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ANTIDEPRESSANT ACTIVITY OF AQUEOUS EXTRACT OF CITRULLUS LANATUS

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ABSTRACT

Depression is a heterogenous mood disorder. The causes of depression are decreased brain levels of monoamines like noradrenaline, dopamine and serotonin. Due to the high cost of antidepressant synthetic drugs and their accompanying side effects, the discovery of safer antidepressant herbal remedies is on the rise. With this background, the present study was carried out to elucidate the antidepressant effect of citrullus lanatus in standardized mouse models of depression.Aqueous extract of *Citrullus Lanatus* (CLE) were prepared, and phytoconstituents were determined using appropriate chemical analytical methods. The aqueous extracts(300mg/kg and 600mg/kg) were administered to Male Wistar rats for evaluating antidepressant activity using forced swim test (FST) and tail suspension test (TST). Animals were divided into four groups: Groups 1 and 2 served as vehicle control and fluoxetine (20 mg/kg) standard control, respectively. Groups 3 and 4 served as treatment groups and were orally administered aqueous extract at doses of 300 mg/kg and 600 mg/kg, respectively.The results obtained from our study reveals that the aqueous CLE at higher concentration showed significant reduction in immobility in tail suspension and forced swim model of depression comparable to standard drug fluoxetine. It was observed that the extract shows dose dependent effect on immobility suggesting its antidepressant potiential.

INTRODUCTION

According to world health report, about 450 million people suffer from a mental or behavioral disorder.[1] by the year 2020, depression is expected to constitute the second largest source of global burden of disease after heart disease.[2] In Indian context, a recent large sample survey with rigorous methodology reported an overall prevalence of 15.9% for depression[3]. Depression is a chronic, pervasive and disabling illness, which can result from a combination of various biological and psychosocial factors.A constant state of depression results from continuous stress or central nervous system (CNS) neurochemical imbalance[4]. Oxidative stress (OS) is considered to be a major factor in the causation of anxiety and depression. The evidence suggests that OS causes imbalance between the production of oxygen-derived free radicals and the antioxidant ability of neuronal cells and tissues, consequently contributing to the neuropathology and psychiatric diseases, including mild and major depression[5]. Medications such as tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), selective reversible inhibitors of monoamine oxidase A (RIMAs), and specific serotonin–noradrenaline reuptake inhibitors (SNRIs) are clinically employed for drug therapy [18]. Each drug used to treat this disorder has a success rate of about 60%. In addition, most therapies require several weeks of treatment before improvement of signs and symptoms is observed and there are numerous

side effects caused by antidepressants [6].However, these drugs can impose a variety of side-effects including cardiac toxicity, hypopiesia, sexual dysfunction, body weight gain, and sleep disorder [7]. Medical plant therapies may be effective alternatives in the treatment of depression, and has progressed significantly in the past decade [8]. *Citrullus lanatus* (CL) is commonly known as watermelon and belongs to the family Cucurbitaceae. In India, it is used as food and for medicinal purposes. Considering the nutritional profile, consumption of 100 g watermelon provides 30 kcal. It contains almost 92 % water and 7.55 % of carbohydrates out of which 6.2 % are sugars and 0.4 % dietary fiber. It is enriched with carotenoid, vitamin C, citrulline, carotenoids and flavonoids and fat and cholesterol free, thus considered as low caloric fruit [9,10]. Additionally, watermelon is rich source of ß-carotene acts as an antioxidant and precursor of vitamin A. Besides the presence of lycopene, it is a source of B vitamins, especially B_1 and B_6, as well as minerals such as potassium and magnesium [11]. Watermelon contains phenolics quite comparable with that of other fruits [12]. CL possess antioxidant, anti-inflammatory, antimicrobial, antidiabetic, antigiardial, antisecretory, laxative, Anti-ulcerogenic, hepatoprotective activities[13]. The present studies were designed to assess the antidepressant activity of *citrullus lanatus*(CL) in the mouse model. This investigation was done in mice dosed with aqueous extract of CL (CLE) in various experiments of depression, and CL-induced effects were compared with reference antidepressant drug, fluoxetine.

