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Screening of Antistress activity of Ficus benghalensis Fruit extract (AbstractView.aspx?PID=2020-13-1-39)

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Screening of Antistress activity of *Ficus benghalensis* Fruit extract

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ABSTRACT:
Ficus benghalensis fruit (Moraceae) is a medicinal plant used in the treatment of many clinical conditions in India. Its anti-stress activity has been investigated in this study using albino mice and rats. The methanolic extract of *Ficus benghalensis* fruit was prepared and subjected to preliminary qualitative phytochemical screening. Flavanoids, Tannins and Alkaloids were found to be present. Acute toxicity studies were carried out in albino mice. The methanolic extract did not show the lethal effect at a dose of 2000mg/kg body weight with no signs of abnormalities or any mortality observed for 14 days period. Anoxia stress tolerance test, Swimmingendurance test, Immobilisation stress models were used to investigate the anti-stress potential of title plant. The results indicate that pretreatment with methanolic extract of fruit of *Ficus benghalensis* exhibited significant anti-stress activity at the tested doses of 125mg/ kg and 250 mg/ kg 500 mg/kg. On the basis of results, it was concluded that *Ficus benghalensis* fruit possess anti-stress activity.

KEYWORDS: *Ficus benghalensis* fruit, anoxia stress tolerance test, swimming endurance test model, Immobilization stress.

INTRODUCTION:

Stress is a common phenomenon that is experienced by all individual. When stress becomes extreme, it is harmful for the body and hence needs to be treated. Stress plays an major role in the pathogenesis of a variety of diseases including hypertension, peptic ulcer, immunodepression, reproductive dysfunction and behavior disorder[1]. Evidence related that stress impairs learning and memory and encounter several disorders including anxiety and depression[2]. Stress mapping or screening of biologically active constituents of natural origin, mainly from plant kingdom[3]. Drugs having Antistress properties induce a state of non-specific resistance against stressful conditions.

Empirical use of medicinal herbs has been widely disseminated since ancient times to treat a wide range of diseases. In current days, the interest in alternative therapies has raised markedly across the globe[4].

Drugs derived from plant source are emerging as alternative therapies in the treatment of many diseases including psychiatric disorders[5]. Medicinal plants playing significant role in the search of novel pharmacotherapy to treat psychiatric illnesses[6]. Plants have always been an exemplary source of drugs and many of the available drugs have been derived directly or indirectly from them[7].

Drugs like benzodiazepines, certain CNS stimulants such as amphetamines and caffeine as well as some anabolic steroids are routinely used by people to combat stress. The incidence of toxicity and dependence has limited the therapeutic usefulness of these drugs[8]. Alternative are clearly needed because of the inability of the current therapies to manage condition of disease[9]. The first drugs used to treat pathologic condition of the CNS were based on natural resources[10]. People from different area of world using herbal medicine to alleviate affective disorders[11]. The herbal formulations claimed to enhance

physical endurance, mental functions and non-specific resistance of the body have been termed as adaptogen[8].

Various plants are being used in complementary and alternative medicines for management of stress[12]. The potential utility of safer and cheaper herbal medicines as Antistress agents have been reported as they can withstand stress without altering the physiological functions of the body.

Ficus benghalensis (FB) commonly known as Alada mara in Kannada is a rich source of medicinal value having multidimensional curative properties. Different parts of the tree have been found to possess medicinal properties; leaves are used for treating ulcers, aerial roots for gonorrhea whereas seeds and fruits are cooling and tonic. In India, milky juice (latex) of stem bark of *F. benghalensis* is used for the treatment of rheumatism and other inflammatory diseases[13].

Photochemical investigation of *Ficusbenghalensis* explored wide variety of constituents which are responsible for its wide range of pharmacological activities. They include ketones, flavonoids, flavonols, sterols, pentacyclic triterpenes and triterpenoids, furocoumarin, tiglic acid ester and some other esters[14]. Flavanoids have been acknowledged for their interesting medicinal properties[15]. Different parts of *Ficus benghalensis* reported to possess anthelmentic, analgesic, immunomodulator, hypolipidemic, antidiabetic and antiallergic activities[16]. Flavonoids isolated from leaves of *Ficus benghalensis* has been reported for Antistress activity[17]. However, Antistress (adaptogenic) activity of fruits of title plant has not been scientifically validated till date. Hence the present study was undertaken to evaluate antistress activity.

MATERIALS AND METHODS:

Plant material:

The fruits of *Ficus benghalensis* was identified and authenticated by Dr. Paramanna D (Needagi) Professor & HOD, Dept. of Botany S.B Arts & KCP Science College, Vijayapur, Karnataka. Then sufficient amount of fruits of *Ficus benghalensis* were collected from in and around the garden of Vijayapur city, Karnataka and the sample has been preserved in the herbarium of the college.

