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ANTIOXIDANT AND ANALGESIC ACTIVITY OF TOTAL FLAVONOIDS OF FORSSKAOLEA TENACISSIMA.

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ABSTRACT

BACKGROUND: Total flavonoids of Forsskaolea tenacissima was screened for possible antioxidant and analgesic activities. It was an attempt to isolate total flavonoids from the aerial parts of plant and subject it for further pharmacological screenings. **METHODS:** Whole plant of *Forsskaolea tenacissima* was collected from Institute of basic medical sciences, Khyber medical university and the voucher specimen was sent in to the pharmacology department. Hot plate method, writhing method and tail immersion methods were used to investigate the possible analgesic activity and DPPH was used for the determination of antioxidant activity of *Forsskaolea tenacissima* while ascorbic acid was used as a reference. **RESULTS:** Mice were divided into six groups and the lethality was zero percent in animals of first three groups while there was 25 %, 50 % and 100 % lethality in animals of group 4 to group 6 respectively. Total flavonoids of *Forsskaolea tenacissima* has analgesic activity which is ascertained by the hot plate, writhing, and tail immersion procedures. The antioxidant activity was less than the EC50, or 37.20 percent, at a concentration of 62.5µg/ml. Which gradually increases and it reached to 74.10 % at 1000 µg/ml which shows that the plant has antioxidant activity. **CONCLUSION:** The study shows that the total flavonoids of *Forsskaolea tenacissima* has antioxidant activity as well as analgesic activity mediated through Hot plate method, writhing method and tail immersion methods. Crude methanolic extract's initial phytochemical tests that the plant includes flavonoids, which may be what give it its antioxidant properties.

KEYWORDS: *Forsskaolea tenacissima*, Flavonoids, Analgesic, Writhing, Antioxidant, DPPH

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INTRODUCTION

From 60,000 years human beings are using plants for the treatment of different ailments. Mostly village people are using medicinal plants because of the unavailability of synthetic drugs and because they have good knowledge of their utilization for their survival. Reference 1+2 even today 80 % of South Asian population rely on traditional use of herbs because they cannot afford primary health care cost [1-3].

The base of the medicines which we are using today is traditional Arab medicine. It was Arabs who has done lots of work in the field of Philosophy and science which was translated into Latin by many European scholars, also the credit of progress of Hospitals and medical schools in twelfth century especially in Baghdad also goes to Arabs [4, 5].

Among the Muslim scholars and writers who contributed in the field of medicine are Ibn-Sina or Avicenna who wrote many books like "Alkanoon Fi Altib" The roles of Medicine and "Canon of medicine" which was considered the best work done by Muslims till the end of sixteenth century, Jaber Bin Hayan who works in purifying different chemicals like nitric acid, sulphuric acid, royal acid and alcohol, Abu Bakr Rhazes who used animals in the laboratory for the first time, Al Zahrawi used surgical equipments for the first time in surgery and the concept of eye sight checking was introduced by Al Haitham[5-9]. *Forsskaolea tenacissima* belongs to Urticaceae family which is commonly called Nettle family [10, 11]. The plants of Urticaceae family grow in soil which is rich in nitrogen. Urticaceae family has 48 genera and 1050 species distributed throughout the world, mostly in tropical regions while in Pakistan It has six genera and nine species [12]. The plants of urticaceae family has allergic pollen grains which when transported through air and when come in contact with human beings causes "Asthma" and "Allergic rhinitis" [13].