MATERIALS AND METHODS:

Preparation of plant extract: Fruits of CL were purchased from a local market in Hyderabad. They were identified and authenticated by the Department of Life Sciences, Osmania University, Hyderabad. The fruit was washed, and then the exocarp was removed using a sterile knife. After which, the fruit juice was extracted using a manual juice extractor. The resultant juice collected was retained in a sterile vessel and stored at 4°C till used.

Preliminary Phytochemical Screening: The aqueous extract of CL was screened for the presence of various phytoconstituents like tannins, amino acids, steroids, alkaloids, glycosides, flavonoids, carbohydrates, proteins and phenolic compounds (14).

Animal husbandry of experimental animals: The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) .Male Wistar rats weighing 250-300 gm, were procured from the central animal facility of the Institute and maintained under the standard conditions, Room temperature (25±3) ℃, humidity 45%–55%, 12 /12 hr-light/dark cycle. %). During the experiments, animals were provided with standard feed and drinking water *ad libitum* in polycarbonate feeder bottles with a stainless steel nipple. The animals were acclimatized for a period of 7 days before the study.

Acute toxicity study: The dose-finding acute toxicity study of CLE was carried out in mice using the OECD Guidelines 423(15). Animals were observed for four hours hourly for behavior changes and daily for fourteen days. The extract was devoid of any toxicity in rats when given in dose up to 2000 mg/kg by oral route. Hence, for further studies 300-600 mg/kg doses of extract were used.

Drugs and preparations: Fluoxetine was used as a standard drug while aqueous CL extract was used as a test agent. These solutions were administered orally by gavage in a dosing volume not exceeding 1 ml in both cases.

Treatment plan for acute dosing

The groups assigned for acute dose study were as follows:

Group 1: Control group (distilled water)

Group 2: Fluoxetine alone (20 mg/kg)

Group 3: CLE-1 (300 mg/kg)

Group 4: CLE-2 (600 mg/kg)

In the acute treatment study, a single dose was administered 30 min prior to testing. Different standardized depression models were used for

behavioral tests to evaluate the antidepressant activity, such as forced swim test (FST) and tail suspension test (TST).

Forced swim test: FST was carried out according to the method described by Porsolt *et al* [16].Depression was produced by forcing the animal to swim individually in a glass jar containing fresh water of 15cm height and maintained at 25ºC. This constituted pretest session. Twenty-four hour later each animal was again forced to swim. After an initial 2 min period of vigorous activity, each animal assumed a typical immobile posture. The total duration of immobility was recorded in next 4 min of a total 6 min test. The change in the immobility period was calculated after administering drugs to the groups as mentioned

Tail suspension test: The total duration of immobility induced by tail suspension was measured according to the method described by Steru et al[17] . Depression was produced by suspending the animal from the edge of a table 50 cm above the floor by an adhesive tape placed approx. 1cm. from the tip of the tail. Immobility time was recorded during a 6 min. period. Changes in the immobility duration were studied after administering drugs in separate groups of animals. The antidepressant activity was expressed as reduction in the immobility duration between the control, standard and animals treated with test drug.

Statistical analysis: All the data was recorded and expressed as Mean ± SEM values were presented in the table I and II for FST and TST respectively. The response time were analysed by means of one-way analysis of variance (ANOVA) followed by Dunnet‟s Test. The result ware regarded as statistical significant at pvalue < 0.05.

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

On preliminary phytochemical analysis of CLE showed the presence of flavonoids, saponins, glycosides, terpenoids, amino acids, alkaloids, carbohydrates, phenolic compounds and proteins. (Table1)

Acute Toxicity Studies: In acute toxicity studies, 2000 mg/kg dose of CLE did not cause any mortality, or overt clinical signs of toxicity observed over 14 days period. Thus, 2000 mg/kg dose was considered as a safe dose of CLE in mice.

Antidepressant Activity

Forced swim test: The antidepressant effects of CLE (300 and 600 mg/kg) and fluoxetine were studied by observing the changes in the duration of immobility in Forced swim test (FST). The observations showed produced significant reduction ($p<0.01$) in the immobility period when compared with that of control group animals that received only the vehicle. [Table2] [Figure1].

