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Pharmacological evaluation for anxiolytic and antidepressant effect of Hydroalcoholicroot extract of *Caesalpinia pulcherrima* in Swiss Albino mice

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Abstract
Experience of mental illness is as old as human existence. Studies have reported that anxiety and depression may occur together with the association of sub threshold depressive symptoms. Anxiety may also predispose depression or symptoms of anxiety and depression may be external manifestations of one under cause. Thus, depression and anxiety issues are difficult enough to deal without the added concern of side effects and cost. Herbal extracts is useful in anxiety relief. In preliminary studies of *Caesalpinia pulcherrima* extracts we identified the enough efficacy for the mental illness. Qualitative studies have shown the presence of carbohydrates, proteins and phenolic compounds in *Caesalpinia pulcherrima* root extract. The extracts of *Caesalpinia pulcherrima* roots are very effective on swimming endurance of mice. This investigation was done as described by the previous investigators, using four groups of albino mice and swimming survival time was recorded. In this method, the ant stress effect of extracts was evaluated by determining the improvement in swimming endurance period and overall performance of the animals. This test is widely used to evaluate antistress activity of drugs, since swim endurance reflects physical endurance. Pretreatment with extracts of *Caesalpinia pulcherrima* have very significantly ($p<0.01$) increased the survival time or swimming endurance of mice at the tested doses of 200 and 400 mg per kg body weight, when compared to the untreated mice. *Caesalpinia pulcherrima* at 400 mg per kg body wt of animal showed maximum increase in swimming survival time.

Keywords: Hydroalcoholicroot extract, anxiolytic, antidepressant, *Caesalpinia pulcherrima*

Introduction
Anxiety is a normal emotional behavior. When it is severe and/or chronic, however, it becomes pathological and can precipitate or aggravate cardiovascular and psychiatric disorders. Although many drugs are available in allopathic medicine to treat anxiety disorders, they produce various systemic side effects or exhibit tolerance upon chronic use [1]. In ayurvedic medicine, many plant products have been claimed to be free from side effects and less toxic than synthetic drugs. Depression is an etiologically heterogeneous group of chronic psychiatric illness with a high prevalence rate (21%) and the second leading cause of the loss of human disability-adjusted life year [2-4]. The prevalence rate of depression is 6-8% in female and 3-5% in male. Clinically characterized by a wide range of symptoms that reflect alternation in cognitive, psychomotor, biological, motivational, behavioral, emotional process and refers to either negative effect or absence of positive effects. The studies have suggested, depression also affects the quality of life of many people and has become a major cause of suicidal death. An anxiolytic is a substance or treatment that reduces anxiety, while an antidepressant is a substance or treatment that helps alleviate symptoms of depression. Both are used to treat various mood and anxiety disorders, but they work through different mechanisms and are often distinct in their primary effects. Anxiolytics are substances or treatments that are primarily used to reduce symptoms of anxiety. They can be categorized into various classes, including benzodiazepines, SSRIs (Selective Serotonin Reuptake Inhibitors), SNRIs (Serotonin-Nor epinephrine Reuptake Inhibitors), and others [5-9].
Treatment of depression entirely depends on clinically available synthetic antidepressants (that based on serendipitous, act via monoamine neurotransmitters, such as serotonin and noradrenalin).

Only certain portion of the patients shows full remission in response to these antidepressants and other hand, associated with more side-effects and the chronic toxicity that affect almost every organ system. These available drugs have potential for adverse effects on cognition and behavior [6-7]. To obtain better therapeutic benefits and minor adverse reactions, there is a pressing need for alternative antidepressant from natural source i.e., herbal remedies, used traditionally, now documented with safe profile. Recently, it has been reported the use of poly herbal formulation exhibiting synergistic activity achieving maximum beneficial potency as compared to single herb. *Caesalpinia pulcherrima* is a large shrub, generally grown in gardens for it showy reddish-yellow flowers, found throughout India. Vernacular name of *Caesalpinia pulcherrima* are: English, Barbados pride, Paradise Flower, Peacock Flower. Hindi, Guletura, Gulutora. Sanskrit-Ratanagandhi, Krishnachure. Kannada-Kenjige, Kamari, Kenjigida [10]. According the review of literatures it was found that the leaves, flowers and fruits contains tannins, gums, resins, benzoic acid and a red coloring matter3. Root contains three new caesalpin-type diterpenoids, hydroxyvouacapen-5α-ol (C20H26O2) along with sitosterol3. Stem contains pertogynoids, pulcherrimin and 6-methoxypulcherrimin, two homoisoflavonoids, bonducellin and 8-methoxybonducellin besides 2, 6-dimethoxybenzoquinon, 4cmethylisoliquiritigenin and a new diterpene ester, pulcherralpin, two ellagitannins, *viz*. tannin1 and tannin 2 beside gallic, ellagic and sebacic acid and β-sitostero [11, 12].