ForsskaoleaTenacissima is 65 centimetres 26 Inches fleshy, stiff-haired woody annual herb which is found in Pakistan in Makerwal and Gulla Khel which are situated partly in tehsil Isakhel district Mianwali Punjab province and partly in tehsil karak, district karak Khyber Pakhtunkhwa province [14]. Caidbeja adhaerens Forssk, *Forsskaolea Cossoniana* Webb and Chamaedryfolia Dill are the synonyms of Forsskaolea tenacissima [15, 16] while it has different names in different languages like in Spanish it is called Forsskaolea while in Arabic it is called Tubbaq and Lazzaq, in Hindi it is called Qishda and Safarjal while in Malakand district of Khyber PakhtunKhwa Pakistan it is called stiker botey and in semi- Tribal area of Makerwal & Gullah KhelPakistan It is called saarr booti [17]. Various phytochemicals have found in *Forsskaolea tenacissima* like Glycosides, Flavonoids, Tannins, Saponins, Sterols, Terpenes, Alkaloids, Resins, Chlorides and Sulphates while Flavonoids are polyphenolic compounds which are present in fruits, nuts, seeds, bark, flowers, vegetables, honey, tea, wine etc. and are present in cells of plants as well as in human diet 18 and In US daily dietary intake of mixed flavonoids is 500 – 1000 mg [18,19].

Table 1 Various phytochemicals are present in *Forsskaolea tenacissma 20*

Constituents	Present/Absent
Glycosides /Carbohydrates	+
Flavonoids	+
Tannins	+
Saponins	-
Sterols	+
Terpenes	+
Alkaloids	-
Resins	-
Chlorides	+
Sulphates	+

Forsskaolea Tenacissima is used as antioxidant, antimicrobial, cytotoxic, diuretic, spasmogenic, spasmolytic, antihypertensive, antifungal and in treating kidney diseases. In Baluchistan Pakistan it

is used to cure cough and headache while in Peshawar Pakistan it is used as anti-inflammatory, anti-diabetic, and antipyretic [20-23].

We have already reported that the plant containing tannins, saponins and alkaloids. The current work is an attempt to isolate total flavonoids from the aerial parts of plant and subject it for further pharmacological screenings.

MATERIALS AND METHODS

Plant Materials

Whole plant of *Forsskaolea tenacissima* was collected from Institute of basic medical sciences, Khyber medical university. The plant taxonomist and head of the university of Peshawar's botany department, recognized and verified the species. The voucher specimen was sent in to the pharmacology department of IBMS, KMU, Khyber Pakhtunkhwa, Pakistan.

Extraction

Forsskaolea tenacissima whole plant was dried in shade at room temperature which was then macerated in commercial grade methanol for about 6 weeks with intermittent filtering through filter paper when shaking at ambient temperature. After this procedure was carried out three times, the filtrates were mixed and evaporated using a rotary evaporator under reduced pressure. The concentrated extract was then transferred into petri dishes and allowed to dry at room temperature in the open until a dark greenish extract was produced.

Fractionation and Isolation

Distill water was added to the crude methanolic extract of *Forsskaolea tenacissima* which was then fractionated with n-hexane and then with ethyl acetate till it gave n-hexane fraction and ethyl acetate fraction. The fractions were then stored in refrigerator.

Drugs and standards

Analytical grade chemicals were used in the bioassay techniques. The chemicals were purchased from Moosa G and sons Peshawar and Northwest General Hospital & Research Centre, Hayatabad, Peshawar, Pakistan. Freshly made solutions were utilized in the studies, and analytical-grade substances and medications were bought. Sigma-Aldrich Chemicals, St. Louis, U.S., utilized DPPH, ascorbic acid, diclofenac sodium, and tramal. All the solutions were freshly prepared in distill water on the same day of experiments.[24]

Animals and data recording

Animals used in this study were Swiss Albino mice having 30 gm- 35 gm body weight, Dowly Wister rats having 180 – 350 gm body weight were purchased from National Institute of Health NIH, Islamabad, and Peshawar University of Pakistan's pharmacy department During their time at IBMS, KMU's animal home, they were housed at temperatures between 20 and 25 degrees Celsius. usage of experimental animals and methodology for their usage was authorized by the ethical committee of the KMU. Before the experiment began, the animals were fed only water and starved for the whole night24. This experimental investigation was conducted in accordance with the rules established by the Institute of Basic Medical Sciences' Animal Ethics Committee at Khyber Medical University in Pakistan.