Preparation of extract:

The fruits were shade dried at room temperature and ground to coarse powder and then extracted with methanol by Soxhlet's extraction method. Thereafter, the extract was concentrated using rotary flash evaporator. The yield of the extract obtained was 7.4 %. The obtained crude extract was stored in airtight container in refrigerator below 10 ^{0}C for further studies.

Preliminary phytochemical screening:

Preliminary phytochemical screening was carried out on test extract for the detection of phytoconstituents by following literature reported methods[18,19].

Experimental animals:

The Wistar albino rats of 150 - 200 gm and Swiss albino mice 20 - 30 gm of either sex was used in the experimentation. After randomization into various groups, animals were acclimatized for period of 10 days under standard husbandry condition as follows.

- Room temperature: 27 ± 3°
- Relative humidity: 65 ± 10%,
- 12 hr light/dark cycle

All the animals were fed with rodent pellet diet (VRK Nutritional Solutions, Pune, India) and water *ad libitium*under strict hygienic condition. Study protocol was approved from Institutional Animals Ethics Committee (IAEC) before initiation of the experiment

Committee (IAEC) before initiation of the experiment.

Acute toxicity study (LD_{50}):

An acute toxicity of methanolic extract of *Ficus benghalensis* fruits was performed on female albino mice (20-30 gm). The animals were fasted overnight prior to the experiment. Fixed dose (OECD Guideline No. 423) method was adapted for toxicity studies[20]. $1/40^{th}$, $1/20^{th}$ and $1/10^{th}$ LD_{50} cut off value of the extract were selected as screening doses for the anti- stress activity.

Evaluation models for anti-stressactivity of methanolic extract of *Ficus benghalensis* fruit extract

Anoxia Stress Tolerance Test

Albino mice of either sex weighing 20-30 gm were selected and divided into five groups of six each.

Group I - Control, received distilled water

Group II - Std. (*Withania somnifera*, 100mg/kg,p.o.)

Group III - MEFBF (125mg/kg, p.o.)

Group IV - MEFBF (250mg/kg, p.o.)

Group V -MEFBF (500mg/kg, p.o.)

Animals were treated as shown above for the three weeks. At the end of 1^{st}, 2^{nd} and 3^{rd} week i.e. on 7^{th}, 14^{th} and 21^{st} day 1 hr. after the treatment. Stress was induced in all the groups of animals by placing each mouse individually in the air tight bottle of 250 ml capacity to record anoxia time. The moment when the animal showed the first convulsions removed immediately from the bottle and resuscitated if needed. The time duration of animal entry into the air tight bottle and the appearance of the first convulsion were recorded as anoxia time. Appearance of convulsion was very sharp end point, as delay by minute of removal of the animal from the vessel may lead to death of the same.

Swimming Endurance test in mice:

Albino mice of either sex weighing 20-30gm divided into five groups of six animals each for the test as below

Group I -Control, received distilled water

Group II - Std. (*Withania somnifera,* 100mg/kg, p.o.)

Group III - MEFBF (125 mg/kg, p.o.)

Group IV – MEFBF (250 mg/kg, p.o.)

Group V – MEFBF (500 mg/kg, p.o.)

Treatment was given to mice for 7 days. On seventh day 1 hr. after treatment, all the mice were subjected to swimming endurance test. The mice were allowed to swim individually in swimming tank 30cm

height with 20cm diameter containing water of 25 cm height maintained at 26+ 1^0 C temperature. The end point was taken when the animals remained at the bottom of swimming tank for 10 sec. The mean swimming time for each group was calculated.

Immobilization Stress in rats[21]

In the present study, adult albino rats of either sex weighing 150 – 200gm were divided into six groups of six animals each.

Group I - Normal control

Group II - Stress control

Group III - Standard (*Withaniasomnifera*, 100mg/kg, p.o.)

Group IV - MEFBF (125 mg/kg, p.o.)

Group V - MEFBF (250 mg/kg, p.o.)

Group VI - MEFBF (500 mg/kg, p.o.)

The treatment was made as stated above for 10 days 1hr. before the exposure of stress. Stress was induced by immobilizing rats with head down, supine position by fixing the forelimbs and hind limbs to a wooden board inclined at an angle of 60^0, daily 2hrs. for a period of ten days.

