# Evaluation of Anti-depressant Potential of Boswellic Acid in Mice

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**Abstract-** Depression is a prevalent, frequently recurrent and often chronic syndrome that affects quality of life and overall productivity. About 18 million Americans and 340 million people worldwide are affected from depression. Fourteen groups (n=6) were employed in the present study. Boswellic acid (25 mg/kg/day, *p.o*) was administered for 12 consecutive days as a test drug. Imipramine (15 mg/kg, *p.o.*) was injected for 12 days as a standard anti-depressant drug for evaluation of behavioural parameters. The present study concluded Boswellic acid as anti-depressant as suggested by the results obtained. Boswellic acid administration reduces immobility period in TST and FST models reverse Sucrose preference in CUMS group, when compared to normal control. The microsomal lipid peroxidation of polyunsaturated fatty acids (PUFA) produced malondialdehyde (MDA), lipid hydroperoxides. The restraint stress enhances the level of lipid peroxidation, hence increased Malondialdehyde, nitrite and significantly decreased reduced glutathione levels. Several studies reveal Boswellic acid as anti-oxidant. Proving its antioxidant effects, Boswellic acid potentially reduces oxidative stress, by altering malondialdehyde (MDA), nitrite/nitrate and reduced glutathione (GSH) levels, when compared to normal control.

Key words- Malondialdehyde, anti-depressant, peroxidation, gluthathione

## Introduction

Health is defined as a state of mental, physical and social well being and not merely absence of disease or infirmity. A sound mind in a sound body has been recognized as a social ideal for the well being [1]. Mental disorders such as depression anxiety, stress and schizophrenia, are the major cause of morbidity and mortality and is equally debilitating as other somatic illness. The magnitude of psychiatric and other central nervous system disorders appear day by day [2]. Depression is a prevalent, frequently recurrent and often chronic syndrome that affects quality of life and overall productivity. About 18

million Americans and 340 million people worldwide are affected from depression [3, 4]. Depression is the leading cause of disability worldwide. According to World Mental Health Survey conducted in seventeen countries, about one in twenty people were reported for having an episode of depression in the year of 2012 [5]. Major depressive disorder (MDD) may begin at any age, most commonly, it starts in the mid-20s, but exceptions occur; its age of onset occurs in a broad range [4]. Patients have intense feelings of sadness, hopelessness, and guilt, accompanied by sleep difficulties, impaired concentration, decreased energy, and possible suicidal ideation [6]. Several forms of depressive disorders are better understood by getting the knowledge of pathophysiology of depression, and evaluating anti-depressants, which facilitate the neurotransmission of 5-hydroxytryptamine/serotonin (5-HT), norepinephrine (NE) and dopamine (DA) [7]. There are many neurochemical and biochemical changes involved in pathophysiology of depression [8]. Transmission of important neurotransmittors 5-HT, DA and NE requires several steps including their synthesis, storage in secretory vesicles, and their regulated release into the synaptic cleft between pre and post synapses by $Ca^{2+}$ dependent process [2, 9, 10]. The synaptic effects of these neurotransmitters are terminated their by binding to specific transporter proteins, reuptake into the pre-synapse and their metabolism by enzymes, such as monoamine oxidase (MAO) [11]. Concomitant increase in the activity of the hypothalamic pituitary adrenal system, with a consequent elevated release of glucocorticoids such as cortisol as well as of corticotropin-releasing hormone (CRH) from brain neurons and brain derived neuronal factor (BDNF) are mediator of depression which play an important role in the growth and regulation of neurons during childhood and adolescence [12, 13]. BDNF protein synthesis is induced by calcium-calmodulin-dependent-kinase translocation to cell which in turn phosphorylate the transcription factor cAMP response element binding (CREB). Increased level of Cyclic adenosine mono phosphate (cAMP) activates the N methyl–d-Aspartate (NMDA) receptor, which increases the nitric oxide (NO) level having pvitol role in depression. [14] The conventional system of allopathic medicine is available for the effective treatment of depressive disorder. Tri-cyclic anti depressant like Amitriptyline, clomipramine, Imipramine, etc inhibit the reuptake of $Na^{+}$ dependent transport of 5-HT and act as anti-depressant [15]. As the disorder requires a long term treatment with frequent dosing, this may produce adverse effects with allopathic medicine. Therefore, herbal therapy is novel system to treat depression [16]. In India, herbal drugs are used officially for a long period as recognized alternative systems of health [17]. Millions of Indians use herbal drugs regularly, as spices, home-remedies, health foods as well as over-the-counter drugs prescribed in the non-allopathic systems *i.e.* Ayurvedic, Homeopathic [18]. The system of Herbal medicines are gaining interest among the population worldwide as these are exclusively obtained from plants and are safe & effective [17]. Boswellic acids, a gum resin is obtained from the plant *Boswellia serrata* family Burseraceae, which is native to Arabia and India [19]. *Boswellia serrata* and Boswellic acid resin is popularly used in Indian systems of number of activities (Unani, Ayurvedic & Sidoswellic acha). Several studies reveal Boswellic acid as an anti-inflammatory, anti-asthmatic, anti-arthritic [20-26], immunomodulatory [27, 28], antimicrobial [29], and an anti-oxidant [20, 30]. Boswellic acid has the potential of