Acute toxicity

Total flavonoids of *Forsskaolea tenacissima* were tested for acute toxicity. The mice, in first phase were given 1, 10, 100 mg/kg and in second phase 1000, 2000, 3000 mg/kg of total flavonoids. Distill water was given to the control group. [25, 26.] For a whole day, every animal was monitored for harmful effects and fatalities. The LD50 was established.[2]

Antioxidant Activityof total flavonoids

2, 2-diphenyl-1-picrylhydrazyl DPPH has free radical scavenging activity [27]. DPPH solution was prepared in methanol by dissolving 24 mg of DPPH in 100 ml of methanol. Test samples of different concentrations like 62.5 µg/ml, 125 µg/ml, 250 µg/ml, 500 µg/ml and 1000 µg/ml were prepared and kept in dark at 23 °C

for 30 minutes. Each test sample is of 2 ml and to these test samples 2 ml DPPH 2% was added. The test samples and DPPH were prepared in methanol. All test samples were compared with ascorbic acid. This experiment was performed three times. Absorbance of the sample and methanol which is used as a blank was noted at a wavelength of 517 nm. Free radical scavenging activity of the test samples at respective concentrations was plotted as % of ascorbic acid [28].

Statistical Analysis

Mean S.D values were calculated using graph pad prism. Level of significance was at 95% C.I P less than or equal to 0.05.

Hot plate method

Six sets of Wister rats were used in this experiment to evaluate various flavonoid concentrations. Rats weighing 180–350 g were used. Six groups of thirty rats were created. Each group consisted of five rats. Prior to the experiment, the animals were pre-treated with total flavonoids 60 minutes in advance. The reference standard drug Diclofenac Na was administered at a dose of 50 mg/kg. The rats were then placed on a hot plate maintained at a constant temperature of 55±0.5°C. The reaction time, defined as the latency to flick the hind paw or jump from the hot plate, was recorded at intervals of 0, 15, 30, 45, 60, 75, 90, and 120 minutes. Group 1 was taken as negative control and was given distill water plus carboxy methyl cellulose CMC orally. Group 2 was the positive control group and rats in this group were given diclofenac Na 50 mg/kg. The group 3 to 5 were given oral doses of 10mg/kg, 30 mg/kg and 100 mg/kg total flavonoids of *Forsskaolea tenacissima* respectively. Group 6 received tramadol. The typical dosage of this narcotic analgesic is 12.5 mg/kg. Thermal pain was induced in rats by placing them on a hot plate maintained at a temperature of 55±0.5°C. The reaction time, defined as the time taken for the rat to flick its hind paw or jump off the hot plate, was recorded at specified intervals 0, 15, 30, 45, 60, 75, 90, and 120 minutes following administration of the test sample. Time was recorded by stop watch.

Writhing Test Induced by Acetic Acid

In the Writhing test, irritants such as formalin, acetic acid, or phenyl Quinone were injected into experimental animals to cause peripheral discomfort. The reduction in writhing is then used to hypothesize analgesic potential. Abdominal writhing was defined by a distinctive set of behaviors, including hind limb extension, abdominal muscle contraction, and dorsal arching. It is a reflexive test [29]. In this study writhing was induced by intraperitoneal administration of acetic acid due to which prostaglandin was released which increase sensitivity to nociceptors [30]. Rats were divided into 6 groups, each group had 5 rats and the total number of writhes were counted for 15minutes, starting after 5 minutes of intraperitoneal administration of Acetic acid 0.6% V/V in normal saline, 10 ml/kg. Group 1 rats were given distill water plus Carboxy methyl cellulose CMC at a 10 ml/kg dosage. Diclofenac Na was administered to Group 2 rats at a dose of 10 mg/kg body weight. Rats in groups three and five received doses of 10 mg/kg, 30 mg/kg, and 100 mg/kg of total flavonoids of Forsskaolea tenacissima, respectively. All animals were given an acetic acid solution to induce writhing after 30 minutes of medication. 30 minutes before the acetic acid injection, oral dosages of 10 mg/kg, 30 mg/kg, and 100 mg/kg of total flavonoids were given. Number of writhing and stretching was observed and percent inhibition was determined from the data.

