IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
December 2020 Vol.:20, Issue:1

Phytochemical and Pharmacological Evaluation of *Ficus carica* Linn Leaves Extract for Antidepressant Activity

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ISSN 2349-7203
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Submitted: 12 November 2020
Revised: 02 December 2020
Accepted: 22 December 2020

Keywords: *Ficus carica Linn*, depression, despair swim test, antioxidant activity, antidepressant activity

ABSTRACT

Objective: The present study was undertaken to evaluate the phytochemical and pharmacological activity of *Ficus carica* leaves extract. The efficacy of the fractions was compared with the standard reference drug imipramine. **Methods:** All the studies were conducted according to the ethical guidelines of CPCSEA Antidepressant activity of *Ficus carica Linn* leaves extract obtained from two different Solvents i.e. Chloroform (FCCH) and Ethanol (FCETH) was evaluated in Albino Wistar rats by using despair swim test (DST) model at the doses of 100mg/kg as lower dose and 200mg/kg as higher dose. Imipramine (25mg/kg) was used as standard drug for antidepressant activity. Antioxidant assay like DPPH Scavenging assay was also carried out to support the antidepressant activity. The leaves of FC were defatted with petroleum ether and fractionated with chloroform and ethanol. All the fractions were subjected for preliminary phytochemical screening, using various qualitative and quantitative tests. **Results:** The preliminary phytochemical screening of FC has revealed the presence of carbohydrates, tannins, phenols and flavonoids in ethanolic fraction. Chloroform fraction showed positive results toward Phenols, flavonoids, alkaloids, tannins, glycosides, and steroids. Ethanol and chloroform extract (100 and 200 mg/kg p.o.) of FC administered orally for 1st, 8th and 15th successive days had decreased the immobility periods significantly in a dose-dependent manner in DST, showing significant antidepressant-like activity. The activities of the extracts were found to be comparable to imipramine in despair swim test. **Conclusion:** FCCH and FCETH exhibit significant antidepressant activity at the dose of 100 and 200mg/kg which was comparable to imipramine which could be attribute to its effect on neurotransmitters and antioxidant activity respectively.

INTRODUCTION

Depression is a state of low mood and aversion to activity that can affect a person's thoughts, behavior, feelings and sense of well-being. People with depressed mood can feel sad, anxious, empty, hopeless, helpless, worthless, guilty, irritable, ashamed or restless. They may lose interest in activities that were once pleasurable and experience overeating, loss of appetite, have problems concentrating, remembering details or making decisions and may contemplate, attempt or commit suicide. Along this insomnia, excessive sleeping, fatigue, aches, pains, digestive problems or reduced energy may also be present. Depressed mood is a feature of some psychiatric syndromes such as major depressive disorder, but it may also be a normal reaction to life events such as bereavement, a symptom of some bodily ailments or a side effect of some drugs and medical treatments. Depression is more common in women than men. The report on Global Burden of Disease estimates the point prevalence of unipolar depressive episodes to be 1.9% for men and 3.2% for women and the one-year prevalence has been estimated to be 5.8% for men and 9.5% for women. In view of the morbidity, depression as a disorder has always been a focus of attention of researchers in India[1].

Ficus carica (Moraceae) is a deciduous tree grows in tropical and subtropical regions and is commonly known as fig tree. In medicine the roots are used in of leucoderma and ringworms and its fruits which are sweet have antipyretic, purgative, aphrodisiac properties and have shown to be useful in inflammations and paralysis. *Ficus carica* is claimed to be useful in liver and spleen disorders, to cure piles and in treatment of gout. Locally the leaves are being used in the treatment of jaundice (personal information from users). Earlier chemical examination of this plant have shown the presence of psoralen, bergapten, umbelliferone, β-sitosterol, campesterol, stigmasterol, fucosterol, fatty acids, 6-(2-methoxy-Z-vinyl)-7-methyl-pyranocoumarin and 9,19-cycloarlane triterpenoid as an anticancer (8,9) and antiproliferative agent: 6-O-acyl-β-Dglucosyl-β-sitosterol, calotropenyl acetate and lupeol acetate. Previously it was reported that the leaf extracts of *Ficus racemosa* and *Ficus hispida* possess significant hepatoprotective activity against carbon tetrachloride and paracetamol induced hepatotoxicity in rats, respectively[2].