Root, leaf, flower and seed used variously in indigenous medicines for stomachache, gall bladder problems, kidney stones, to accelerate childbirth, as an abortifuge, among other ailments. It also traditional & folklore medicine it is found to be used in treatment of ulcer, cholera, bronchitis, asthma, malarial fever and as abortifacient.1etc. In Indo-China, the plant is considered tonic, stimulant and emmenagogue. *Caesalpinia pulcherrima*, commonly known as Pride of Barbados or Red Bird of Paradise, is a plant that is often used in traditional medicine for various purposes, including its potential as an antidepressant and anxiolytic. However, it's important to note that the scientific evidence supporting its effectiveness for these purposes is limited, and it should not be considered a primary or first-line treatment for clinical depression or anxiety disorders. In some traditional medicinal systems, such as Ayurveda, different parts of *Caesalpinia pulcherrima*, including the leaves and flowers, have been used for their potential calming and mood-enhancing properties. They are often prepared as herbal remedies or teas. While there may be anecdotal reports and traditional use suggesting potential benefits, there is a lack of rigorous clinical studies and scientific evidence to establish the efficacy and safety of *Caesalpinia pulcherrima* as an antidepressant or anxiolytic. Balakrishna, *et al*. (2021) [14] *Caesalpinia pulcherrima* Swartz native to India referred to as Guletura is widely distributed in South India, and its leaves, flower, bark, and seeds are employed in Indian medicine. The plant contains many active elemental fractions like caesalpin-type diterpenoids, sitosterol, pulcherrimin, lupeol, lupeol acetate, myricetin, quercetin and rutin, flavonoids, carotenoids, glycosides, peltogynoids, phenols, and steroids. The current study was designed to gauge the anti-anxiety activity of varied extracts *viz* n-hexane, chloroform, ethyl acetate, and methanol of the leaves of *Caesalpinia pulcherrima* by using elevated plus maze (EPM) model in albino mice. Albino mice have ministered orally with different doses of the extracts (i.e. 200 and 400mg/kg) and behavior was observed on the EPM. Diazepam (2mg/kg, P.O) was used as a standard (positive control). Results indicate that the methanol extract of Caesalpinia leaves showed maximum and significant dose-dependent effect at 200 and 400mg/kg on EPM, the results were just like the standard antianxiety agent diazepam (2mg/kg). In the Actophoptometer model, two different doses of *Caesalpinia pulcherrima* (200 and 400mg/kg) showed a dose-dependent decrease within the locomotor activity, compared to the control animals. As the phytochemical screening of methanol extract exhibited the presence of polyphenols could be liable for the anxiolytic potential of C.pulcherrima. Hence this plant could also be developed as a potentially useful anti-anxiety agent [14].

Material and methods

Collection of the sample

Plant Material Collection and Extraction: Root samples of caesalpinza pulcherrima will be sourced from reputable suppliers and authenticated by botanist in Janta PG Collage APS University Rewa.

Preparation of crude powder: The collected plant material will be manually cleaned to remove course impurities and then air-dried in shade at a well-ventilated place in the laboratory. Further drying will be done in the incubator to remove moisture at a temperature of 40 °C. The dried bark will be crushed and grounded in electric mixer-grinder to form crude powder and stored in air tight container.

Preparation of hydro alcoholic extracts: The authenticated aerial parts of *Caesalpinia pulcherrima* will be extracted successively with hydro alcoholic solution (Ethanol 70%: Water 30%)

Animal Models: Adult male mice will be obtained from an accredited animal facility. They will be acclimated to laboratory conditions for a specified period before the experiments commence. The animals will be housed in standard cages with controlled temperature and a 12 Hour light-dark cycle. Standard rodent chow and water will be provided ad libitum. Animal will be kept at a temperature of with a 12 hour light/dark cycle and a relative humidity of 50-60%. Free access to food and water will be allowed at all the time. Experiment approval will be taken from IAEC (Institutional Animal Ethical Committee) for investigate anti-anxiety and antidepressant effect of extract against in anxiety and depressant in albino mice. The mice will be randomly divided into multiple groups, including control, treatment, and standard drug groups. Treatment groups will receive oral doses of extract *Caesalpinia pulcherrima*. While the control group will receive a placebo. A standard drug group will receive a reference anxiolytic / anti-depressant drug.