reducing oxidative stress, scavenging reactive oxygen and nitrogen species, able to change the malondialdehyde (MDA) and reduced glutathione (GSH) level and stimulating $Ca^{2+}$ mobilization in human polymorphonuclear leucocytes (PMNL) [19, 25, 30, 31]. Further, study carried out on *Boswellia serrata* suggests its anti-depressant activity [32]. Considering multifarious beneficial effects of Boswellic acid and the fact that the anti-depressant activity of *Boswellia serrata* might be due to the presence of Boswellic acid, motivates us to evaluate the possible effect of Boswellic acid as an anti-depressant in mice.

## Materials and Methods

### Experimental Protocol

Fourteen groups (n=6) were employed in the present study. Boswellic acid (25 mg/kg/day, *p.o*) was administered for 12 consecutive days as a test drug. Imipramine (15 mg/kg, *p.o.*) was injected for 12 days as a standard anti-depressant drug for evaluation of behavioural parameters. Prazosin (62.5 µg/kg, *p.o.*), Sulpiride (50 mg/kg, *p.o.*), Baclofen (10 mg/kg, *p.o.*) and Para-chlorophenylalanine (*p*-CPA, 100 mg/kg, *i.p.*) were administered once on $12^{th}$ day to mice for exploration of the probable mechanism of anti-depressant action. Biochemical estimation of MAO-A, MAO-B, total protein, MDA, GSH, nitrite/nitrate levels in brain homogenate and corticosterone levels in blood plasma were carried out to evaluate the anti-depressant effect of Boswellic acid.

Solubility of Drugs: Boswellic acid was dissolved in 0.5% carboxymethyl cellulose (CMC). Imipramine, Prazosin, Sulpiride, and Baclofen were dissolved in normal saline (0.9% NaCl). *p*-CPA was dissolved in minimum quantity of 0.1 N sodium hydroxide solution and pH was adjusted to 7.0 with 0.1 N hydrochloric acid.

### Laboratory models employed for testing anti-depressant activity:

**Tail suspension test:** TST is commonly employed behavioral model for screening anti-depressant like activity in mice, was first given by Steru *et al.* [33]. Animals were moved from their housing colony to laboratory conditions for 1-2 hour. Each mouse was individually suspended to the edge of a table, 50 cm above the floor, by adhesive tape placed approximately 1 cm from tip of the tail. Each animal under test was both acoustically and visually isolated from other animals during test. The total duration of immobility induced was measured manually for 6 min as a means of evaluating potential anti-depressants [34, 35]. Immobility time was recorded during a 6 min period. Animal was considered to be immobile, when it did not show any body movement, hung passively and completely motionless.

**Forced swim test:** FST is frequently used behavioural model for anti-depressant like activity in mice, was proposed by Porsolt *et al*. [36]. Mice were forced to swim individually in glass jar (25 x 12 x 25 $cm^3$) containing fresh water at 15 cm height and maintained at $25^oC \pm 3^oC$. After an initial 2 min period of vigorous activity, each animal assumed a typical immobile posture. A mouse was considered to be immobile, when it remained floating in the water without struggling, making minimum movements of its

limbs necessary to keep its head above water. The total duration of immobility was recorded during the next 4 min of total 6 min test. The changes in immobility duration were studied after administration of drugs in separate groups of animals

**Locomotor activity:** Closed field activity was measured to rule out any interference in locomotor activity by the investigational drug, which may affect the process of depression, in FST and TST. Gross behavioral activity was assessed by digital Actophotometer. Each animal was observed over a period of 5 min in a square (30 cm) closed arena equipped with infrared light-sensitive photocells and values expressed as counts per 5 min [37]. The beams in the actophotometer, cut by the animal, were taken as measure of movements. The apparatus was placed in a darkened, sound-attenuated and ventilated testing room.