MATERIALS AND METHODS

Animals

Albino Wistar rats weighing around 160-180 g were selected. The animals had free access to food and water and were housed in an animal room with alternating light–dark cycle of 12 hrs each. The animals were acclimatized for at least 5 days to the laboratory conditions before the commencement of behavioral experiments. Experiments were carried out between 9:00 am and 11:00 am. The Institutional Animal Ethics Committee (IAEC) approved the experimental protocol, and the care of laboratory animals was taken as per the guidelines of CPCSEA: NIN Hyderabad (154/Go/RBi/SL/99/CPCSEA); Wockhardt, Aurangabad (13/Po/RcBi/SL/99/CPCSEA).

Collection of plant material

The Fresh leaves of plant *Ficus carica* was collected from known source from Nanded region. The Morphological study identification is carried out and further processed for authentication. The authentication was done by Dr. S. S. Bodke, HOD, Dept. of Botany, Yeshwant Mahavidyalaya, Nanded. Authentication of plant *Ficus carica* was done using the allotted plant specimen/Herbarium No H-05[3].

Preparation of extract

Dried powdered leaves were defatted with Petroleum ether (60-80°C) and extracted with chloroform and ethanol using Soxhlet apparatus. The dried extract obtained was used for further studies. Both the extracts for oral administration was prepared in hydroalcoholic solution using distilled water and methanol[3].

Preliminary phytochemical screening of FC

Various qualitative tests were performed for the detection of phytochemical constituents present in all three fractions, for the presence of carbohydrates, tannins, phenols, flavonoids, steroids, glycosides, alkaloids, and saponins etc[4].

TLC Fingerprinting of FC

TLC Fingerprinting were also carried out for all the extracts with different solvent systems using different locating agents[5][6][]7].

Total Phenolic content

Total phenolic content was determined by the Folin-ciocaltue reagent assay. An aliquot (1ml) of extract or standard solution of Gallic acid (5, 10, 15, 20 and 25µg/ml) was added to 10ml volumetric flask containing 9 ml of distilled water. A reagent blank using distilled water was prepared. 0.5 ml of Folin-ciocaltue phenol reagent was added to the mixture and shaken. After 5 min 1.5 ml of 7% Na_2CO_3 solution was added to the mixture. The volume is then made up to the mark. After incubation for 90 minutes at room temperature, the absorbance against the reagent blank was determined at 550 nm with a UV-Visible spectrophotometer. Total phenolic content was expressed as mg Gallic Acid Equivalents per gram (mg GAE/ gm)[8][9][10].

Total Flavonoid content

Total flavonoid content was measured by the Aluminium chloride colorimetric assay. An aliquot (1ml) of extract or standard solution of Quercetin (5, 10, 15, 20 and 25µg/ml) was added to 10 ml volumetric flask containing 4 ml of distilled water. To the flask was added 0.30 ml 5% $NaNO_2$, after 5 minutes 0.3 ml 10% $AlCl_3$ was added, after 5 min 2 ml 1 M NaOH was added and the volume was made up to 10 ml with distilled water. The solution was mixed and absorbance was measured against the blank at 510 nm. The total flavonoid content was expressed as mg Quercetin equivalent per gram (mg QE/gm)[8][9][11].

***In vitro* antioxidant activity**

DPPH radical scavenging assay

The reaction mixture (3.0 ml) consists of 0.5 ml of 0.135mM DPPH solution in methanol was mixed with 2.5 ml of extract solution of *Ficus carica* and 1.0 ml of methanol. The reaction mixture was vortexes and left in the dark at room temperature for 30 min. The various concentration of extract (50 and 100µg/ml) were prepared. A reaction mixture without test sample was served as control. The absorbance was measured at 514 nm and (%) inhibition was calculated against control. The assay was performed in triplicates[12].

Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the formula[13][14].

$$\% \text{ inhibition} = [(Abs_{control} - Abs_{test})]/Abs_{control.}$$

Preparation of standard

Imipramine was procured from Nanded medical store as sample. Stock solution was prepared by dissolving 25 mg tablet in 10 ml of distilled water, and then diluted to required dilutions.