Biochemical estimations:

At the end of 10^{th} day one hour after drug treatment the blood was collected from retro orbital plexus in sodium citrated tubes under mild ether anesthesia using disposable syringe and needle for estimation of biochemical parameters, such as serum glucose (GOD-POD method), cholesterol (CHOD-PAP method), triglycerides (GPO-Trinder method), BUN (Blood Urea Nitrogen, GLDH-UREASE method) using Erba Chem Semi-auto analyzer and ready reagent kits.

The rats then scarified and their organs such as liver, spleen and adrenal glands were removed. The weight of organs such as liver, spleen and adrenal glands after washing with alcohol was recorded per 100g body weight of animal.

RESULTS:

Preliminary phytochemical screening:

Preliminary phytochemical investigation on methanolic extract of *Ficus benghalensis* fruit indicated the presence of flavonoids, alkaloids and tannins.

Acute toxicity study:

MEFBF was studied for acute toxicity at dose of 2000 mg/kg i.p. in female albino mice. The extract did not cause any mortality (0/3 mice died) of the animals at dose of 2000mg/kg, even at repeated dosing using 3 new mice. Hence, 5000mg/kg was taken as LD_{50} cutoff value as per fixed dose method of OECD guideline number 423.

Anoxia stress tolerance time in mice:

In the anoxic tolerance test, the time taken for the mice to exhibit clonic convulsions was taken as the end point. The graded doses (125, 250, 500mg/kg) of the test extract demonstrated dose and duration dependent significant delay in clonic convulsions on 7th, 14th and 21st day compared to control group. Antistress effect of the higher dose (500 mg/kg) of the test extract was found closer to that of the standard drug. The results are presented in Table-1.

Table 01. Effect of MEFBF on anoxia stress tolerance time in mice

Groups	Treatment	Dose (mg/kg)	Duration of anoxia stress tolerance time (min)		
			7th Day	14th Day	21st Day
I	Control	--	30.2 ± 0.87	36.5 ± 1.12	39.9 ± 1.58
II	Std. (*Withania somnifera*)	100	56.5± 2.4***	59.0 ± 1.45***	64.5 ± 2.0***
III	MEFBF	125	36.4 ± 1.5***	41.5 ± 1.5***	44.3 ± 1.7***
IV	MEFBF	250	39.4 ± 1.2***	43.3± 1.6***	47.5± 1.3***
V	MEFBF	500	46.0 ± 1.1***	48.2± 1.9***	51.1± 1.4***

The values are expressed as Mean ± SEM, (n=6).

Where * $p< 0.05$, ** $p< 0.01$, *** $p< 0.001$ as compared to control.

Swimming endurance test in mice:

There was dose dependent significant increase in swimming performance time observed in mice seven days pretreated with graded doses (125, 250 and 500 mg/kg) of the test extract. The percentage increase in swimming performance time was found to be 22 to 74. However, the effect of test extract on swimming performance time was found to be less potent than the reference standard drug, *Witheniasominifera*. The results are tabulated in Table -2.

Immobilization Stress:

Effect on biochemical parameters:

Immobilization stress adversely affected the serum concentration of various biochemical parameters. The induction of Immobilization stress significantly elevated the serum cholesterol, triglycerides, BUN and glucose levels in stress control rats compared to normal control group. Animals pretreated for ten days with test extract at different dose levels (125, 250 and 500 mg/kg) showed significant and dose dependent fall in all the biochemical parameters, as compared to the stress control animals. The results are displayed in Table -3.

Effect on weight of organs:

Immobilization stress significantly increased the weight of liver, adrenal glands and decreased the spleen weight. Ten days pretreatment with graded doses of MEFBF significantly and dose dependently ameliorated the Immobilization stress induced altered organs weight. The results are represented in Table –4.

Table 02. Effect of MEFBF on swimming endurance time in mice

Groups	Treatment	Dose mg/kg	Swimming endurance time (min)	% increase in swimming time
I	Normal control		----	---
II	Stress control		230.23 ± 11.85$^{@}$	----
III	Standard (W S)	100	418.56 ± 5.71***	81
IV	MEFBF	125	282.43 ± 4.15***	22
V	MEFBF	250	346.51± 4.86***	50
VI	MEFBF	500	402.80 ± 6.14***	74

The values are expressed as Mean ± SEM, (n=6),

Where $^{@}p< 0.001$ * $p< 0.05$, ** $p< 0.01$, *** $p< 0.001$ as compared to stress control.