Tail immersion method

For the determination of central antinociceptive activity of total flavonoids of *Forsskaolea tenacissima,* rats were divided into six groups. To first group rats distill water was given. Rats from group two to group five were given different concentrations of total flavonoids before

the immersion of tail in hot water while to group 6 rats opioid analgesic morphine was administered in order to investigate the possible involvement of opioid receptor. Reaction time was noted at 15, 30, 45, 60, 75, 90 and 120 minutes after administration of sample.

Data analysis

All the data are expressed as mean ± SEM standard error mean, n= 5. Applied one-way ANOVA test for comparing the treatment group with the control groups.

RESULTS

Yield of total Flavonoids of *Forsskaolea tenacissima*

Approximately 55 grams of total flavonoids were obtained from 10 Kg of *Forsskaolea tenacissima.*

Table 2: Acute Toxicity Study Dose mg/kg body weight

Dose mg/kg body weight			
1st Phase	Group 3 100 mg/kg	Group 2 10 mg/Kg	Group 1 1 mg/Kg
	All Alive	All Alive	All Alive
2nd Phase	Group 6 3000 mg/kg	Group 5 2000 mg/kg	Group 4 1000 mg/kg
	All Died	Two Died	One Died

Figure 1 An illustration of the acute toxicity test of Forsskaolea tenacissima's total flavonoids in a mouse model.

Mice were divided into six groups and the lethality was zero percent in animals of first three groups while there was 25 %, 50 % and 100 % lethality in animals of group 4 to group 6 respectively.

Table 3: Results of Antioxidant activity of Ascorbic acid

Antioxidant activity of Ascorbic acid						
Concentration µg / ml	Absorbance I	Absorbance II	Absorbance III	Mean	Standard Deviation	%DPPH Scavenging activity
62.5	0.089	0.091	0.088	0.089	0.002	73.51
125	0.082	0.081	0.082	0.081	0.002	75.89
250	0.077	0.079	0.078	0.078	0.001	76.78
500	0.068	0.067	0.069	0.068	0.001	79.76
1000	0.056	0.055	0.057	0.056	0.001	83.34