Tail suspension test:

The antidepressant effects of CLE (300 and 600 mg/kg) and fluoxetine were studied by observing the changes in the duration of immobility in Tail suspension test (TST). The observations produced significant reduction ($p<0.01$) in the immobility period when compared with that of control group animals that received only the vehicle. [Table] [Figure 2]

DISCUSSION

The present study was designed to evaluate the antidepressant activity of aqueous extract of fruits of citrullus lanatus in mice using behavioral tests of depression. From the above results, it is concluded that aqueous extract of fruits of citrullus lanatus showed significant antidepressant activity. Watermelon is a good source of carotenoid and lycopene. Watermelon is also expectedly high in citrulline; an amino acid the body make use of to make another amino acid, arginine (used in the urea cycle to remove ammoniacal from the body) [21]. The antidepressant activity may be attributed to the presence of lycopene, has been reported to possess cytoprotective effects with its antioxidant activity by reducing lipid peroxidation and also significantly scavenges superoxide as well as inhibits its generation. polyphenols, flavanoids in the extract responsible for the attenuation of oxidative stress produced during depression. . Antioxidant protects against many ailments caused by reactive oxygen species(ROS) [23,24].

General procedure for qualitative phytochemical analysis			
S.No	Chemical tests	Observation	Inference
1	Test for alkaloids a.Dragendorff's test To the filtrate few drops of dragendroff's reagent was added. b.Mayer's test To the filtrate few drops of Mayer's reagent was added. c.Wagner's test To the filtrate few drops of Wagner's reagent was added. d.Hager's test To the filtrate few drops of Hager's reagent was added.	reddish-brown precipitate is formed cream colour precipitate is formed Brown colour precipitate yellow colour precipitate	Presence of Alkaloids. Presence of alkaloids Presence of alkaloids Presence of alkaloids
2	Test for flavanoids To the extract (5 ml), 0.5g of Mg and conc. sulphuric acid was added	pink coloration that disappear on standing.	Presenceof flavanoids
3	Test for carbohydrates a.Molisch's test: to the 2ml extract, few drops of α-Napthol solution in alcohol was added followed by addition of conc.H2SO4 from the sides of the test tube. b.Fehling's test:To the extract, equal quantities(1ml) of fehling's reagent A and B was added and heated for 5min.	Violet ring is formed at the junction of two liquids Brickred precipitate is formed	presence of carbohydrate presence of reducing sugars
4	Test for Proteins a.Biuret Test To 2ml of extract, 4% NaOH and few drops of 1% CuSO4 Solution was added. b.Millon's Test To 2ml of extract, 2ml millons reagent was aded	Violet or Pink colour develops pink color is formed	Presence of Proteins presence of proteins
5	Test for Aminoacids Ninhydrin test To the 3ml extract , 4 drops of Ninhydrin reagent(triketohydrindene hydrate) was added and heated in waterbath for 10 min.	Purple colour was formed	Presence of Amino acids
6	Test for phenols To the 3ml extract, 1ml of neutral ferric chloride solution was added.	violet colour develops	presence of phenols
7	Test for Triterpenoids: Liebermann Burchard test: 3ml of the extract was mixed chloroform and 5-6 drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was then added from the sides of the test	purple ring at the junction of two layers And deep red color in the lower layer was formed	Presence of triterpenoids
8	Test for tannins To the 3ml of extract, 1ml of ferric chloride solution was added.	bluish black color develops	Presence of tannins
9	Test for saponins To 1 ml of aqueous extract, distilled water was added and shaken vigorously.	Develops Persistent foam	Presence of saponins
10	Test for glycosides:	Develops Reddish	Presence of

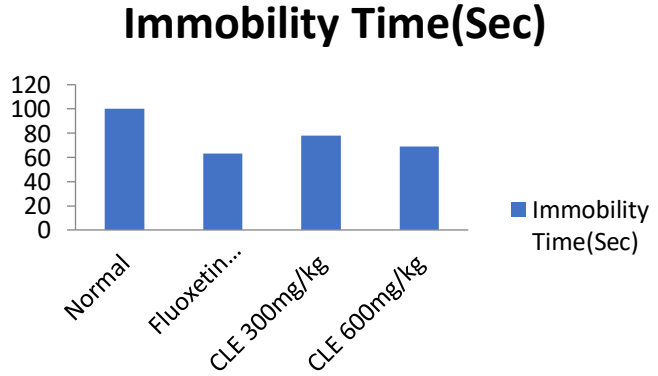
	Keller Kiliani test To 2ml of extract, 1ml glacial acetic acid and 2 drops of ferricchloride added, followed by the addition of concentrated sulphuric acid.	brown ring at the junction of two liquids	cardiac glycosides