**Chronic unpredictable mild stress:** CUMS was performed according to the procedure adapted by Dhingra *et al.* and Lin P. *et al.* with slight modifications [13, 38]. Briefly, the weekly stress regimen consisted of food and water deprivation, exposure to an empty bottle, soiled cage, light/dark succession every 2 hour, space reduction, 45ºcage tilt, overnight illumination and rat exposure. All stressors were applied individually and continuously, day and night. CUMS group animals were housed in separate room and had no contact with the stressed groups. They were deprived of food and water for 24 hour preceding SPT, but otherwise food and water were freely available in the home cage.

Table 1: Stress regimen employed on mice for 3 weeks during CUMS period

| Day | Monday | Tuesday | Wednesday | Thursday | Friday | Saturday | Sunday |
|---|---|---|---|---|---|---|---|
| Food Deprived | 9:30 am → 9:30 am | | | | | | |
| Water deprived | | 9:30am-10:30am | | | | | |
| Soiled cage | | 10:30 am → 10:30 am | | | 8:30pm- 8:30 pm | | |
| Light and dark | | | 10:30am-8:30pm | | | 8:30am-8:30pm | |
| Space reduction | | | 8:30 pm → 8:30 am | | | 8:30 pm → 9:30 am | |
| 45º cage | | | | 8:30am-8:30 pm | | 8:30pm → 8:30am | |

| | | | |
|---|---|---|---|
| Overnight illumination | | 8:30 pm → 8:30 am | |
| Rat exposure | | 8:30am-8:30pm | |

**Sucrose preference test:** Sucrose preference test was carried out at the end of 3-week CUMS [39]. According to Li *et al.* before carrying the test, mice were trained to adapt to sucrose solution (1%, w/v). Two bottles of sucrose solution were placed in each cage for 24 hour, and then one bottle of sucrose solution was replaced with water for 24 hour. After the adaptation, mice were deprived of water and food for 24 hour before the test. Mice were housed in individual cages and were free to access to two bottles containing 100 ml of sucrose solution (1% w/v) and 100 ml of water, respectively. On the test day after 24 hour, the weights of consumed sucrose solution and water were recorded, and sucrose preference was calculated as

$$\text{Sucrose preference (\%)} = \frac{\text{Sucrose consumption}}{\text{[Sucrose consumption + Water consumption]}} \times 100\%$$

**Biochemical Estimation:**

Estimation of Brain MAO-A and MAO-B levels:- On 13$^{th}$ day, after exposure to TST, mice were sacrificed under light anesthesia. The brain samples were collected on an ice plate. The collected brain samples were washed with cold 0.25M Sucrose- 0.1M Tris-0.02M EDTA buffer (pH 7.4), weighed and homogenized in a glass homogenization tube. The whole procedure of brain samples collection was completed within five minutes. Mouse brain mitochondrial fractions were prepared following the procedure of schurr *et al*. [40]. The MAO activity was assessed spectrophotometrically. Briefly, the buffer washed brain sample was homogenized in 9 volumes of cold 0.25M Sucrose- 0.1M Tris-0.02M EDTA buffer (pH 7.4) and centrifuged twice at 800 rpm for 10 min at 4°C in cooling centrifuge (REMI cooling centrifuge, Mumbai). The pellet was discarded. Supernatant was then centrifuged at 12000 rpm for 20 min at 4°C in cooling centrifuge. The precipitates were washed twice with about 100 ml of sucrose-tris-EDTA buffer and suspended in 9 volumes of cold sodium phosphate buffer (10 mM, pH 7.4, containing 320 mM sucrose) and mingled well at 4$^{o}$C for 20 min The mixture was then centrifuged at 15000 rpm for 30 min at 0$^{o}$C and the pellets were re-suspended in cold sodium phosphate buffer. For estimating MAO-B activity, 2.75 ml sodium phosphate buffer (100 mM, pH 7.4) and 100 µl of 0.1 M benzylamine were mixed in a quartz cuvette which was then placed in double beam spectrophotometer (Lab India, Mumbai). This was followed by the addition of 150 µl solution of mitochondrial fraction to initiate the enzymatic reaction and the change in absorbance was recorded at wavelength of 249 nm for 5 min against the blank containing sodium phosphate buffer and benzylamine. For estimating

MAO-A activity, 2.75 ml sodium phosphate buffer (100 mM, pH 7.4) and 100 µl of 4 mM 5-hydroxy-tryptamine (5-HT) were mixed in a quartz cuvette which was then placed in double beam spectrophotometer (Lab India, Mumbai). This was followed by the addition of 150 µl solution of mitochondrial fraction to initiate the enzymatic reaction and the change in absorbance was recorded at wavelength of 280 nm for 5 min against the blank containing sodium phosphate buffer and 5-HT.

