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Evaluation of Antidepressant-like Effects of *Carissa spinarum* Root Bark Extract in Wistar Rats via Serotonergic and Noradrenergic Pathways.

Pratiksha Jetithor [1], Ms. Rutuja Mule [2], Dr. Rani Divekar [3], Dr. Swati Jogdand [4], Dr. Swati Deshmukh [5]
CAYMET's Siddhant college of Pharmacy, Sudumbare, Pune, Maharashtra, India.

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ABSTRACT:
Depression is a major neuropsychiatric disorder associated with disturbances in monoaminergic neurotransmission, oxidative stress, and neuroinflammation. The present study was designed to evaluate the antidepressant-like activity of hydroalcoholic extract of *Carissa spinarum* root bark in Wistar rats using behavioral and biochemical parameters. Preliminary phytochemical screening revealed the presence of flavonoids, alkaloids, tannins, saponins, glycosides, and phenolic compounds. Depression was induced using the Chronic Unpredictable Mild Stress (CUMS) model. Behavioral assessment was performed using Tail Suspension Test (TST), Open Field Test, and lithium-induced head twitch models. Oxidative stress parameters including Superoxide Dismutase (SOD), Catalase (CAT), Reduced Glutathione (GSH), and Lipid Peroxidation (LPO) were evaluated. Fluoxetine was used as the standard drug. The extract significantly reduced immobility time and improved locomotor activity compared with stressed control animals. Furthermore, treatment restored antioxidant enzyme levels and reduced oxidative stress markers. The antidepressant-like effect may be attributed to the presence of flavonoids and other phenolic compounds capable of modulating serotonergic and noradrenergic pathways. The findings scientifically support the traditional use of *Carissa spinarum* in stress-related and neurological disorders.

INTRODUCTION:
Depression is a serious neuropsychiatric disorder characterized by persistent sadness, loss of interest, reduced motivation, impaired concentration, disturbed sleep, fatigue, and emotional instability. It affects the emotional, behavioral, cognitive, and physical well-being of an individual and significantly interferes with daily life activities and social functioning. Depression is recognized as one of the leading causes of disability worldwide and has emerged as a major public health concern due to its increasing prevalence and association with suicide, chronic illness, and reduced quality of life. According to the World Health Organization, millions of individuals globally suffer from depressive disorders, and the incidence continues to rise because of stress, urbanization, lifestyle

changes, and socioeconomic pressures.

The pathophysiology of depression is highly complex and involves disturbances in multiple neurotransmitter systems, neuroendocrine dysfunction, oxidative stress, inflammation, and impaired neuronal plasticity. Among these mechanisms, the monoamine hypothesis remains one of the most accepted theories explaining depression. This theory suggests that reduced levels of monoamine neurotransmitters such as serotonin (5-HT), dopamine (DA), and norepinephrine (NE) in the brain contribute significantly to depressive symptoms. Serotonin regulates mood, appetite, and sleep; dopamine controls pleasure and motivation; whereas norepinephrine influences alertness, stress response, and emotional regulation. Deficiency of these neurotransmitters impairs neuronal communication and leads to symptoms such as hopelessness, anhedonia, and fatigue.

Conventional antidepressant drugs including Selective Serotonin Reuptake Inhibitors (SSRIs), Tricyclic Antidepressants (TCAs), and Monoamine Oxidase Inhibitors (MAOIs) are widely used for the management of depression. SSRIs such as fluoxetine and sertraline act by blocking serotonin reuptake, thereby increasing serotonin levels in the synaptic cleft. gastrointestinal disturbances, sexual dysfunction, sedation, cardiovascular complications, and poor patient compliance. Furthermore, approximately 30–40% of patients fail to achieve complete remission with currently available antidepressant therapies, indicating the need for safer and more effective alternative treatments.

In recent years, medicinal plants have gained considerable attention as alternative therapeutic agents for the management of neurological and psychiatric disorders. Herbal medicines have been extensively utilized in traditional systems of medicine such as Ayurveda, Siddha, Unani, and traditional Chinese medicine because of their therapeutic efficacy, affordability, and relatively lower adverse effects. Several medicinal plants have been scientifically reported to possess antidepressant activity through modulation of monoaminergic neurotransmission, antioxidant defense systems, and neuroprotective pathways. Phytoconstituents such as flavonoids, alkaloids, terpenoids, tannins, glycosides, saponins, and phenolic compounds are known to exhibit antidepressant-like effects by enhancing serotonergic and noradrenergic signaling and reducing oxidative stress–mediated neuronal damage.

Among various medicinal plants, *Carissa spinarum* belonging to the family Apocynaceae has attracted scientific interest because of its broad spectrum of pharmacological activities. The plant is a thorny evergreen shrub widely distributed in tropical and subtropical regions of Asia and Africa. Traditionally, different parts of *Carissa spinarum* have been used for the treatment of fever, inflammation, epilepsy, stress, anxiety, microbial infections, diabetes, and gastrointestinal disorders. Phytochemical investigations of the plant have revealed the presence of several biologically active constituents including flavonoids, alkaloids, tannins, saponins, terpenoids, steroids, and phenolic compounds. These phytochemicals possess antioxidant, anti-inflammatory, neuroprotective, and central nervous system modulatory properties that may contribute to antidepressant-like activity.

Previous studies on medicinal plants have demonstrated that natural antioxidants significantly improve depressive symptoms by reducing oxidative stress and restoring endogenous antioxidant enzymes such as superoxide dismutase, catalase, and glutathione. Several herbal extracts rich in flavonoids and polyphenols have shown promising antidepressant effects in animal models including Tail Suspension Test (TST), and Chronic Mild Stress (CMS) paradigms. Literature surveys have also reported that *Carissa spinarum* possesses potent antioxidant and neuroprotective activities. However, despite its extensive traditional use in nervous disorders and the presence of neuroactive phytoconstituents, there is very limited scientific evidence regarding the antidepressant potential of *Carissa spinarum* root bark and its possible involvement in serotonergic and noradrenergic pathways.

This lack of detailed pharmacological evaluation represents an important research gap. Most available studies focus primarily on the antimicrobial, anti-inflammatory, and antioxidant properties of the plant, while systematic investigation of its antidepressant-like activity remains insufficient. In addition, mechanistic studies exploring its influence on monoaminergic neurotransmission and oxidative stress markers are scarce. Therefore, scientific validation of the traditional use of *Carissa spinarum* in stress-related and depressive disorders is necessary.

The present study was therefore undertaken to evaluate the antidepressant-like effects of hydroalcoholic extract of *Carissa spinarum* root bark in Wistar rats using established behavioral models such as Tail Suspension Test. The study also aimed to investigate the possible involvement of serotonergic and noradrenergic pathways and

assess oxidative stress parameters including Superoxide Dismutase (SOD), Catalase (CAT), Reduced Glutathione (GSH), and Lipid Peroxidation (LPO). The findings of the study may provide scientific evidence supporting the traditional medicinal use of *Carissa spinarum* and contribute toward the development of safer plant-based antidepressant therapies with improved efficacy and reduced adverse effects.