Acute Oral Toxicity study

In accordance with OECD guideline no. 423, acute oral toxicity study of FC was performed in rats. The extracts were found to be safe till a dose of 2000 mg/kg and hence doses of 100 mg/ kg as lower dose and 200 mg/kg as higher dose were chosen for *in vivo* pharmacological evaluation[15][16][17].

Experimental protocol

Animals were divided into 6 groups, and each group consists of 6 rats.

The treatment groups were as follows:

Group I: Control will receive water for inj /normal saline solution (0.9% NaCl).

Group II: Standard will receive standard drug Imipramine (25mg/kg).

Group III: Test will receive Lower dose (100mg/kg) of FCCH.

Group IV: Test will receive Higher dose (200mg/kg) of FCCH.

Group V: Test will receive Lower dose (100mg/kg) of FCETH.

Group VI: Test will receive Higher dose (200mg/kg) of FCETH.

The rats were randomly assigned into six groups (n=6) and the rats were pre-treated for 10 days before the experiment[18].

Antidepressant activity:

The Despair swim test described by Porsolt *et al*. was slightly modified for the conduct of the experiment. Each mouse was positioned in a glass cylinder (height 30 cm, diameter 22.5 cm), containing 15 cm of water maintained at 25°C. Animals were pre-screened on the previous day by placing the animals individually in the water filled glass cylinder. After 5-6 min, immobility reaches a plateau where the mice remained immobile for approximately 80% of the time. After 15 min in the water, the mice were removed and allowed to dry before being

returned to their home cages. On the day of experiment, 60 min prior to the test, the drugs were administered. The mice were again placed individually in the water filled glass cylinder and the duration of immobility was recorded during the last four min of a six min test. A mouse was considered immobile when floating motionless or making only those movements necessary to keep its head above water surface. The water was changed after each test[19][20][21].

Statistical analysis

All the results were expressed as S.E.M. mean ± standard error. Data were analyzed using one-way ANOVA followed by Tukey's test. In all the tests, the criterion for statistical significance was *p<0.05 to **p<0.001.

RESULTS AND OBSERVATIONS

Table No. 1: Preliminary phytochemical screening

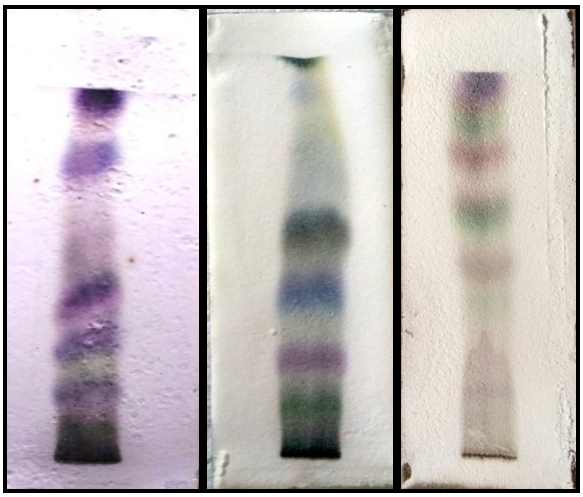
Sr. No.	Test	Pet. Ether Extract	Chloroform Extract	Ethanol Extract
1	Carbohydrates	+	-	+
2	Proteins	-	+	-
3	Alkaloids	+	+	+
4	Flavonoids	-	+	+
5	Phenols	-	-	+
6	Steroids	+	+	-
7	Amino acids	-	-	-
8	Gums	+	+	+
9	Mucilage	-	+	+

(+) Present, (-) Absent

Above observation, table shows the presence of phytoconstituents in the extracts of *Ficus carica Linn.* It reveals that all three (Pet. Ether, Chloroform and Ethanol) extracts contains Carbohydrates, Proteins, Glycosides, Tannins, Alkaloids, Amino acids, Steroids, Phenols and Flavonoids were present or absent.