Behavioral Assays: A series of established behavioural assays will be conducted to assess antidepressant and anxiolytic effects. Forced Swim Test: Mice will be placed in a water-filled cylinder, and their mobility and immobility periods will be recorded [9-14].

Results

Qualitative studies on *Caesalpinia pulcherrima* root extract exhibited presence of carbohydrates, proteins and phenolic compounds. Tests for Phytosterols and Triterpenoids were found to be positive revealing the presence of steroids and triterpenes. The voucher specimen for plant authentification is J/Not/CI-L/125 dated 16.11.2023.

Table 1: Preliminary qualitative studies of extracts by phytochemical investigation.

Phytochemical constituents	Tests	*Caesalpinia pulcherrima*
Carbohydrates	Molisch' stest	+
	Barfoed' stest	+
	Fehling' stest	+
	Benedict' stest	+
Proteins and freeaminoacids	Biurettest	+
	Millon'stest	+
	Ninhydrintest	+
Alkaloids	Mayer'stest	-
	Dragendroff'stest	-
	Hager's test	-
	Wagner'stest	-
Glycosides	Borntrager'stest	-
	Legaltest	-
Phytosterols and Triterpenoids	Liebermann-Burchard'stest	+
	Salkowski'stest	+
Phenolic compounds and tannins	Ferricchloridetest	+
	Leadacetatetest	+
	Gelatintest	-
Flavonoids	ShinodaTest	-
	Sodiumhydroxide test	-
	Leadacetatetest	-
Saponins	Foamtest	-
Fixed oils and fats	Staintest	-

Animal (Acute toxicity) study

The pharmacological investigations were conducted on albino mice and albino rats of either sex. Ethical clearance was obtained for procuring of animals and for evaluating antistress activity of *Caesalpinia pulcherrima*. (Approval No. IAEC/2024/22/038).

Animal maintenance (Housing and feeding condition)

Experimental studies were carried out using normal adult Albino mice (24±2 g) and Albino rats (200±20 g) of either sex of Wistar strain. Animals were housed in clean and sanitized polypropylene cages under standard environmental conditions of relative humidity (50±5%), room temperature (25±2 °C) and photocycle (12:12h of light/dark period with lights on 0700h). Feed of animals was dietary pellets (pellets of Amruth Lab. Bangalore). Drinking water was maintained *ad libitum*. All animals were acclimatized and habituated to laboratory conditions, for seven days prior to experiment, to minimize nonspecific stress conditions.

In this study, a single dose of the drug was administered orally to each animal for the determination of gross behavior and median lethal dose (LD_{50}), which can be expected to cause death in fifty percent of animals. An acute toxicity study was conducted for *Caesalpinia pulcherrima* extracts, as per guidelines set by Organization for Economic Co-operation and Development (OECD guideline No. 425) received from CPCSEA. Limit test at 5000 mg/kg body weight was considered to determine acute toxicity study of both the extracts. As per guidelines of OECD, this limit test would have a direct relevance for protecting human and animal health. As per this principle, animal was administered with the extract at the dose of 5000 mg per kg body weight. After the survival of the first animal, then two additional animals were administered with the extract at the dose of 5000 mg per kg body weight. Test was terminated after the survival of additional animals. Observation of animals was carried for 14 days.

Effect of extracts of *Caesalpinia pulcherrima* roots on swimming endurance of mice

In this method, the anti-stress effect of extracts was evaluated by determining the improvement in swimming endurance period and overall performance of the animals, when subjected to swim in restricted space like water vessel. Experimental investigation was carried out using albino mice of either sex, weighing between 22 to 26 g. Experiment was conducted in compliance with the guidelines provided by CPCSEA. Testing drugs were *Caesalpinia pulcherrima* roots extracts suspended in distilled water using 1% w/v gum acacia. Geri forte was used as standard drug suspended in distilled water using 1% w/v gum acacia. Vehicle was 1% w/v gum acacia prepared in normal saline. The dose of plant extracts selected in this investigation was 200 and 400 mg/kg body weight of animals. The dose of Geri forte was 100 mg/kg body weight of animal, selected form previous reported studies of Geri forte as antistress agent. The dose of vehicle was 10 ml/kg body weight of animal. Albino mice of either sex were randomly assigned into six groups, and each group consisted of six mice as follows:

Group 1: Animals of this stress control group were administered with vehicle and subjected for swimming stress.