MATERIALS AND METHODS:

Materials

The root bark of *Carissa spinarum* was collected, authenticated, shade dried, and powdered for experimental use. Analytical grade chemicals and reagents such as methanol, petroleum ether, chloroform, ethyl acetate, DPPH, hydrogen peroxide, nitro blue tetrazolium, Folin–Ciocalteu reagent, ferric chloride, aluminium chloride, and phosphate buffer were used throughout the study. Standard drugs including fluoxetine, imipramine, lithium chloride, and haloperidol were procured from standard pharmaceutical suppliers. Instruments used included Soxhlet apparatus, rotary evaporator, UV–Visible spectrophotometer, centrifuge, homogenizer, hot air oven, analytical balance, open field apparatus, tail suspension apparatus, and forced swim tank.

Collection and Authentication of Plant Material

The root bark of *Carissa spinarum* was collected from a local herbal source and authenticated by a qualified taxonomist. The collected material was washed with distilled water to remove impurities and shade dried at room temperature. The dried bark was powdered using a mechanical grinder and stored in airtight containers until further use.

Physicochemical Evaluation

Physicochemical parameters such as total ash value, acid insoluble ash, water soluble ash, loss on drying, and extractive values were determined according to WHO guidelines for crude drugs. Total ash value was determined by incinerating the powdered material in a silica crucible at 500–600°C until carbon-free ash was obtained. Acid insoluble ash was measured after boiling the ash with hydrochloric acid, while water soluble ash was estimated using distilled water. Moisture content was determined by drying the sample at 105°C until constant weight was achieved. Water-soluble and alcohol-soluble extractive values were determined by maceration with water and methanol respectively.

Fluorescence Analysis

The powdered drug was treated with different chemical reagents and observed under visible and ultraviolet light to study fluorescence characteristics. This analysis was carried out for authentication and quality evaluation of the crude drug.

Preparation of Extracts

Approximately 1 kg of powdered root bark was subjected to successive Soxhlet extraction using solvents of increasing polarity including petroleum ether, chloroform, ethyl acetate, and methanol. Extraction was continued until complete exhaustion of the plant material. The obtained extracts were filtered and concentrated under reduced pressure using a rotary evaporator to obtain dry extracts, which were stored in airtight containers for further studies.

Preliminary Phytochemical Screening

Preliminary phytochemical screening was carried out to detect the presence of alkaloids, flavonoids, tannins, glycosides, carbohydrates, proteins, saponins, steroids, terpenoids, phenolic compounds, fats, and oils using standard qualitative chemical tests. Wagner's, Dragendorff's, Mayer's, Shinoda's, Froth, Molisch's, Benedict's, Fehling's, Salkowski, and ferric chloride tests were employed for phytochemical identification.

In Vitro Antioxidant Assays

DPPH Radical Scavenging Assay

The antioxidant activity of the extracts was evaluated using DPPH free radical scavenging assay. Different concentrations of plant extracts and standard ascorbic acid were mixed with DPPH solution and incubated in dark for 30 minutes. Absorbance was measured at 517 nm using a UV spectrophotometer, and percentage inhibition was calculated.

Hydrogen Peroxide Scavenging Assay

Hydrogen peroxide scavenging activity was determined spectrophotometrically at 230 nm. Different concentrations of extracts were mixed with phosphate buffer and hydrogen peroxide solution. Reduction in absorbance indicated antioxidant activity.

Superoxide Radical Scavenging Assay
Superoxide radical scavenging activity was evaluated using NADH, nitro blue tetrazolium, and PMS system. Absorbance was measured at 560 nm and percentage inhibition of superoxide radicals was calculated.

Reducing Power Assay
The reducing power of extracts was assessed by mixing the extracts with phosphate buffer and potassium ferricyanide followed by incubation at 50°C. Ferric chloride was added after centrifugation, and absorbance was measured at 700 nm. Increased absorbance indicated greater reducing power.

Quantitative Estimation of Phytoconstituents
Total phenolic content was estimated using Folin–Ciocalteu reagent with gallic acid as standard. Total flavonoid content was determined using aluminium chloride colorimetric method with quercetin as standard. Total alkaloid content was estimated using bromocresol green method, whereas total saponin content was measured using vanillin-sulphuric acid assay. Absorbance values were recorded using a UV–Visible spectrophotometer.

Experimental Animals
Healthy Wistar rats of either sex weighing approximately 150–200 g were used for the experimental study. Animals were housed in polypropylene cages under standard laboratory conditions at temperature 25 ± 2°C with relative humidity of 45–55% and 12 h light/dark cycle. Animals were supplied with standard pellet diet and water ad libitum. All experimental procedures were performed according to CPCSEA guidelines and approved by the Institutional Animal Ethics Committee.

Experimental Design
Animals were divided into five groups containing six animals in each group. Group I served as normal control and received vehicle only. Group II served as disease control and received chronic unpredictable mild stress with vehicle treatment. Group III received standard drug imipramine hydrochloride (10 mg/kg). Group IV , Group V and Group VI received methanolic extract of *Carissa spinarum* at doses of 200 mg/kg , 300 mg/kg and 400 mg/kg respectively through oral route.

Evaluation of Antidepressant Activity
Tail Suspension Test (TST)
In tail suspension test, rats were suspended individually using adhesive tape fixed approximately 1 cm from the tip of the tail. The animals were observed for 6 minutes and the duration of immobility was recorded. Decrease in immobility time indicated antidepressant-like effect.

Lithium-Induced Head Twitch Test
Lithium chloride was administered to induce head twitch responses in rats. The frequency of head twitches was recorded for one hour to evaluate the involvement of serotonergic pathways in antidepressant activity.

Haloperidol-Induced Catalepsy
Haloperidol-induced catalepsy test was performed to assess dopaminergic modulation. Rats were placed with forepaws on a horizontal glass bar and duration of catalepsy was recorded at different time intervals. Reduction in cataleptic duration indicated possible dopaminergic activity of the extract.

Open Field Test
Open field test was used to evaluate locomotor and exploratory behavior. Rats were placed in an open field apparatus divided into equal squares, and locomotor activity, rearing, and grooming behavior were recorded for 6 minutes. Increased locomotor activity was considered as improvement in behavioral performance.

Statistical Analysis
All data obtained from experimental studies were expressed as mean ± SEM. Statistical analysis was carried out

using one-way Analysis of Variance (ANOVA) followed by Tukey's multiple comparison test. Values of $p < 0.05$ were considered statistically significant.

RESULTS:

Standardization of Plant Material (Preliminary Pharmacognostical Studies)

The safety and efficacy of herbal products depends upon the standardization of the herbs used[1-3]. Therefore physicochemical characterization of plant was done for the standardization of crude drug. The physicochemical parameters of *Carissa spinarum L.* were studied and the results are enlisted in tables 7.1 and 7.2.

The total ash value obtained from *Carissa spinarum L.* was 7.1±0.09% which clearly indicated the presence of lesser amount of inorganic substances like carbonate, silicates, oxalates, and phosphates etc.