Table No. 2: TLC Fingerprinting

Sr. No.	Extract	Mobile phase	Spraying reagent
1	Petroleum ether	Benzene: Chloroform(4.9:0.1)	10 % dil. H_2SO_4
2	Chloroform	Benzene: E.A.: Acetone (4.6:0.2:0.2)	Vanillin sulphuric acid
3	Ethanol	Benzene: Chloroform: E.A(4.5:0.3:0.2)	10 % dil. H_2SO_4



Pet. Ether Extract Chloroform extract Ethanol extract

Figure No. 1: TLC of different extracts of *Ficus carica*

Total Phenolic and Flavonoid Content

Table No. 3: Observation of Total Phenolic and Flavonoid content of standard

Sr. No.	Conc. (µg/ml)	Absorbance for TPC Gallic acid	Absorbance for TFC Quercetin
1	5	0.307±0.002	0.370±0.001
2	10	0.414±0.001	0.552±0.004
3	15	0.690±0.004	0.729±0.002
4	20	0.852±0.001	0.871±0.003
5	25	1.016±0.003	1.039±0.002

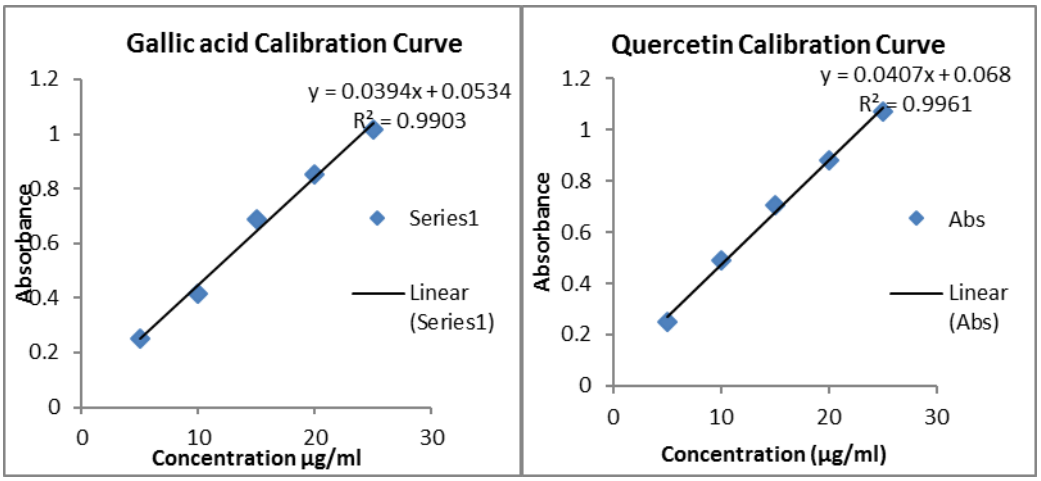


Chart 1: Calibration curve of standard G.A. **Chart 2: Calibration curve for Quercetin**

Table No. 4: Result of Total Phenolic and Flavonoid Content of *Ficus carica*

Sr. No.	Extract	Conc. (µg/ml)	TPC Absorbance	TPC of test FC (mg GAE/gm)	TFC Absorbance	TFC of test FC (mg QE/gm)
1	Pet. Ether	100	2.482±0.0022	58.651	1.314±0.0012	29.585
2	Chloroform	100	1.673±0.0024	39.538	0.7380.0024	16.617
3	Ethanol	100	2.150±0.0012	50.804	0.549±0.0023	12.361