Group 2: Albino mice of this group were administered with *Caesalpinia pulcherrima* roots extract at the dose of 200 mg/kg body weight and subjected for swimming stress.

Group 3: Albino mice of this group were administered with *Caesalpinia pulcherrima* roots extract at the dose of 400 mg/kg body weight and subjected for swimming stress.

Group 4: In this standard group, animals were administered with Geri forte at the dose of 100 mg/kg body weight and then subjected for swimming stress.

Table 2: Acute toxicity study of *Caesalpinia pulcherrima* roots extract.

Parameters		Time(min)on1st Day								2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th
		30	60	90	120	150	180	210	240													
Behavioral observation																						
	Alertness	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	Stereotypy	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Awareness	Visual placing	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	Passivity	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Vocalization	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Grooming	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Mood	Restlessness	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Irritability	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Fearfulness	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Spontaneous	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	Activity																					
Motor activity	Reactivity	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	Touch response	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	Pain response	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Toxicity																						
Mortality	Acute	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	Delayed	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Moribund status	Acute	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	Delayed	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

The base score for normal signs or effects is 4. Score below or above 4 indicates subnormal or supernormal respectively.

Acute toxicity study

As shown in the table 3, during acute toxicity studies of plant *Caesalpinia pulcherrima* extract, mice showed normal behavioral, neurological and autonomic response. Mice were neither depressed nor excited throughout study. Mice did not show any change in alertness, visual placing, grooming, reactivity, spontaneous activity, touch response, pain response, posture, limb tone, grip strength and abdominal tone. Pupil size, corneal and pinna reflex were normal. There were no signs of CNS or autonomic stimulation, such as vocalization, restlessness, aggression, fearfulness, tremors, convulsion, Straub tail phenomenon, writhing and piloerection. As shown in table 3, animal showed normal body weight gain during 14 days. There was no significant change in body weight as compared to control group. There was no noticeable toxicity and no deaths till the dose tested (up to the dose of 5000 mg per kgbody weight of animal) [14-16].

Table 3: Effect of extract on body weight of mice

SL. No.	Group	Mean body weight of mice (g) ± SEM		
		Before dosing(1st Day)	After1st week (7th Day)	After2nd week(14th Day)
1	Control	23.67±0.71	24.67±.49	27.33±0.61
2	*Caesalpinia pulcherrima*	26.17±1.11^{ns}	27.33±1.12^{ns}	29.33±0.76^{ns}

Values shown for each group as the mean ± S.E.M. obtained from six observations.

Where^{ns} $p>0.05$ represent non-significant, compared to control group.

Effect of extracts of *Caesalpinia pulcherrima* roots on swimming endurance of mice

Improvement in swimming endurance period of mice was used as a parameter to evaluate antistress activity of extracts. As shown in table 4, the swimming endurance or survival time of mice in stress control group without any drug treatment was found to be 187.33± 7.3 min. The swimming survival time of mice in standard drug treated group with Geri forte was found to be 248±12 min. Compared to stress control group value, Geri forte exhibited highly significant ($p<0.001$) increase in swimming survival time. Group of mice (n=6) treated with *Caesalpinia pulcherrima* at the dose of 200 mg per kg body weight of animal, exhibited survival time of 227.33±5.4 min. Compared to the stress control group value, *Caesalpinia pulcherrima* showed statistically very significant ($p<0.01$) increase in swimming survival time. In a dose response study, *Caesalpinia pulcherrima* at 400 mg per kg body weight of animal, exhibited survival time of mice with 243.46±9.2 min. Compared to the stress control group value, *Caesalpinia pulcherrima at* 400 mg per kg body weight of animal showed highly significant ($p<0.001$) increase in swimming survival time. Compared to standard drug treated group, both the test extracts exhibited non significance change in swimming survival time of mice. *Caesalpinia pulcherrima at* 400 mg per kg body wt. of animal showed maximum increase in swimming survival time (statistically highly significant with $p<0.001$).