The acid insoluble ash value of *Carissa spinarum L.* was 1.3±0.18%. The water soluble ash value of *Carissa spinarum L.* was 2.5±0.32% and loss on drying of *Carissa spinarum L.* powder was 10.2±0.82% respectively. The estimation of acid insoluble ash value demonstrated the presence of acid insoluble inorganic substances whereas the estimation of water soluble ash value revealed the presence of water solvent inorganic substances.

Alcohol and water extractive values of *Carissa spinarum L.* were 16.4±0.64% and 21.8±0.72%, respectively. These findings indicated that flowers contains higher amount of highly water soluble phytoconstituents.

The fluorescence investigation was done to determine the quality & appearance of the medication material. The powdered drug showed divergent fluorescence characters in the presence of ultra-violet light because of quality of various constituents in the medication. The results of fluorescence characteristics of drug are displayed in table 7.2.

Table 7.1: Physical Parameters of *Carissa spinarum L.* Powder

Parameters	Values (% w/w)
Total ash	7.1 ± 0.09
Acid insoluble ash	1.3 ± 0.18
Water soluble ash	2.5 ± 0.32
Loss on drying	10.2 ± 0.82
Alcohol extractive	16.4 ± 0.64
Water extractive	21.8 ± 0.72

Values are expressed as mean ± SEM

Table 7.2: Fluorescence Characters of *Carissa spinarum L.* Powder

Treatment	Day light	UV Light
Powder	Pinkish	Pink
Powder + 1 N HCl	Light pink	Dark pink
Powder + aqueous 1 N NaOH	Yellowish pink	Greenish pink
Powder + alcoholic 1 N NaOH	Colorless	Dark pink
Powder + 50% HNO_3	Yellowish orange	Greenish pink
Powder + 50% H_2SO_4	Yellowish pink	Fluorescent pink
Powder + ME	Pink	Pink
Powder + Water	Light Pink	Yellowish Pink

7.1 Extraction of Plant Material

The chemical constituents present in the plants are responsible for their varied therapeutic attributes. Hence the presence of secondary metabolites present in the plant part was determined by following standard methods of phytochemical screening. The flowers were extracted with different solvents namely petroleum ether, chloroform, ethyl acetate and methanol. The % yield of petroleum ether extract (PESC), chloroform extract (CESC), ethyl acetate extract (EASC) and methanolic extract (MESC) were found as 3.5%, 5.8%, 10.6% and 17.3%, respectively as shown in table 7.3

The study also revealed that *Carissa spinarum L.* flowers contain higher amount of semi polar and polar secondary metabolites. The pharmacological activity of these metabolites varies according to their polarity or nature of phytoconstituents.

Table 7.3: % Yield of *S.campanulata* Flowers in Different Solvent Extracts

Extract	Yield (% w/w of powdered drug)
PESC	3.5
CESC	5.8
EASC	10.6
MESC	17.3

7.3 Qualitative Phytochemical Screening of *Carissa spinarum L.*

Preliminary investigation of phytochemicals from *Carissa spinarum L.* Root bark uncovered the presence of flavonoids, tannins, phenolics, alkaloids, glycosides, fats, proteins, saponin and starches in various plant extracts. The results of phytochemical screening of various Root bark are depicted in table 7.4

The results of phytochemical screening of *Carissa spinarum L.* flowers revealed the presence of polyphenols in all extracts, while flavonoids were available only in EASC and MESC as depicted in table 7.4.

Table 7.4: Phytochemicals Present in *Carissa spinarum L.* Root bark

Phytoconstituents	Extracts			
	PESC	CESC	EASC	MESC
Proteins	-	+	-	+
Carbohydrates	-	-	+	+
Flavonoids	-	-	+	+
Glycosides	-	+	+	+
Saponins	+	+	+	+
Polyphenols	+	+	+	+
Tannins and phenolic compounds	+	+	-	+
Alkaloids	-	+	+	+
Steroids	-	-	+	-
Fats and oils	+	-	+	-

+ = Present, □ = Absent

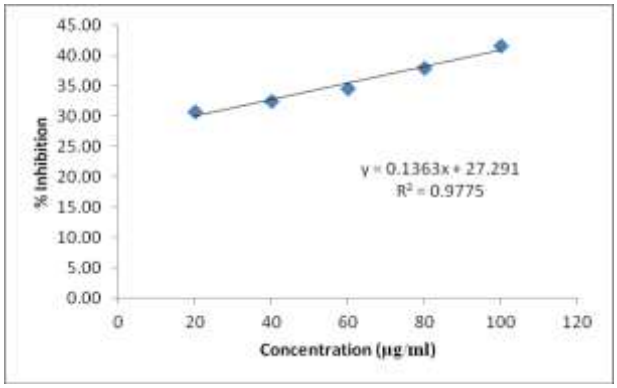
7.4 *In vitro* Antioxidant Activity

Different extracts of *Carissa spinarum L.* flower were subjected to *in vitro* antioxidant studies so as to investigate their free radical scavenging potential using some well-established protocols including hydrogen-donating activity, hydrogen peroxide scavenging assay, superoxide scavenging assay and reducing power assay.

7.4.1 Hydrogen Donating Activity of *Carissa spinarum L.* Root bark

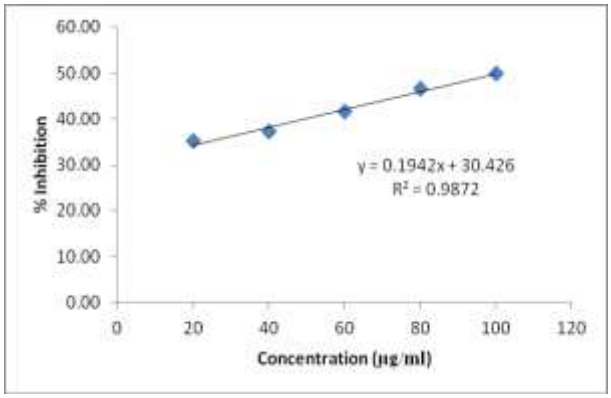
DPPH free radicals were effectively scavenged by PESC, CESC, EASC and MESC of *Carissa spinarum L.* with IC_{50} value as 166.98, 100.92, 80.21 and 58.67µg/ml, respectively as illustrated in table 7.6 to 7.9 and fig 7.1 to 7.4

Carissa spinarum L. extracts demonstrated DPPH scavenging activity in ascending order as MESC > EASC > CESC > PESC in a dose dependent manner. The ascorbic acid was taken as a reference and its IC_{50} was found a 18.36 µg/ml as shown in table 7.10 and fig 7.5. The maximum scavenging property was shown by EASC and MESC as compared to the other extracts.