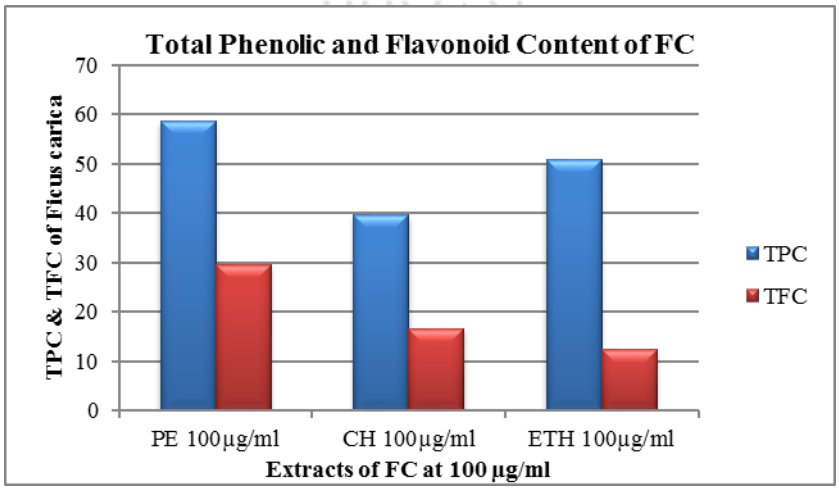


Chart 3: Total Phenolic and Flavonoid content of *Ficus carica*

***In-vitro* Antioxidant activity**

DPPH Radical Scavenging Assay

Table No. 5: DPPH Radical Scavenging assay for Different Standards & their % Inhibition

Sr. No.	Conc. (µg/ml)	λmax	% Inhibition of AA	% Inhibition of GA	% Inhibition of EA	% Inhibition of BHT
1	5	514	69.13±0.44	93.64±0.57	65.51±0.35	88.29±0.20
2	10	514	71.98±0.15	93.98±0.07	68.19±0.18	88.93±0.55
3	15	514	75.32±0.54	94.12±0.08	69.21±0.19	93.41±0.23
4	20	514	81.90±0.12	95.59±0.30	71.87±0.32	93.77±0.47
5	25	514	86.75±0.08	95.59±0.19	76.90±0.01	94.91±0.04

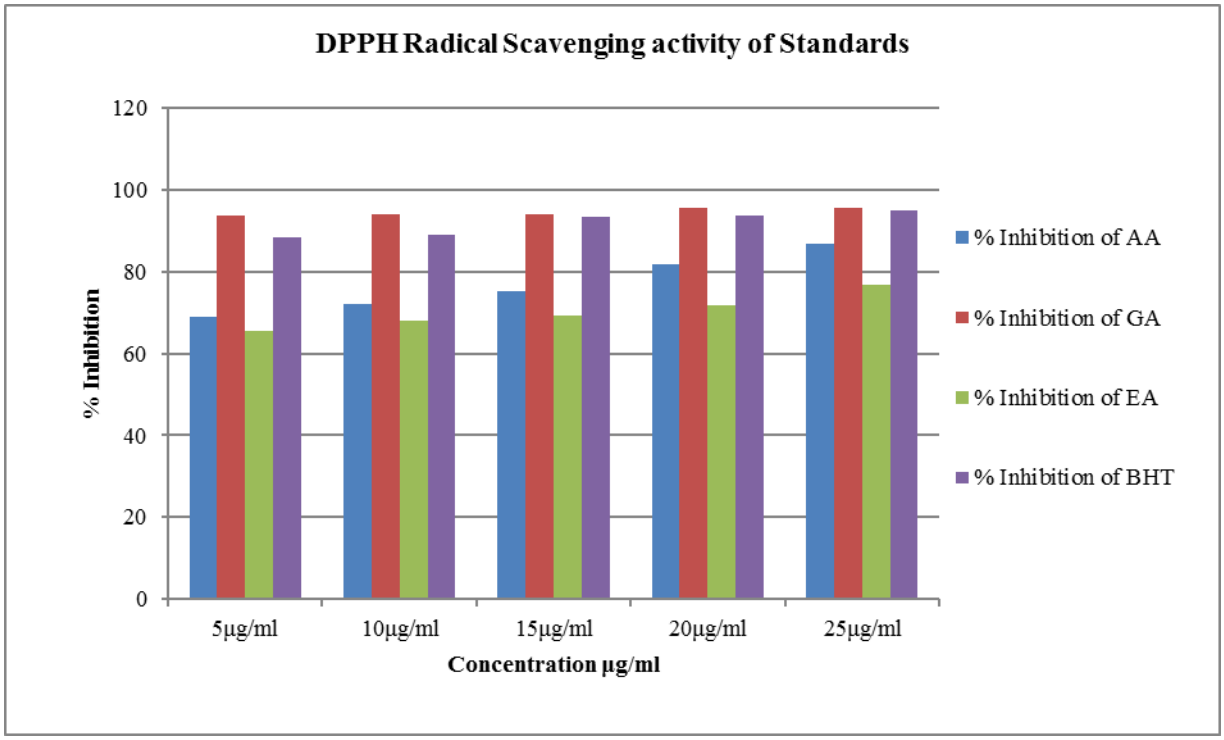


Chart 4: DPPH Radical Scavenging assay for Different Standards & their % Inhibition