Table 4: Effect of *Caesalpinia pulcherrima* root extracts on swimming endurance

SL. No.	Group	Treatment	Dose / kg Body weight	Survival time (min) Mean ± S.E.M.
1	Control	Vehicle + Swimming	0.25ml/mice, P.O.	187.23±7.065
2	Standard	Geri forte + Swimming	100mg, P.O.	248.12± 11.056**
3	CP200	*Caesalpinia pulcherrima* extract + Swimming	200mg, P.O.	227.33±5.469*#
4	CP400	*Caesalpinia pulcherrima* extract + Swimming	400mg, P.O.	243.46±9.291**#

Values shown for each group as the mean ± S.E.M. obtained from six observations. Where *p<0.01 and **p<0.001 represent very significant and highly significant respectively, compared to the control group. Whereas #p>0.05 represent non-significant compared to standard (Geri forte) group.

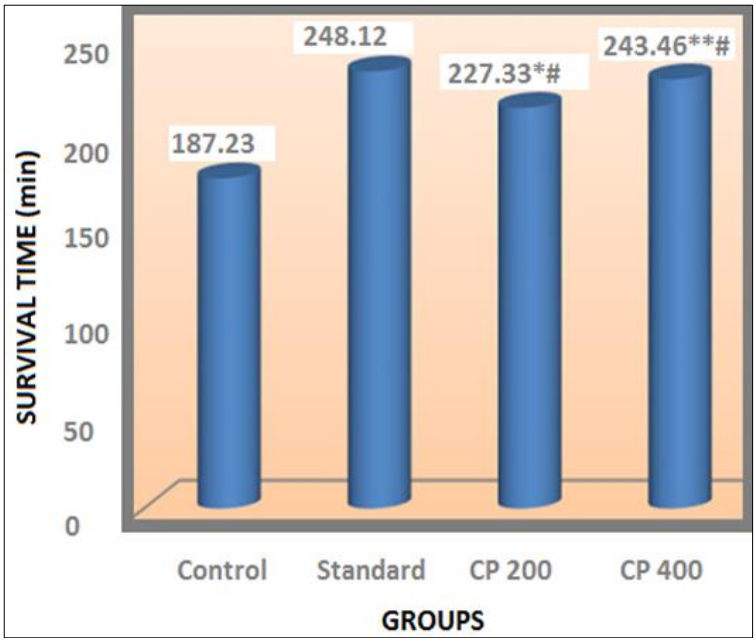

Fig 1: Effect of *Caesalpinia pulcherrima* on swimming endurance of mice

Values shown for each group as the mean ± S.E.M. obtained from six observations. Where *p<0.01 and **p<0.001 represent highly significant compared to control group. Whereas #p>0.05 represent non-significant compared to standard (Geri forte) group.

Effect of *Caesalpinia pulcherrima* root extracts on drug induced narcosis in mice: In this method, the effect of extract on righting reflex of mice was used as a parameter to determine synergism with CNS depressant. As shown in table 5, Phenobarbitone sodium induced narcosis time in normal mice was found to be 31.80±2.2 min.

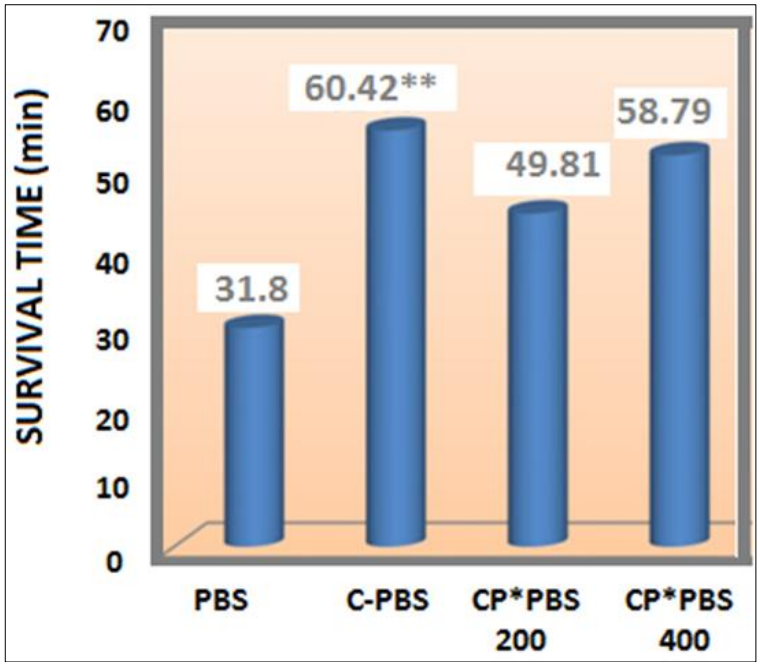

Fig 2: Effect of *Caesalpinia pulcherrima* root extract on Pentobarbitonesodium induced narcosis in mice