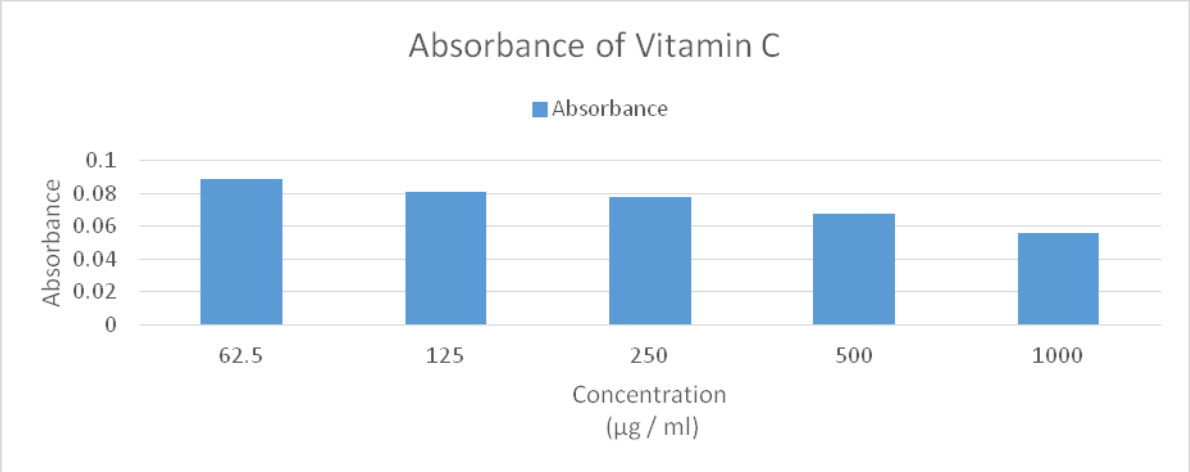

Figure 2: Graphical presentation of Absorbance of Vitamin C

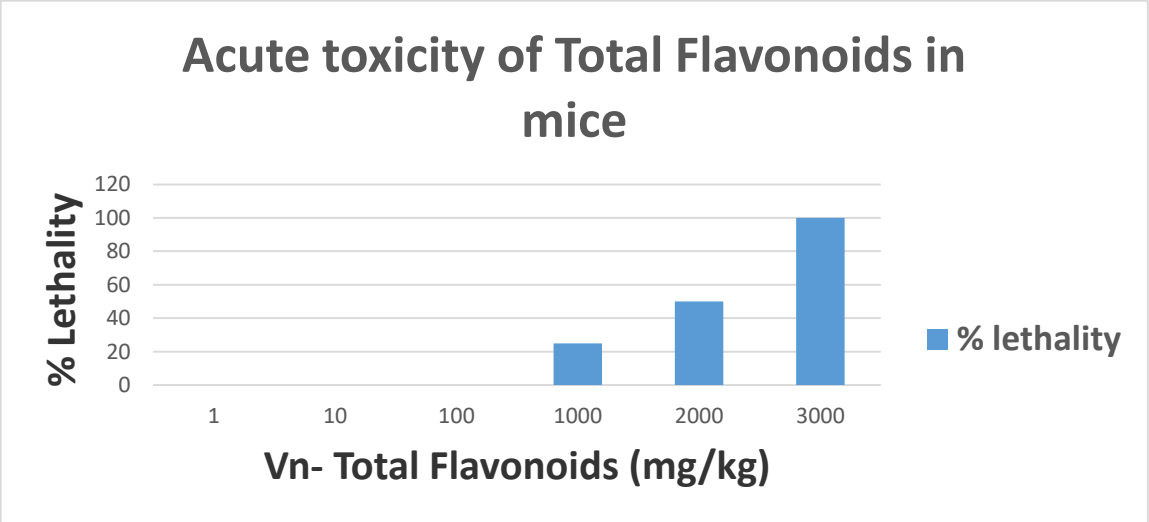

Table 4 Results of antioxidant activity of total flavonoids of *Forsskaolea tenacissima*

Antioxidant activity of Forsskaolea tenacissima						
Concentration µg / ml	Absorbance 1	Absorbance 2	Absorbance 3	Mean	SD	% DPPH Scavenging activity
62.5	0.211	0.211	0.212	0.211	0	37.20
125	0.185	0.188	0.186	0.186	0.001	44.64
250	0.151	0.153	0.151	0.152	0.001	54.76
500	0.121	0.123	0.124	0.123	0.001	63.39
1000	0.087	0.088	0.087	0.087	0	74.10

The antioxidant activity is lower than that of EC5O at a concentration of 62.5 µg/ml 37.20%, but it is 44.64 %, 54.76 %, 63.39 %, and 74.10 % at concentrations of 125 µg/ml, 250 µg/ml, 500 µg/ml, and 1000 µg/ml, respectively. It is clear from the crude methanolic extract's initial phytochemical tests that the plant includes flavonoids, which may be what give it its antioxidant properties.