Table No. 6: DPPH radical scavenging assay of *Ficus carica* leaves extracts

Sr. No.	Extracts	Absorbance	% Inhibition	Absorbance	% Inhibition
		Conc. 50 µg/ml		Conc. 100 µg/ml	
1	Pet. Ether	0.359 ± 0.002	70.95%	0.325 ± 0.003	73.70%
2	Chloroform	0.912 ± 0.001	49.01%	0.238 ± 0.001	80.74%
3	Ethanol	0.905 ± 0.001	50.37%	0.363 ± 0.002	70.63%

Each value represents the mean ± S.E.M., The Pet. Ether extract shows 70.95% and 73.70% of radical scavenging activity at 50µg/ml and 100µg/ml conc. respectively while Chloroform extract shows 49.01% and 80.74% inhibition at 50µg/ml and 100µg/ml conc. respectively likewise Ethanol extract shows 50.37% and 70.63% of scavenging activity at 50µg/ml and 100µg/ml conc. respectively. That observation reveals that all three extracts shows significant antioxidant activity at 50 and 100µg/ml concentrations.

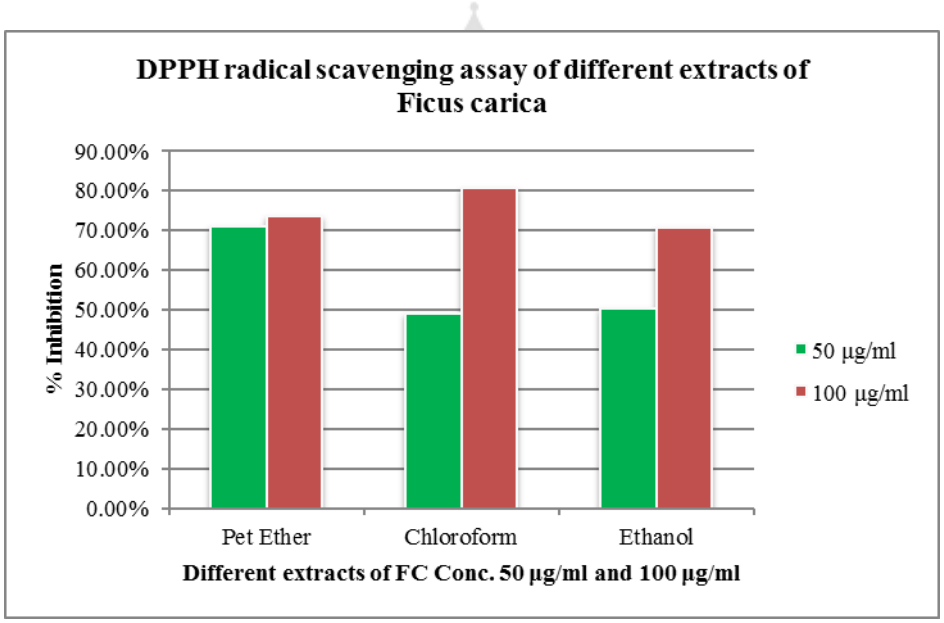


Chart 5: DPPH radical scavenging assay of Test i.e. different extracts of *Ficus carica*

***In-vivo* Antidepressant activity**

Table No. 7: Observation for Antidepressant activity of *Ficus carica* Comparative study:

Groups	Time of Immobility In Second's		
	1st day	8th day	15th day
Control- I Normal Saline	164.66 ± 19.54	167.66 ± 6.31	159.33 ± 16.53
Standard- II Imipramine 25mg/kg	81.66 ± 4.59 **	76.33 ± 4.15 **	72.83 ± 7.74 **
Test FCCH 100mg/kg	104.83 ± 7.08 **#	71 ± 9.07 **#	64.5 ± 7.31 **#
Test FCCH 200mg/kg	95 ± 10.76 **#	48 ± 4.05 **$	50.5 ± 3.89 **$
Test FCETH 100mg/kg	77.5 ± 5.93 **#	62.5 ± 5.07 **$	69.16 ± 6.29 **#
Test FCETH 200mg/kg	61 ± 2.59 **$	53.5 ± 5.10 **$	56.16 ± 8.03 **#