**Estimation of Brain Protein concentration**:- Total proteins were assessed by Biuret method using the commercially available kit (Agappe diagnostics Ltd. Kerala, India). The working reagent containing potassium iodide, potassium sodium tartarate, copper sulphate and sodium hydroxide (1000 µl) was added to 20 µl standard protein and to 20 µl sample to prepare standard (S) and test (T). Blank (B) contains 1000 µl of reagent only. All the tubes were incubated at 37ºC for 10 minutes. The absorbance of Test and Standard were measured against blank (B) at 546 nm spectrophotometrically. Proteins in sample form a blue colored complex, when treated with cupric ions in alkaline solution. The intensity of blue color was proportional to proteins present in sample.

$$\text{Total Protein Conc. (g/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times 6$$

**Estimation of Brain Malondialdehyde (MDA) levels**:- Malondialdehyde (MDA), an index of free radical generation/lipid peroxidation, was determined by the procedure given by wills, 1965 [41]. Briefly, the reaction mixture consisted of 0.2 ml of 8.1% sodium lauryl sulphate, 1.5 ml of 20% acetic acid (pH 3.5) and 1.5 ml of 0.8% aqueous solution of thio-barbituric acid, which was added to 0.2 ml of supernatant of brain homogenate. The mixture was made up to 4.0 ml with distilled water and heated at 95$^{o}$C for 60 min After cooling the contents under running tap water, 5.0 ml of n-butanol and pyridine (15:1 v/v) and 1.0 ml of distilled water was added. The contents were centrifuged at about 3000 rpm for 10 min The organic layer was separated out and its absorbance was measured at 532 nm using double beam UV-Visible spectrophotometer (Lab India, Mumbai) against a blank having reaction mixture minus supernatant of brain homogenate [42].

**Estimation of Brain Reduced Glutathione levels:-** The brain GSH level was estimated using the methods described by Ellman et al., 1959 [43]. The brain homogenate of the mice was mixed with 10% w/v trichloro acetic acid in 1:1 ratio and centrifuged at 4 ◦C for 10 min at 5000 rpm. The supernatant (0.5 ml) was mixed with 2 ml of 0.3 M disodium hydrogen phosphate buffer (pH 8.4) and 0.4 ml of distilled water. Then, 0.25 ml of 0.001 M freshly prepared DTNB [5, 5'-dithiobis (2-nitrobenzoic acid) dissolved in 1% w/v sodium citrate was added to the reaction mixture, and then incubated for 10-min The absorbance of the yellow colored complex was noted spectrophotometrically at 412 nm. A standard curve was plotted using the reduced form of glutathione (0.1–1 µM).

**Estimation of Brain Nitrite/Nitrate levels:-** Greiss reagent (mixture of 250 µL of 1.0% sulphanilamide prepared in 3N HCl and 250 µL of 0.1% N-naphthyl ethylene diamine prepared in water) was kept for 1 hour at 0ºC. 1 part of brain homogenate was added to 1 part of Greiss reagent, and kept in dark for 10 mins at room temperature [44]. The absorbance was measured at 546 nm using UV–visible spectrophotometer.

**Estimation of Plasma Corticosterone levels**:- The quantitative estimation of Corticosterone levels in the blood plasma was performed by the method of Bartos and Pesez, 1979 [45, 46]. To 1.0 ml of sample in 1.0 ml ethanol, 0.50 ml of 0.10% solution of p-nitroso-N,N-dimethylaniline in ethanol was added and the tubes were immersed in ice water for 5 min, and then 0.50 ml of 0.10 M sodium hydroxide was added. The tubes were plugged with cotton-wool and were let to stand at 0ºC for 5 h, protected against light. To the above solution, 2.0 ml of buffer for pH 9.8, 5.0 ml of 0.10% solution of phenol in ethanol and 0.50 ml of 1.0% aqueous solution of potassium ferricyanide were added. The tubes were kept in a water bath at 20 ± 2ºC for 10 min The absorbance was read at 650 nm using UV–visible spectrophotometer (Lab India, India).