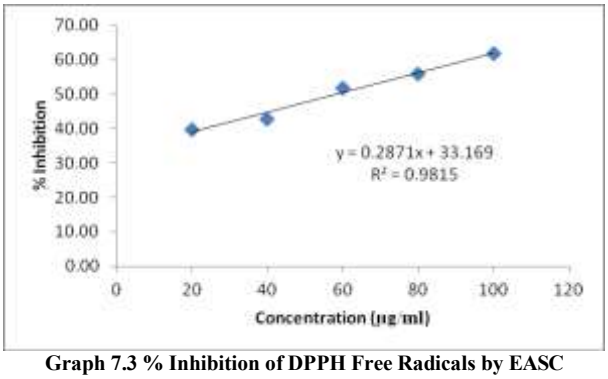


Graph. 7.1: % Inhibition of DPPH Free Radicals by PESC

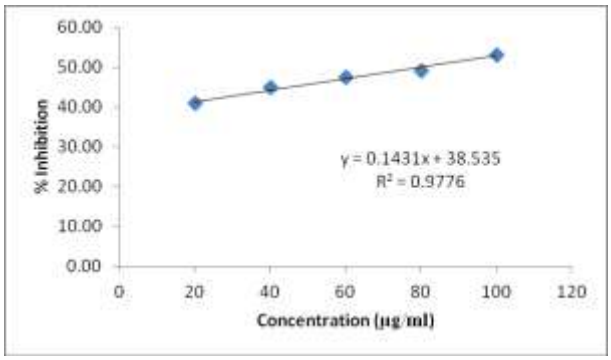
Chinese Journal of Health Management
Volume 20 Issue 6, Year of Publication 2026, Page 1710-1726 | DoI-10.564220/1674-0815.2026.168



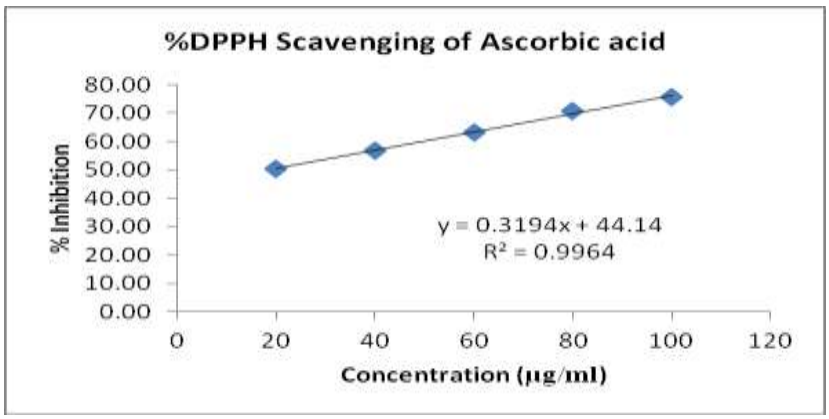
Graph 7.2 % Inhibition of DPPH Free Radicals by CESC



Graph 7.3 % Inhibition of DPPH Free Radicals by EASC



Graph 7.4: % Inhibition of DPPH Free Radicals by MESC

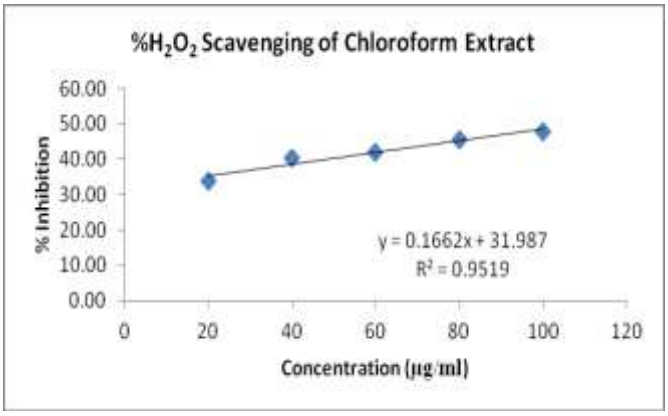


Graph 7.5: % Inhibition of DPPH Free Radicals by Ascorbic Acid

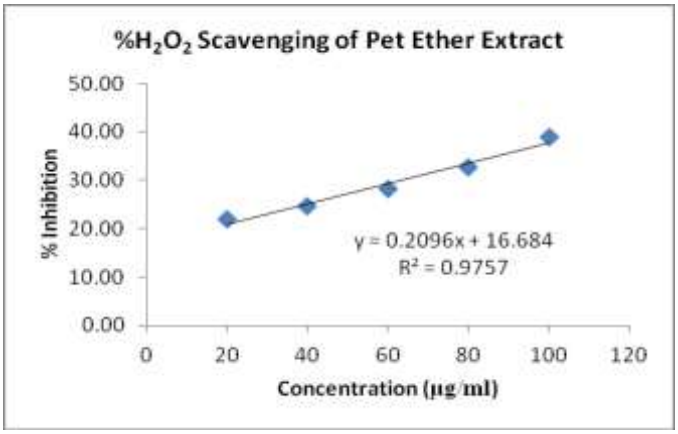
7.4.2 Hydrogen Peroxide Scavenging Activity of *Carissa spinarum L.* Root bark

The hydrogen peroxide scavenging capacity of PESC, CESC, EASC and MESC of *Carissa spinarum L.* flowers was found significant in a dose dependent manner with IC_{50} value of 159.42, 108.55, 59.71 and 45.42µg/ml, respectively as shown in table 7.11 to 7.14 and fig 7.6 to 7.9. Hydrogen peroxide scavenging activity of *Carissa spinarum L.* extracts was found to be in the ascending order as MESC > EASC > CESC > PESC. The ascorbic acid was taken as a reference and its IC_{50} value was 21.65 as depicted in table 7.15 and fig 7.10.

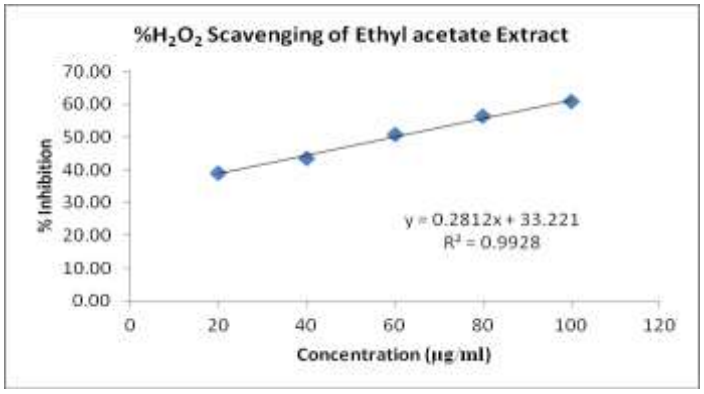
The hydrogen peroxide radicals are not harmful always as that of DPPH radical but occasionally they produce toxic effect to cell. Hence it is essential to neutralize them. The maximum H_2O_2 scavenging activity was shown by EASC and MESC of *Carissa spinarum L.* flowers as compared to the other extracts (137, 138).