Table 03. Effect of MEFBF on serum biochemical changes in immobilization stress in rats

Groups	Treatment	Dose mg/kg	Biochemical estimations			
			Glucose mg/dl	Total Cholesterol mg/dl	Triglyceride mg/dl	BUN mg/dl
I	Normal Control	--	99.5±4.2	53.3±3.5	58.9±2.2	23.1±1.0
II	Stress Control	Vehicle	149.4±6.1$^{@}$	139.8±2.9$^{@}$	90.3±2.5$^{@}$	38.4±0.9$^{@}$
III	Standard (W S)	100	109.1±4.3***	59.6±3.0***	56.6±1.8***	26.9±0.8***
IV	MEFBF	125	129.0±2.4*	126.4±2.5*	80.9±2.1^{ns}	33.6±1.5*
V	MEFBF	250	119.1±3.2***	99.3±3.1***	75.3±2.3***	30.5±0.9***
VI	MEFBF	500	113.0±2.6***	66.4±3.1***	70.4±2.5***	28.9±0.8***

The values are expressed as Mean ± SEM, (n=6),

Where$^{@}p< 0.001$, * $p< 0.05$, ** $p< 0.01$, *** $p< 0.001$ as compared to stress control.

Table 04. Effect of MEFBF on organs weight in immobilization induced stress rats

Groups	Dose mg/kg	Organs weight (gm/100 gm b.w.)		
		Liver	Adrenal glands	Spleen
Normal control		3.58 ± 0.20	0.013 ± 0.001	0.380 ± 0.005
Stress control		5.63±0.17$^{@}$	0.098±0.031$^{@}$	0.231±0.004$^{@}$
Standard (W S)	100	3.53±0.13***	0.017 ± 0.001***	0.370±0.009***
MEFBF	125	4.81±0.12**	0.024±0.001**	0.275 ±0.005**
MEFBF	250	4.62±0.11***	0.021±0.001**	0.310±0.004***
MEFBF	500	4.13±0.15***	0.018±0.001**	0.348±0.006***

Values are expressed as Mean ± SEM, (n=6)

Where, *p< 0.05, **p< 0.01, ***p< 0.001 as compared to stress control and

@p< 0.001 compared to normal control.

DISCUSSION:

In the current study, anti-stress activity of MEFBF was demonstrated at different dose levels (125, 250 and 500 mg/kg) against anoxia stress tolerance test, swimming endurance time and immobilization stress models in experimental animal like mice and rat.

Anoxia is a more severe stress. All the body functions including cellular respiration depends on oxygen supply to them. Any lack of this vital element plays major role on all body mechanisms. Increase in adaptation during anoxic stress by any drug could be considered as its major anti-stress effect[22-24]. The results of the anoxic tolerance test showed that MEFBF significantly delayed the latency of post anoxic convulsions in experimental animals, thereby confirm its anti-stress activity.

Increase in swimming endurance time has reported in mice when pre-treated with anti-stress agents[25] and the test has been utilized to investigate the adaptogenic activity of different agents, based on the fact that swim endurance reflects physical endurance[26]. In the present investigation the results indicate clearly that the extract of *Ficus benghalensis* have the properties whereby they increased the physical endurance as well as the overall performance in mice.

Experimental animals exposed to an Immobilisation stress resulted in hyperglycemia, this is because during stressful condition adrenal cortex secrets excess cortisol[26]. Excessive secretion of cortisol maintains the internal homeostasis through the process of gluconeogenesis and lipogenesis[27.] The results of the current study revealed that the extract of the *Ficus benghalensis* exhibited promising effect in controlling hyperglycemia indicating the ability to prevent the alterations on adrenal cortex and helping in maintaining the homeostasis.

The mechanism by which stress rises serum cholesterol is likely to be related to the enhanced activity of hypothalamo-hypophyseal axis (HPA) resulting in liberation of catecholamines and corticosteroids. This could lead to increase in blood cholesterol level, since epinephrine is known to mobilize lipids from adipose tissues. The effect of stress on serum triglycerides has been shown to be variable. The increase in release of catacholamines leads to elevated levels of glucose and BUN[28-29]. In Immobilisation **s**tress model, the test extract reduced the elevated levels of serum biochemical parameters in dose dependent manner.

Stress induces adreno-medullary response in man. Adrenaline in turn stimulates Beta 2 receptors on the pituitary glands causing greater release of ACTH, which can stimulate the adrenal medulla as well as cortex. So adrenal gland weight increases. Cortisol increases mRNA levels in liver cells, this lead to increase in weight of liver. Spleen constricts to release more red blood cells (RBC) during stress, so its weight decreases during stress[30-32]. The rats pretreated with *Withenia Somnifera* and MEFBF significantly reversed altered organs weight of adrenal glands, liver and spleen thus supports the anti-stress effect of MEFBF against immobilization stress model.