**Groups employed for Tail suspension test (TST) and Forced swim test (FST):**

Group I: (*Normal control*), Mice were administered vehicle (0.5% w/v CMC, 10 ml/kg, *p.o.*) for 12 days and on 12$^{th}$ day; the animals were subjected to TST after 60 min of the vehicle administration. This group was subjected to FST on 13$^{th}$ day after 60 min of vehicle administration.

Group II: (*Imipramine per se*), Mice were administered Imipramine (15 mg/kg/day, *p.o.*) for 12 days and on 12$^{th}$ day; the animals were subjected to TST after 60 min of the drug administration. This group was subjected to FST on 13$^{th}$ day after 60 min of Imipramine administration.

Group III: (*Boswellic acid per se*), Mice were administered Boswellic acid (25 mg/kg/day, *p.o.*) for 12 days and on 12$^{th}$ day; the animals were subjected to TST after 60 min of the drug administration. This group was subjected to FST on 13$^{th}$ day after 60 min of Boswellic acid administration.

**Groups employed for Chronic unpredictable mild stress (CUMS) and Sucrose preference test (SPT)**

Group IV: (*CUMS*), Mice were subjected to CUMS for consecutive 21 days; animals were trained and subjected to SPT on 24$^{th}$ day.

Group V: (*CUMS + Imipramine*), Mice were subjected to CUMS for consecutive 21 days. Imipramine (15 mg/kg/day, *p.o.*) was administered for 24 days and on 24$^{th}$ day, these mice were subjected to SPT after 60 min of Imipramine administration.

Group VI: (*CUMS + Boswellic acid*), Mice were subjected to CUMS for consecutive 21 days. Boswellic acid (25 mg/kg/day, *p.o.*) was administered for 24 days and on 24$^{th}$ day; the mice were subjected to SPT after 60 min of Boswellic acid administration.

**Groups employed for Mechanism of action**

Group VII: (*Sulpiride Control*), Vehicle (CMC 0.5%, 10ml/kg, *p.o.*) was administered orally for 12 successive days in mice. Sulpiride (50 mg/kg, *p.o.*) was administered 60 min after vehicle administration on 12$^{th}$ day. Animals were subjected to TST after 60 min of Sulpiride administration.

Group VIII: (*Sulpiride and Boswellic acid),* Boswellic acid *per se* (25 mg/kg, *p.o.*) was administered orally for 12 successive days in mice. Sulpiride (50 mg/kg, *p.o.*) was administered 60 min after Boswellic acid *per se* administration on 12$^{th}$ day. Animals were subjected to TST after 60 min of Sulpiride administration.

Group IX: (*Baclofen Control*), Vehicle (CMC 0.5%, 10ml/kg, *p.o.*) was administered orally for 12 successive days in mice. Baclofen (10 mg/kg, *p.o.*) was administered 60 min after vehicle administration on 12$^{th}$ day. Animals were subjected to TST after 60 min of Baclofen administration.

Group X: (*Baclofen and Boswellic acid*), Boswellic acid *per se* (25 mg/kg, *p.o.*) was administered orally for 12 successive days in mice. Baclofen (10 mg/kg, *p.o.*) was administered 60 min after Boswellic acid administration on 12$^{th}$ day. Animals were subjected to TST after 60 min of Baclofen administration.

Group XI: (*Prazosin Control*), Vehicle (CMC 0.5%, 10ml/kg, *p.o.*) was administered orally for 12 successive days in mice. Prazosin (62.5 µg/kg, *p.o.*) was administered 60 min after vehicle administration on 12$^{th}$ day. Animals were subjected to TST after 60 min of Prazosin administration.

Group XII: (*Prazosin and Boswellic acid*), Boswellic acid (25 mg/kg, *p.o.*) was administered orally for 12 successive days in mice. Prazosin (62.5 µg/kg, *p.o.*) was administered 60 min after Boswellic acid administration on 12$^{th}$ day. Animals were subjected to TST after 60 min of Praosin administration.

Group XIII: (*p*-CPA *Control*), Vehicle (CMC 0.5%, 10ml/kg, *p.o.*) was administered orally for 12 successive days in mice. *p*-CPA (100 mg/kg, *i.p.*) was administered 60 min after vehicle administration on 12$^{th}$ day. Animals were subjected to TST after 60 min of *p*-CPA administration.

Group XIV: (*p-CPA and Boswellic acid*), Boswellic acid (25 mg/kg, *p.o.*) was administered orally for 12 successive days in mice. *p*-CPA (100 mg/kg, *i.p.*) was administered 60 min after Boswellic acid administration on 12$^{th}$ day. Animals were subjected to TST after 60 min of *p*-CPA administration.