Graph 7.6: % Inhibtion of H_2O_2 Activity by PESC

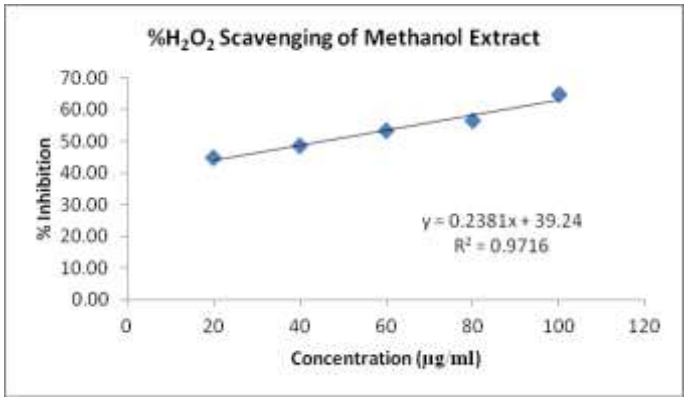


Graph 7.7: % Inhibition of H_2O_2 Activity by CESC

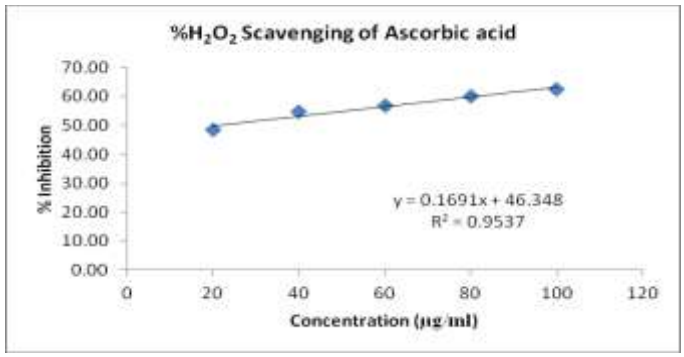


Graph 7.8: % Inhibition of H_2O_2 Activity by EASC

Chinese Journal of Health Management
Volume 20 Issue 6, Year of Publication 2026, Page 1710-1726 | DoI-10.564220/1674-0815.2026.168



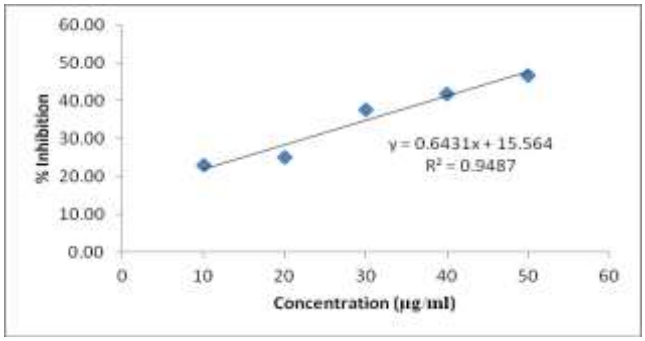
Graph 7.9: % Inhibition of H_2O_2 Activity by MESC



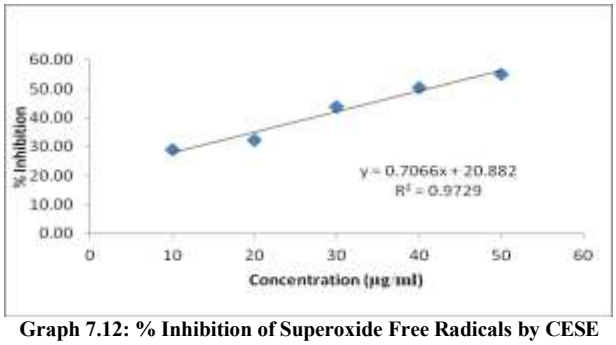
Graph 7.10: % Inhibition of H_2O_2 Activity by Ascorbic Acid

7.4.3 Superoxide Scavenging Activity of *Carissa spinarum L.* Root bark

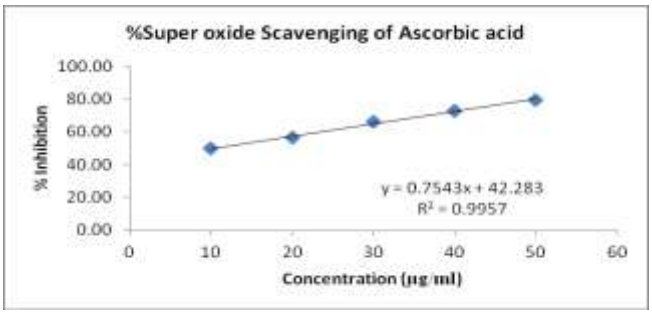
The IC_{50} value of superoxide scavenging free radicals by PESC, CESC, EASC and MESC was found to be 53.56, 41.24, 27.24 and 20.04 µg/ml, respectively, as depicted in table 7.16 to 7.19 and fig 7.11 to 6.14. Superoxide radical scavenging activity of *Carissa spinarum L.* Root bark were in the ascending order as MESC > EASC > CESC > PESC. The ascorbic acid was taken as a standard and its IC_{50} value was found as10.23 µg/ml as shown in table 7.20 and fig 7.15. The EASC and MESC demonstrated maximum superoxide scavenging property as compared to other extracts. Generation of superoxide anion radicals leads to redox imbalance and associated with harmful physiological consequences. The findings revealed that superoxide radicals were significantly decreased by the extracts in a dose dependent manner thus clearly indicating their protective roles to the cellular components.



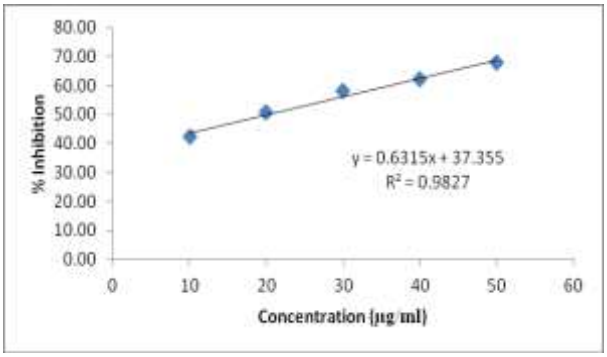
Graph 7.11: % Inhibition of Superoxide Free Radicals by PESC



Graph 7.12: % Inhibition of Superoxide Free Radicals by CESE



Graph 7.14: % Inhibition of Superoxide Free Radicals by MESE



Graph 7.15: % Inhibition of Superoxide Free Radicals by Ascorbic Acid

7.4.4 Reducing Power Assay of *Carissa spinarum L.* Root bark

The reducing power assay of *Carissa spinarum L.* Root bark were screened and impressively exhibited the reducing activities of different extracts in the ascending order: MESC > EASC > CESC > PESC as shown in fig 7.16 and table 7.21**)**.

The MESC and EASC demonstrated potent reducing property, whereas PESC showed least reducing property as compared to the other extract. The results indicated that MESC and EASC act as electron donors and could neutralize free radicals to result formation of stable products.

Previously various scientists have reported that the antioxidant activity of extracts could be attributed to the phenolic and flavonol substance present in the plant extracts. Therefore it can be possible that the reducing power demonstrated by MESC and EASC is due to the presence of high quantity of totals polyphenols and flavonols.