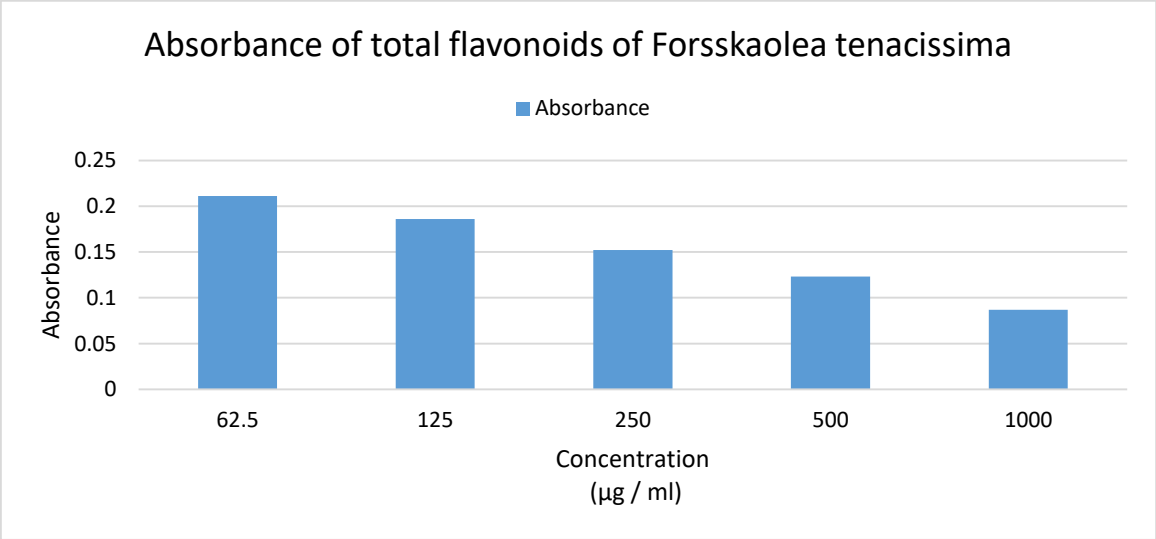

Figure 3 Graphical presentation of absorbance of total flavonoids of Forsskaolea tenacissima

Table 5 Results of Analgesic activity Hot Plate method of total flavonoids of *Forsskaolea tenacissima*

Group	Dosemg/ kg	Time for Rat jumping from Hot plate seconds on different intervals							
		0 min	15 min	30 min	45 min	60 min	75 min	90 min	120 min
Negative Control	0.5% CMC in Distill water	7.2 ± 1.5	7.2 ± 2.6	7.4 ± 1.8	7.6 ± 1.5	8.6 ± 1.5	9 ± 1	9 ± 1.6	9.8 ± 2.1
Diclofenac Sodium	50	11.4 ± 2.7	13.4 ± 2.6	13.4 ± 2	13.6 ± 2.4	14.6 ± 2.6	14.6 ± 2.2	14.8 ± 2.6	15.8 ± 2.2
Flavonoid	10	5.8 ± 1.6	7.2 ± 1.3	7.6 ± 1.3	8 ± 1.5	8 ± 1.2	8.6 ± 2.1	9 ± 2.2	9 ± 1.9
Flavonoid	30	10.6 ± 2	11 ± 1.6	12.2 ± 1.9	12.6 ± 2.9	12.6 ± 2	13.4 ± 0.8	13.8 ± 1.9	11.8 ± 3.4
Flavonoid	100	17.4 ± 3.4	17.6 ± 2.1	17.8 ± 3.1	18.4 ± 0.5	18.4 ± 1.5	19.2 ± 2.8	19.8 ± 2.4	18.4 ± 2.5
Tramal	12.5	19.8 ± 2.8	19.8 ± 3.8	20.6 ± 3.4	20.6 ± 4	20 ± 2.6	20.6 ± 3.6	22.8 ± 3.4	22.8 ± 2.4

When compared the analgesic activity of total flavonoids of *Forsskaoleatenacisima* with Diclofenac sodium and Tramal there is a significant increase in time of stay of rats on the hot plate which showed that the total flavonoids of *Forsskaolea tenacissima* has analgesic activity.