Table 5: Effect of *Caesalpinia pulcherrima* root extracts on Phenobarbitone sodium induced narcosis in mice

Group	Treatment	Dose/kg Bodyweight	Narcosis time (min) Mean± S.E.M.
1	Pentobarbitonesodium	50mg;i.p.	31.8±2.262
2	Chlorpromazine+ Pentobarbitonesodium	3mg;i.p.+ 50mg;i.p.	60.42±4.094**
3	*Caesalpinia pulcherrima* extract+ Pentobarbitone sodium	200mg;*p.o.*+ 50mg;i.p.	49.81±4.448*#
4	*Caesalpinia pulcherrima* extract+ Pentobarbitone sodium	400mg;*p.o.*+ 50mg;i.p.	58.79±5.3*#

Values shown for each group as the mean ±S.E.M. obtained from six observations. Where $^{*}p<0.01$ and $^{**}p<0.0001$ represent highly significant and extremely significant respectively compared to control group (Pentobarbitone sodium). Whereas $^{\#}p>0.05$ represent non-significant compared to standard (Chlorpromazine+ Pentobarbitone sodium) group.

Discussion

Preliminary qualitative studies of extracts. Qualitative studies have shown the presence of carbohydrates, proteins and phenolic compounds in root extract. Even tests for Flavonoids were found to be positive for extract, indicating its presence in the roots extract of plant. *T*he presence of steroids and triterpenes. During acute toxicity studies of both plant extracts, mice showed normal behavioral, neurological and autonomic response. Mice were neither depressed nor excited throughout study. Mice did not show any change in alertness, visual placing, grooming, reactivity, spontaneous activity, touch response, pain response, posture, limb tone, grip strength and abdominal tone. Pupil size, corneal and pinna reflex were normal. There were no signs of central nervous system or autonomic stimulation, such as vocalization, restlessness, aggression, fearfulness, tremors, convulsion, straub tail phenomenon, writhing, piloerection, salivation and urination. There was no significant change in body weight as compared to control group. During acute toxicity studies of both plant extracts, there was no noticeable toxicity and no deaths till the dose tested (up to the dose of 5000 mg per kg body weight of animal) [14, 15]. Effect of ethanolic extracts of *Caesalpinia pulcherrima* roots on swimming endurance of mice was very good. This investigation was done as described by the previous investigators, using four groups of albino mice and swimming survival time was recorded. In this method, the antistress effect of extracts was evaluated by determining the improvement in swimming endurance period and overall performance of the animals, when subjected to swim in restricted space like water vessel. This test is widely used to evaluate antistress activity of drugs, since swim endurance reflects physical endurance. Pretreatment with extracts of *Caesalpinia pulcherrima* have very significantly ($p<0.01$) increased the survival time or swimming endurance of mice at the tested doses of 200 and 400 mg per kg body weight, when compared to the untreated mice. *Caesalpinia pulcherrima* at 400 mg per kg body wt of animal showed maximum increase in swimming survival time (highly significant with $p<0.001$). The swimming endurance of mice was increased by *Caesalpinia pulcherrima* extracts, thereby showing antistress activity of this plant extracts. Effect of extracts of *Caesalpinia pulcherrima* roots on drug induced narcosis in mice. This investigation was done as described by the previous investigators, using seven groups of albino mice and sleeping time (Narcosis time) was recorded. In this method, the antistress effect of extracts was evaluated by determining their (Adaptogen) ability to synergize with CNS depressant such as barbiturates, and it could be the mechanism of antistress activity.

Conclusion

Pharmacological evaluation of a polyherbal formulation for its anti-anxiety and antidepressant effects is a crucial step in the development of herbal remedies for mental health disorders. The primary goal of pharmacological evaluation was to determine whether the polyherbal *Caesalpinia pulcherrima* root extract based formulation indeed possesses anti-anxiety and antidepressant effects. This involves conducting rigorous scientific studies, such as animal models to assess its efficacy in alleviating anxiety and depression symptoms. These results can provide insights into the biological pathways involved and help refine the formulation or guide the development of similar remedies in the future. The pharmacological evaluation of a polyherbal formulation for its ant anxiety and antidepressant effects involves rigorous testing to establish its safety, efficacy, and mechanism of action. This process is crucial for developing evidence-based herbal remedies and integrating them into clinical practice for mental health disorders.

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