Chinese Journal of Health Management
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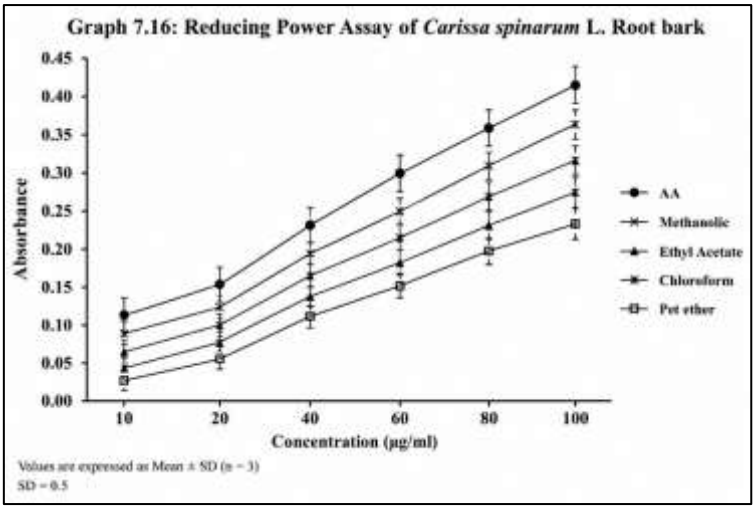


Graph 7.16: Reducing Power Assay of *Carissa spinarum L.* Root bark

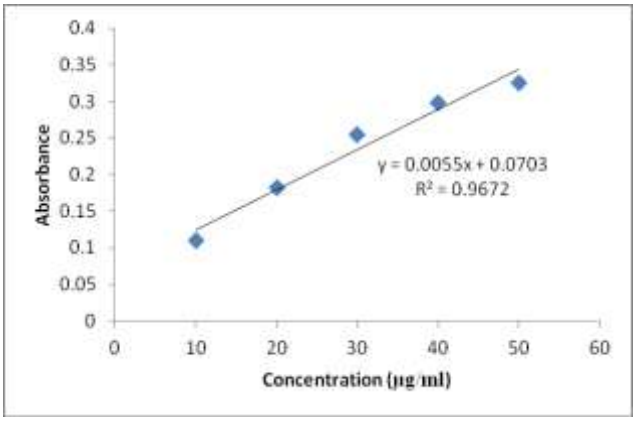
From the results of DPPH, hydrogen peroxide, superoxide radical scavenging and reducing power assay, it was confirmed that MESC and EASC demonstrated higher antioxidant activity. The results indicate the presence of different antioxidant components in crude extracts of *Carissa spinarum L.* flower**.** From these findings, it can be assumed that antioxidant activity of extracts relies upon the nature of active constituents present in them because each *in vitro* antioxidant assays has different mechanism to reduce the free radicals.

7.5 Quantitative Analysis of *Carissa spinarum L.* Root bark

As phenols and flavonoids were present in EASC and MESC**,** so total phenolic content (TPC) and total flavonoid content (TFC) were determined. The total alkaloids and saponins content were also determined additionally.

7.5.2 Estimation of Total Phenolic Content (TPC)

TPC of extracts was determined using Folin-Ciocalteu technique and gallic acid was taken as a standard. Line of regression got from standard curve of gallic acid was utilized so as to estimate absolute phenolic content in extracts. The findings are illustrated in table 6.22 and fig 7.17. The total phenolic content in EASC, and MESC were found as 192.73±0.503 and 217.00±0.916 mg/gm extract equivalent to gallic acid respectively as shown in table 7.23.



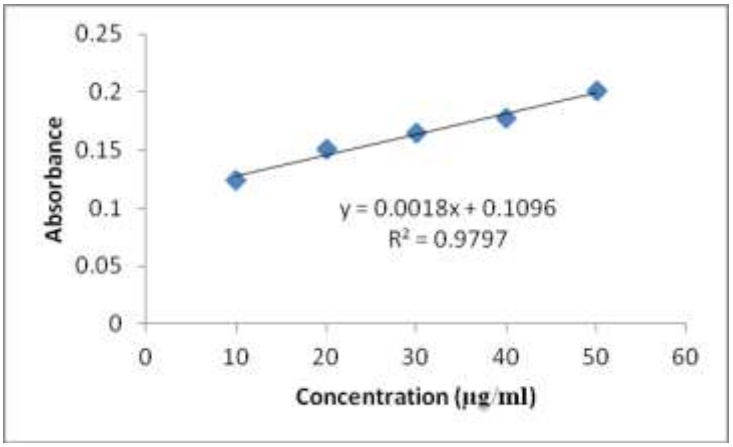
Graph 7.17: Standard Curve of Gallic Acid in Distilled Water

7.5.3 Estimation of Total Flavonoid Content (TFC)

The TFC was determined by using line of regression obtained from standard curve of rutin as shown in table 7.24 and fig 7.18. The TFC in EASC and MESC were found as 45.66±4.041 and 79.33±3.511 mg/gm extract equivalent to rutin respectively as depicted in table 7.25.

Polyphenols and flavonoids are utilized for the prevention and cure of different illnesses, which are basically

associated with free radicals. It has been accounted for example, the flavonoids, which contain hydroxyl ions, are responsible for the radical scavenging effects of plants. The mechanism of action of the flavonoids is through scavenging or chelating processes. It is reported that plant phenolics are profoundly successful in free radicals scavenging activity and they are powerful antioxidants (7–10).



Graph 7.18: Standard Curve of Rutin in Distilled Water

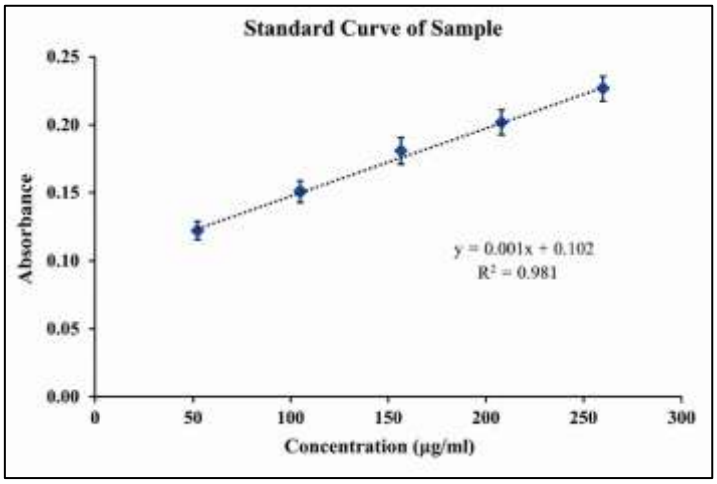
Table 7.24: Determination of TFC form *Carissa spinarum L.* Root bark

Extract	Total flavonol content
EASC	45.66±4.041
MESC	79.33±3.511

Values are expressed as mean ± SEM

7.5.4 Estimation of Total Alkaloid Content

The total alkaloids in *Carissa spinarum L.* Root bark were determined by using standard curve of atropine (the standard curve equation: y = 0.001x + 0.102, R2 = 0.981) as shown in table 7.26 and fig 7.19. The total alkaloid content in EASC and MESC were found to be 19.33±2.081 and 26.66±2.516 mg/gm extract equivalent to atropine as shown in Table 7.27