S.No	Name of the Phytochemicals	Result
1	Alkaloids	Present
2	Phenols	Present
3	Flavonoids	Present
4	Tannins	Present
5	Saponins	Present
6	Glycosides	Present
7	Sterols	Present
8	Carbohydrates	Present
9	Proteins	Present
10	Triterpenes	Present
11	Amino acids	Present
12	Cardiac Glycosides	Present

S.No	Groups	Treatment	Dose	Immobility Time(Sec)
1	I	Distilled Water	10ml/Kg	180(+Or-)3.96
2	II	Fluoxetine	20mg/Kg	125(+Or-)2.45
3	III	CLE-I	300mg/Kg	138(+Or-)3.45
4	IV	CLE-II	600mg/Kg	129(+Or-)6.97

S.No	Group	Treatment	Dose	Immobility Time(Sec)
1	I	Distilled Water	10ml/kg	100(+or-)3.61
2	II	Fluoxetine	20mg/kg	63(+or-)5.22
3	III	CLE-I	300mg/kg	78(+or-)5.63
4	IV	CLE-II	600mg/kg	69(+or-)5.66

Figure 1- Effect of Citrullus lanata extract on duration of immobility forced swim test. Gr 1: Normal Control, Gr 2: Standard Fluoxetine (20 mg/kg), Gr 3: CLE-1 (300 mg/kg), Gr 4: CLE-2 (600 mg/kg).



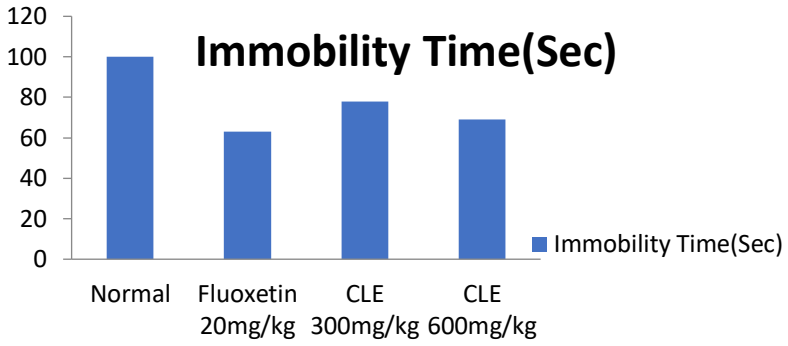


Figure 2- Effect of aqueous *Citrullus lanata* extract on duration of immobility in tail suspension test. Gr 1: Normal Control, Gr 2: Standard Fluoxetine (20 mg/kg), Gr 3: CLE-1 (300 mg/kg), Gr 4: CLE-2 (600 mg/kg).

CL also contains **S -Adenosylmethionine (SAMe) 5-Hydroxytryptophan (5-HTP) and tryptophan and tyrosine.** SAMe is an amino acid derivative, plays a role in many biological reactions by transferring its methyl group to DNA, proteins, phospholipids and biogenic amines. Several scientific studies indicate that SAMe may be useful in the treatment of depression. 5-Hydroxytryptophan (5-HTP) and tryptophan as a natural alternatives to traditional antidepressants. Taking 5-HTP as a supplement may raise serotonin levels. The widely accepted concept that an increased central serotonergic (5-HT) neurotransmission is a key therapeutic factor for depression. tyrosine is utilized in the synthesis of dopamine and increased dopamine level boosts mood. Water melon is fat free and contains a high amount of vitamin B6 that is used by the body to produce neurotransmitters like dopamine. DA may promote neurotrophic processes in the adult hippocampus as 5-HT and NA do.It is thus possible that the stimulation of multiple signalling pathways resulting from the elevation of all three monoamines may account in part for an accelerated and greater antidepressant response.

CONCLUSION

The present study provides the evidence indicating that aqueous extract of *Citrullus lanatus* showed significant antidepressant activity in TST and FST models of depression. Further studies are required to characterize the exact mechanism of anti-depressant effect of *Citrullus lanatus* .

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