Acetic acid induced Writhing test result

Table 6 Results of Analgesic activity Writhing method of total flavonoids of *Forsskaolea tenacissima*

Group	Dose mg/kg	Flavonoid	Flavonoid	Flavonoid	Flavonoid	Flavonoid	Mean
		No. of	No. of	No. of	No. of	No. of	

		Writhing	Writhing	Writhing	Writhing	Writhing	
1	0.5% CMC in Distill water	59	57	61	58	54	50 ± 2.6
2 Diclofenac Na	10	29	31	34	28	25	25 ± 3.4
3	10	57	55	54	51	49	53.2 ± 3.2
4	30	41	39	41	37	37	39 ± 2.0
5	100	30	26	32	25	26	27.8 ± 3.0
6 Morphine sulphate	5	4	6	8	4	8	6 ± 2.0

The values are n=6; p≤0.01 ANOVA against control; mean ±SEM

Total flavonoids of *Forsskaolea tenacissima* showed a significant effect while performing the acetic acid induced writhing essay. There was sequential decrease in number of writhing as the concentration of total flavonoids was increased and also when compared with the standard drug, Diclofenac sodium and Morphine sulphate which is an opioid analgesic which means that the total flavonoids of *Forsskaolea tenacissima* has analgesic activity.

Table 7: Results of Analgesic activity Tail immersion method of total flavonoids of *Forsskaoleatenacissima*

Group	Dose mg/kg	Time for tail withdrawing seconds on different intervals							
		0 min	15 min	30 min	45 min	60 min	75 min	90 min	120 min
Negative control	Distill water	5.4 ± 0.1	3.4 ± 0.1	4.6 ± 0.2	4.9 ± 0.1	3.4 ± 0.1	3.4 ± 0.2	3.8 ± 0.1	5.3 ± 0.1
Diclofenac sodium	50	5.9 ± 0.2	6.1 ± 0.2	6 ± 0.1	6.5 ± 0.1	5.4 ± 0.2	5.9 ± 0.2	5.7 ± 0.1	6.3 ± 0.1
Flavonoid	10	3.4 ± 0.1	3.2 ± 0.2	3.4 ± 0.2	3.7 ±0.1	3.6 ± 0.1	4 ± 0.2	3.5 ± 0.2	3 ± 0.2
Flavonoid	30	3.9 ± 0.2	4.1 ± 0.1	4.2 ± 0.1	4 ± 0.2	3.9 ± 0.2	4.3 ± 0.2	4.2 ± 0.1	4 ± 0.2
Flavonoid	100	5 ± 0.1	4.9 ± 0.2	5.5 ± 0.2	5.1 ± 0.1	5.2 ± 0.1	7.1 ± 0.1	6.5 ± 0.1	4.7 ± 0.1
Morphine	5	7.3 ± 0.2	7.5 ± 0.1	6.8 ± 0.2	7.1 ± 0.2	7.4 ± 0.2	6.6 ± 0.2	6.4 ± 0.1	6.8 ± 0.2

Values are mean ± SEM, n= 6; ANOVA followed by Dennett's test vs control

CONCLUSION

Concerning the lethality of aerial parts of *Forsskaolea tenacissima*, the lethality was 25% at the test dose of 1000mg/kg which gradually increases and at a test dose of 3000mg/kg the lethality was 100 %. The crude extract of *Forsskoaleatenacissima* showed significant antioxidant activity which may be due to the flavonoid content of the plant. The total flavonoids of *Forsskaolea tenacissima* possess potent antinociceptive activity which is determined through Hot plate method, Writhing method and Tail immersion method, this proves the use of the plant in folk medicine as analgesic agent.

Abbreviations

Ft.Cr: Crude methanolic extract of *Forsskaolea tenacissima*; DPPH: 2, 2-diphenyl-1-picrylhydrazyl

SZ carried out experimental work as M.Phil Scholar. Also prepared the 1st draft of manuscript. NA extensively revised the manuscript. He also designed the study. MN helped in experimental work. All authors approved the final version of manuscript.

ETHICS APPROVAL: The ERC gave ethical review approval.

CONSENT TO PARTICIPATE: written and verbal consent was taken from subjects and next of kin.

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AUTHORS' CONTRIBUTIONS:

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated in the work to take public responsibility of this manuscript. All authors read and approved the final manuscript.

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