of brain antioxidant capacity and preservation of cortical and hippocampal neuronal architecture. At 400 mg/kg the extract matched fluoxetine on every outcome measured. To our knowledge this is the first neuropharmacological evaluation of the flower of this species, an organ that has been consistently overlooked in favour of the fruit and root.

Validity of the disease model is the necessary starting point for interpreting any of these effects. Cold water immersion imposed for 21 consecutive days generated the full behavioural signature expected of a stress-based depression paradigm: locomotor activity declined progressively rather than abruptly, immobility in the forced swim test lengthened in parallel, brain SOD and GSH fell, and histology showed unequivocal neuronal degeneration. The progressive nature of the decline is important. An acute stressor can transiently suppress activity through fatigue or hypothermia alone; a deficit that deepens between day 8 and day 21 while the daily stress dose remains constant is more consistent with cumulative neurobiological adaptation. This is precisely the pattern reported by Agrawal and colleagues in rats exposed repeatedly to cold water immersion, and by Shalyapina and co-workers, who documented stable behavioral passivity in Wistar rats after water immersion stress. Mechanistically, cold immersion is a compound physical and psychological stressor: it triggers sustained corticotropin-releasing hormone and corticosterone release, and chronic glucocorticoid exposure is itself sufficient to deplete hippocampal glutathione, promote lipid peroxidation and impair mitochondrial function.

The behavioral results require careful interpretation because immobility and locomotion are not independent readouts. A compound that sedates an animal will increase immobility; a compound that stimulates it will reduce immobility without any effect on affective state. The forced swim test is therefore uninterpretable in isolation, a point emphasized repeatedly in critical appraisals of the paradigm. In the present study the two measures moved in opposite directions in the treated groups — locomotor activity rose while immobility fell — which excludes the sedative explanation. Nor can the reduction in immobility be dismissed as generalized psychostimulation, since the behavioral improvement developed gradually over two weeks rather than appearing acutely, and was accompanied by biochemical and structural normalization that a stimulant would not produce.

One observation warrants explicit comment. At 400 mg/kg, locomotor activity exceeded the normal control significantly and by day 21 reached 333.0 ± 8.5 against 290.4 ± 2.3 in unstressed animals. The temporal profile of the immobility data is one of the more instructive features of the study. On day 10, immobility in all three treated groups remained close to that of the disease control; separation appeared only from day 14 and was maximal at day 21. This latency mirrors the two-to-four-week onset characteristic of SSRIs in clinical practice, which is generally attributed to downstream neuroadaptive change — receptor desensitisation, enhanced BDNF–TrkB signalling and restored synaptic plasticity — rather than to the immediate elevation of synaptic serotonin. That the extract followed the same time course rather than acting acutely argues against a simple stimulant or anxiolytic mechanism and is consistent with a slower, plasticity-dependent action.

The biochemical findings supply the most direct mechanistic evidence available from this dataset. Superoxide dismutase catalyses the dismutation of superoxide anion to hydrogen peroxide and is the first enzymatic barrier against oxidative injury; reduced glutathione is the principal non-enzymatic thiol buffer and the cofactor for glutathione peroxidase. Depletion of both in stressed animals indicates that the antioxidant system was overwhelmed rather than merely challenged.

The histopathological data close the causal chain between biochemistry and behaviour. Cortical and hippocampal sections from stressed animals showed shrunken hyperchromatic neurons, vacuolation and disrupted lamination the morphological endpoint of sustained glucocorticoid and oxidant exposure. Both regions are directly implicated in human depression: hippocampal volume loss is one of the most replicated structural findings in MDD, and prefrontal dysfunction underlies the cognitive and executive deficits that accompany the mood disturbance. The dose-graded preservation of architecture by the extract, near-complete at 400 mg/kg, indicates that the antioxidant effect translated into structural protection rather than remaining a

biochemical epiphenomenon. It also suggests that the treatment prevented, rather than merely masked, the neuronal consequences of chronic stress.

Fluoxetine performed as expected, reversing behavioural, biochemical and structural changes. Its restoration of SOD and GSH is worth noting in itself, since it reinforces evidence that chronic SSRI treatment exerts antioxidant and neuroprotective actions extending beyond serotonin transporter blockade. That the extract achieved comparable outcomes at 400 mg/kg does not imply equivalent clinical.

The clinical relevance of these findings should be framed conservatively. Depression associated with high oxidative load, chronic inflammation and prominent somatic symptomatology is the phenotype most likely to respond to an antioxidant-based intervention, and it is also the phenotype that responds least reliably to SSRI monotherapy. A well-tolerated botanical adjuvant with a favourable safety profile could plausibly find a place in that setting, either as an add-on to conventional therapy or as an option where standard agents are poorly tolerated. Any such application, however, is several steps removed from the present data.

4. Conclusion

Repeated cold water immersion for 21 days produced a reproducible depressive-like phenotype in Wistar rats, characterised by progressive suppression of locomotor activity, prolonged immobility in the forced swim test, depletion of brain superoxide dismutase and reduced glutathione, and histologically evident neuronal degeneration in the cerebral cortex and hippocampus. Oral administration of Solanum xanthocarpum flower extract at 200 and 400 mg/kg attenuated all of these changes in a dose-dependent manner, and the higher dose produced behavioural, biochemical and histological outcomes comparable to those of fluoxetine 10 mg/kg. Because locomotor activity was preserved rather than depressed, the reduction in immobility reflects genuine antidepressant-like activity rather than a non-specific motor effect.

The concordance between behavioural recovery, restoration of endogenous antioxidant defences and preservation of neuronal architecture supports the interpretation that the antidepressant-like activity of the extract is mediated, at least in part, through attenuation of oxidative stress and consequent neuroprotection. These findings identify the flower of S. xanthocarpum — an organ previously unexamined pharmacologically — as a source of neuroactive constituents worth pursuing. They do not, however, establish a molecular mechanism, identify an active principle or demonstrate safety. Chemical standardisation, mechanistic studies addressing Nrf2 signalling, neurotrophic factors, HPA axis activity and monoaminergic transmission, and formal toxicological evaluation are required before any therapeutic claim can reasonably be advanced.

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