Graph 7.19: Standard Curve of Atropine in Distilled Water

Table 7.26: Determination of Total Alkaloid Content from *Carissa spinarum L.* Root bark

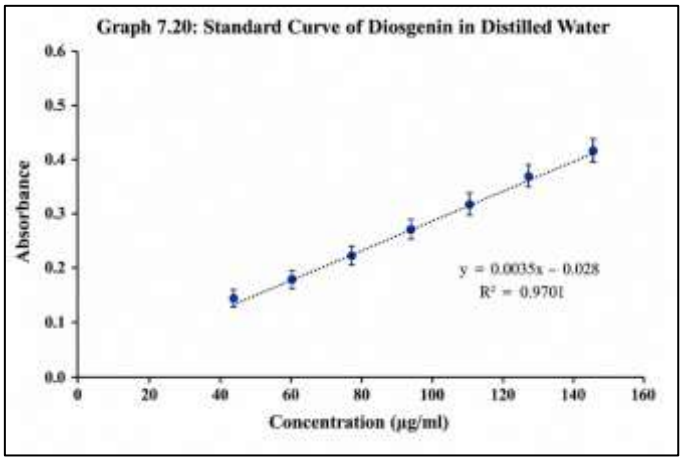
Extract	Total alkaloid content (mg/g equivalent of Atropine)
EASC	19.33±2.081
MESC	26.66±2.516

Values are expressed as mean ± SEM

7.5.5 Estimation of Total Saponin Content

The total saponin content was determined by using vanillin reagent. Diosgenin was taken as a reference compound and total saponin contents were expressed as mg/g diosgenin equivalent by utilizing the standard curve equation: $y = 0.0035x + 0.028$, $R^2 = 0.9701$ as shown in table 6.28 and fig 6.20.

Total saponin content in the EAE and ME of *Carissa spinarum L.* flower was found as 31.44±0.838 and 51.88±1.347 mg/g equivalent of diosgenin as depicted in table 6.29.



Graph 7.20: Standard Curve of Diosgenin in Distilled Water

Table 7.28: Determination of Total Saponin Content from *Carissa spinarum L.*
Root bark

Extract	Total saponin content (mg/g equivalent of diosgenin)
EASC	31.44±0.838
MESC	51.88±1.347

Values are expressed as mean ± SEM

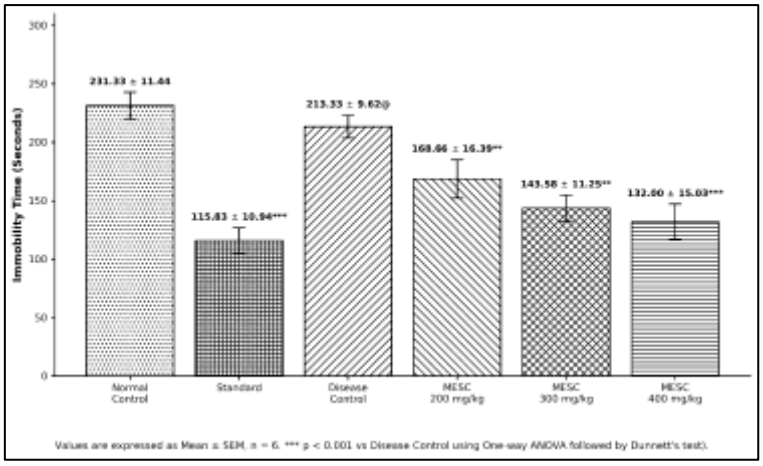
From the results of antioxidant studies and quantitative phytochemical screening, it is confirmed that MESC and EASC showed remarkable antioxidant activity as compared to PESC which could not reduce the oxidative stress effectively. MESC and EASC exhibited better activities in antioxidant assays, they both were selected for the evaluation of *in-vivo* antidepressant activity in Wistar rat models.

7.6 *In vivo* Assay

7.6.1 Assessment of Antidepressant Activity

I. TST

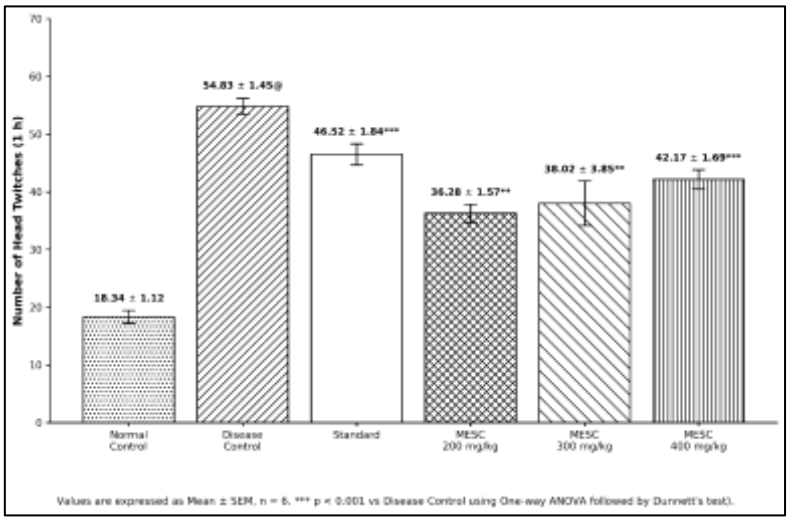
The behavioral score of immobility in control, standard and extract-treated groups has been depicted in [Fig. 1]. In the TST model, the Wistar rat treated with MESC showed significant ($p<0.001$) reduction in immobility period in dose dependent manner when compared to control group (231.33± 11.44 in TST). MESC demonstrated antidepressant potential (168.66 ±16.39 at 200 mg/kg and 132.00 ± 15.30 at 400 mg/kg) in TST assays respectively and thus it was selected for further study. While, the animals treated with EASC also showed reduction in immobility period at the both the doses (200 mg/kg and 400 mg/kg body weight) when compared to control group. Behavioral score of immobility in control, standard and extract-treated groups has been depicted in Table 6.30.