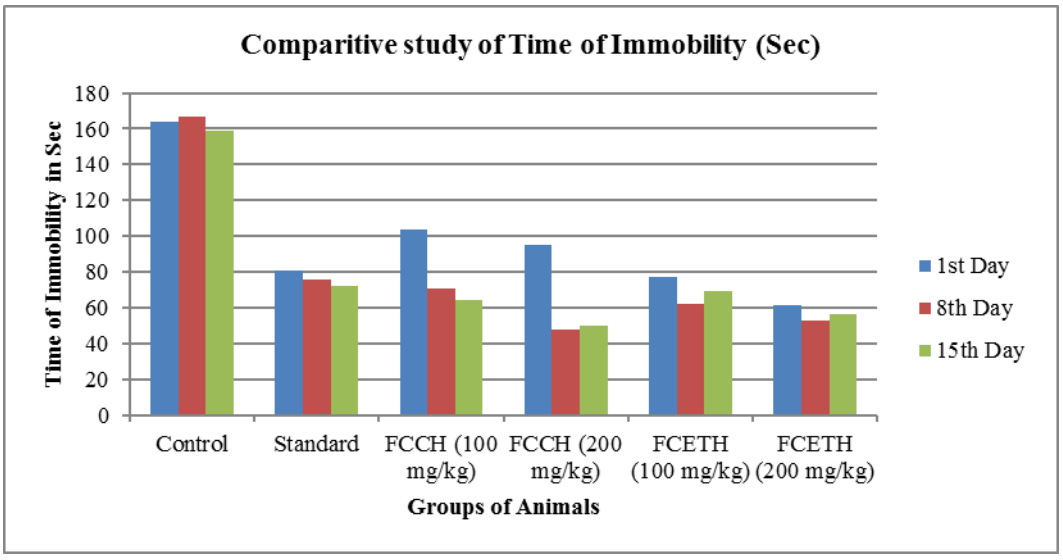


Chart 6: Comparison between all three (1st, 8th and 15th) days data time on Immobility in seconds

The values are represented as mean ± S.E.M. (n=6), for all groups and statistical significance between treated and control group was analyzed using one way ANOVA, followed by Tukey test.

Above observation, table reveals that for all groups and statistical significance between treated and control, *P<0.05- Significant difference when compared to control, **P<0.001- Highly Significant difference when compared to control, #- No significant difference when compared to standard, $ significant difference when compared to standard activity is more than standard.

DISCUSSION

In the present study, the preliminary phytochemical analysis and TLC fingerprinting revealed that the presence of various phytoconstituents such as steroids, tannins, phenols and flavonoids in all fraction. Chloroform and ethanol fractions showed positive results toward phenols, flavonoids, alkaloids, and steroids. From the study of determination of Total phenolic and flavonoid content it was revealed that *Ficus carica* showed presence of both phenols and Flavonoids but major quantity of phenols as compared to flavonoids which was responsible for a significant decrease in immobility time in despair swim test indicating their antidepressant activity.

Based on that data and comparison between all groups on all three days of experiment it is confirmed that both the chloroform and ethanolic extracts showed highly significant result i.e. reduced duration of immobility, activity is more than standard at 8th and 15th day of the experiment as compared to 1st day. And the dose 200 mg/kg of both chloroform and ethanolic extracts was effective and showed highly significant results as compared to 100 mg/kg of both extracts.

SUMMARY AND CONCLUSION

Chloroform fraction of FC showed antidepressant activity, which was comparable to a standard drug, i.e., imipramine (25 mg/kg). As reported earlier, FC contains many bioactive compounds and majority of these compounds are phenols and flavonoids that are responsible for the health benefits. Therefore, this study explores the use of FC in the treatment of depression. Further study can be explored to isolate the active constituents and evaluate the mechanism of antidepressant activity of FC.

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