The literature reports indicated that extracts of medicinal plants containing flavonoids and tannins known to possess significant anti-stress activity[33.] In our study also flavonoid and tannin contents of crude extract of the title plant (evidenced by phytochemical analysis) may be responsible for observed anti- stress (adaptogenic) activity.

CONCLUSION:

In conclusion, the methanolic extract of *Ficus Benghalensis* fruit exhibited dose dependent significant antistress activity by increasing the capacity to tolerate stress in experimental animal as well as restoring the altered biochemical parameters and organs weight. Thus the fruit extract of the title plant acts as an adaptogenic agent in management of stress and stress related diseases.

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CONFLICT OF INTEREST:
The authors do not have any conflict of interest.

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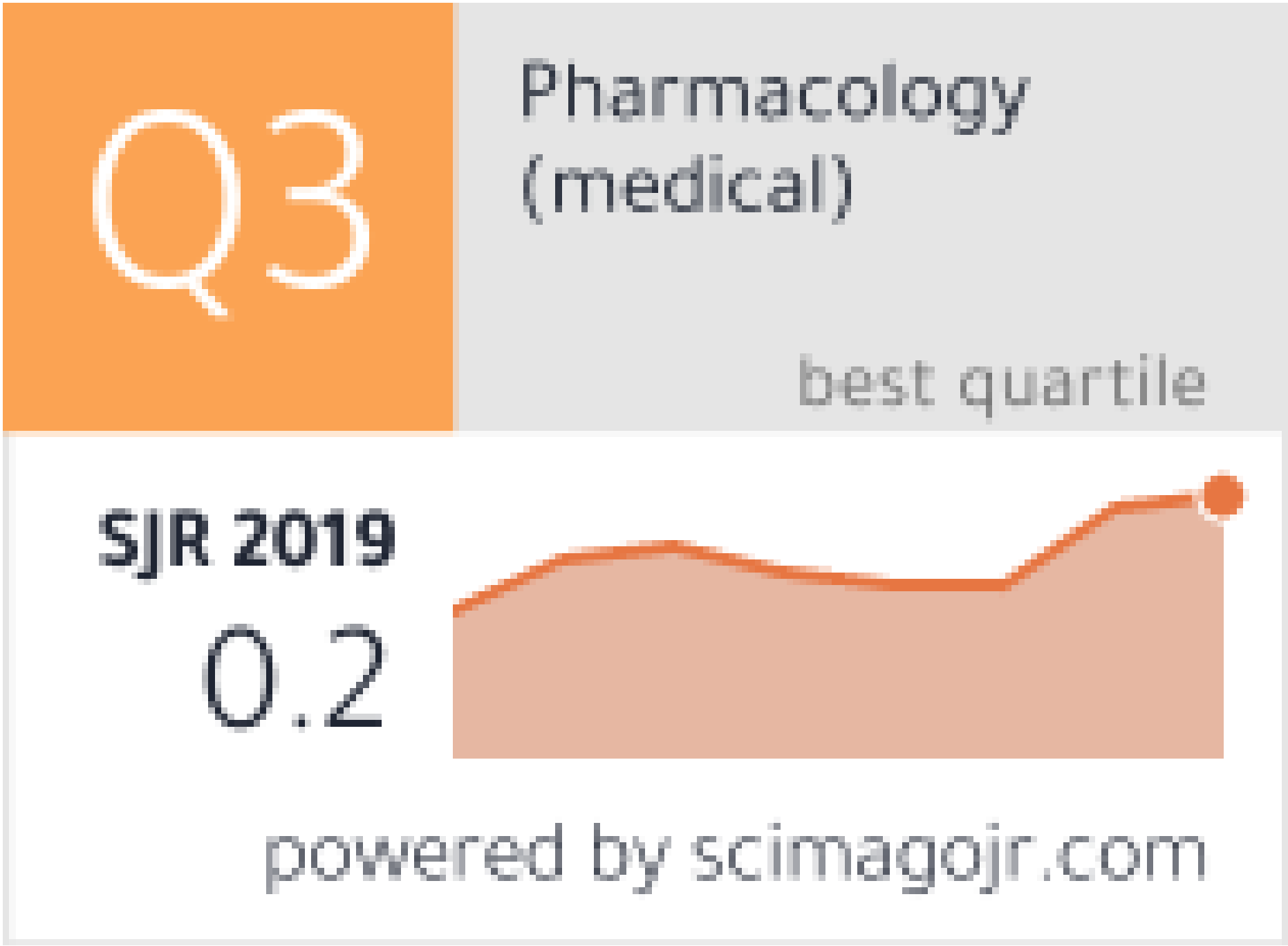

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