Graph 7.21: Efficacy of Extracts on Immobility Period Displayed by the Wistar rat TST Assay

II. Lithium Induced Head Twitches in Wistar rat

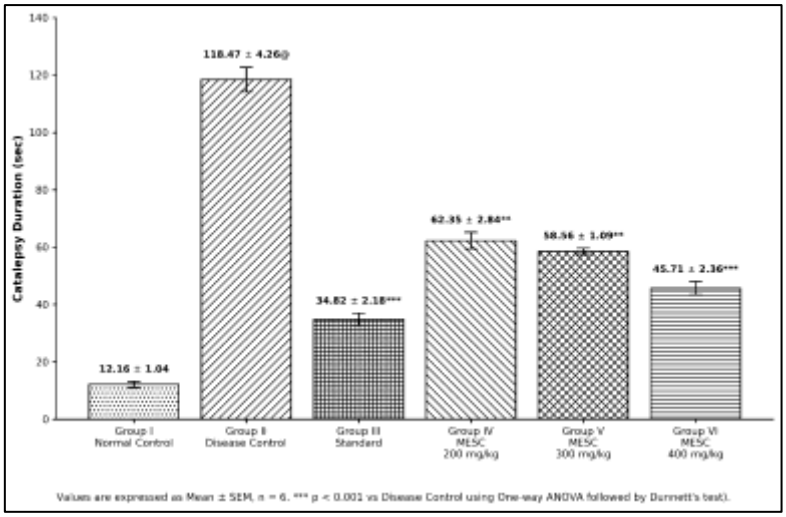
The findings of head-twitches responses after treatment with extract and standard drug are demonstrated in Table 7.30 The Wistar rat treated with MESC at a dose of 200 mg/kg and 400 mg/kg significantly reduced the head twitches as compared to vehicle group Wistar rat. A marked decrease in head twitches was shown by imipramine at a dose of 10mg/kg when compared with vehicle group Wistar rat.



Graph 7.22: Efficacy of Extract on Lithium Induced Head Twitches

III. Haloperidol Induced Catalepsy in Wistar rat

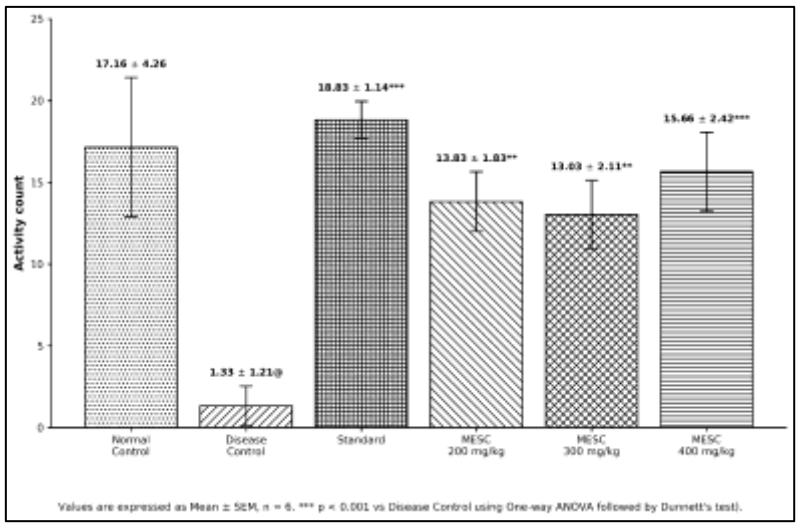
The Wistar rat treated with haloperidol produces peak of catalepsy at 120 minutes. The Wistar rat treated with MESC caused effective reduction in catalepsy at a dose of 200 mg/kg and 400 mg/kg when compared with vehicle group. Likewise, the Wistar rat treated with imipramine at dose of 10 mg/kg have also demonstrated significant decrease in catalepsy as shown in table 7.32. These finding clearly indicates antidepressant potential of test extract in Wistar rat.



Graph 7.23: Effect of Extract on Haloperidol-induced Catalepsy

IV. Open Field Test

The prominent elevation in locomotor activity was observed after the oral administration of MESC at a dose of 200mg/kg (13.83± 1.83) and 400mg/kg (17.66±2.42) in Wistar rat as compared to control group (3.16± 1.21). The imipramine treated group produced a significant ($p<0.001$) increase in locomotor activity as depicted.



Graph 7.24: Effect of Extract on Locomotor Activity

DISCUSSION

The present study demonstrated that the methanolic extract of *Carissa spinarum* root bark (MESC) possesses significant antidepressant-like activity in Wistar rats. Preliminary phytochemical screening revealed the presence of flavonoids, alkaloids, tannins, saponins, terpenoids, steroids, and phenolic compounds, which are known to exhibit antioxidant, neuroprotective, and monoaminergic modulatory effects. Acute toxicity studies indicated that the extract was relatively safe at lower doses, and therefore 200 mg/kg and 400 mg/kg doses were selected for pharmacological evaluation.

Behavioral studies using the Tail Suspension Test (TST) showed that MESC significantly reduced immobility time in a dose-dependent manner, indicating marked antidepressant-like activity. The higher dose (400 mg/kg) produced effects comparable to the standard drug imipramine. The Open Field Test further confirmed that the extract improved locomotor and exploratory behavior without causing nonspecific motor stimulation.

The Lithium-Induced Head Twitch model demonstrated a significant increase in head twitch responses following treatment with MESC, suggesting involvement of serotonergic pathways in its antidepressant action. In addition, MESC significantly reduced haloperidol-induced catalepsy, indicating preservation of monoaminergic neurotransmission and possible modulation of dopaminergic signaling.

Oxidative stress parameters revealed that disease-control animals showed decreased levels of endogenous antioxidants such as SOD, CAT, and GSH with increased lipid peroxidation. Treatment with MESC restored antioxidant enzyme levels and significantly reduced oxidative stress in brain tissues. These antioxidant effects may contribute to neuronal protection and improved neurotransmitter function.

Overall, the findings suggest that the antidepressant activity of *Carissa spinarum* root bark extract may be mediated through enhancement of serotonergic and noradrenergic neurotransmission along with antioxidant mechanisms. The study scientifically validates the traditional use of *Carissa spinarum* in stress-related and neurological disorders and highlights its potential as a promising natural antidepressant agent.

CONCLUSION:

The present study successfully demonstrated the antidepressant-like potential of the methanolic extract of *Carissa spinarum* root bark (MESC) in Wistar rats. Behavioral assessments using the Tail Suspension Test, Open Field Test, Lithium-Induced Head Twitch model, and Haloperidol-Induced Catalepsy model revealed significant improvement in depressive-like symptoms following treatment with the extract. The reduction in immobility time, enhancement of locomotor activity, increase in serotonergic responses, and reduction in cataleptic behavior indicate that the extract exerts marked antidepressant activity through modulation of monoaminergic neurotransmission.

Preliminary phytochemical screening confirmed the presence of important bioactive constituents such as flavonoids, alkaloids, tannins, saponins, terpenoids, steroids, and phenolic compounds. These phytoconstituents are known for their antioxidant and neuroprotective properties and may contribute synergistically to the observed pharmacological effects. The antioxidant studies further demonstrated that MESC significantly restored endogenous antioxidant enzymes including Superoxide Dismutase (SOD), Catalase (CAT), and Reduced Glutathione (GSH), while reducing lipid peroxidation levels. These findings suggest that attenuation of oxidative stress plays an important role in the antidepressant mechanism of the extract.

Among the tested doses, 400 mg/kg exhibited greater efficacy and produced effects comparable to the standard antidepressant drug imipramine. The extract also showed a satisfactory safety profile at therapeutic doses during acute toxicity evaluation.

Overall, the study scientifically validates the traditional use of *Carissa spinarum* in the management of stress-related and neurological disorders. The findings indicate that *Carissa spinarum* root bark may serve as a promising natural source for the development of safer and effective antidepressant agents with antioxidant and monoaminergic modulatory properties.

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