**Groups employed for Biochemical estimations:-** Groups I-VI are used for biochemical estimations.

**Statistical analysis:-** The result data was expressed in mean ± standard error mean. Data was statistically analyzed by using repeated measures of analysis of variance (ANOVA), followed by dunnet T test. '*p*' value less than 0.05 was considered to be significant.

## Results

### Behavioral Parameters:-

**Effect of Boswellic acid on immobility period in TST and FST:-** Boswellic acid (25 mg/kg, *p.o.*) *per se* administered for 12 successive days to mice significantly ($p<0.01$) decreased the immobility periods in both TST and FST, indicating significant antidepressant-like activity. Imipramine (15 mg/kg, *p.o.*) *per se* administered for 12 successive days to mice as standard antidepressant significantly ($p<0.01$) decreased the immobility periods in both TST and FST as compared to the normal control group. (Fig no. 1 & 2)

**Effect of Boswellic acid on locomotor activity:-** Boswellic acid (25mg/kg, *p.o.*) *per se* administered for 12 successive days to mice, indicated no significant change in locomoter activity, when compared with normal control. Imipramine (15mg/kg, *p.o.*) *per se* administered for 12 successive days to mice didn't not showed any significant difference in locomoter activity as compared to the normal control group.

**Effect of combination of Boswellic acid with Sulpiride, Baclofen, Prazosin and *p*-CPA on immobility period in TST:-** Prazosin (62.5 µg/kg, *i.p)*, Sulpiride (50 mg/kg, *i.p.*), Baclofen (10 mg/kg, *i.p.*), and p-CPA (100 mg/kg, *i.p.*) alone significantly ($p<0.05$, $p<0.01$) increased the immobility period as compared to normal control group. Boswellic acid (25 mg/kg, *p.o.*) *per se* significantly ($p<0.01$) reversed the increase in immobility time period elicited by Prazosin, Sulpiride, Baclofen and *p*-CPA, when compared to Boswellic acid *per se* alone. (Table no. 2)

**Effect of Boswellic acid on Sucrose preference after Chronic unpredic-table mild stress:-** Exposure of the mice to chronic unpredictable mild stress (CUMS) for 21 successive days significantly ($p<0.01$) decreased sucrose preference. Boswellic acid (25mg/kg, *p.o.*) and Imipramine (15 mg/kg, *p.o.*) significantly restored the reduced sucrose preference (%) in CUMS groups as compared to CUMS alone group.

### Biochemical estimations:

**Effect of Boswellic acid on Brain MAO-A levels:-**Boswellic acid (25mg/kg, *p.o.*) *per se* and Imipramine (15 mg/kg, *p.o.*) *per se* administered for 12 successive days significantly ($p< 0.01$) reduced the brain MAO-A level in treated mice as compared to the normal control group. The efficacy of Boswellic acid was found to be comparable to Imipramine. (Fig. 3)

**Effect of Boswellic acid on Brain MAO-B levels:-** Boswellic acid (25mg/kg, *p.o.*) *per se* and Imipramine (15 mg/kg, *p.o.*) *per se* administered for 12 successive days significantly ($p<0.01$) reduced MAO-B activity in treated mice as compared to normal control group. The efficacy of Boswellic acid was found to be comparable to Imipramine. (Fig. 4)

**Effect of Boswellic acid on Brain Protein concentration:-** Boswellic acid (25mg/kg, *p.o.*) *per se* and Imipramine (15 mg/kg, *p.o.*) *per se* administered for 12 successive days significantly ($p<0.01$) reduced brain protein concentration in treated mice as compared to normal control group. The efficacy of Boswellic acid was found to be comparable to Imipramine. (Fig. 5)

**Effect of Boswellic acid on Brain MDA levels:-** Boswellic acid *per se* and Imipramine *per se* administered for 12 sucessive days significantly ($p<0.01$ ) decreased MDA levels in treated mice as compared to normal control group. The efficacy of Boswellic acid was found to be comparable to Imipramine. (Fig. 6)

**Effect of Boswellic acid on Brain Reduced glutathione levels:-** Boswellic acid *per se* and Imipramine *per se* administered for 12 successive days significantly ($p< 0.05$, $p<0.01$) increased, reduced glutathione levels in treated mice as compared to control normal group. (Fig. 7)

**Effect of Boswellic acid on Brain Nitrite/Nitrate levels:-** The dose of Boswellic acid (25mg/kg, *p.o.*) *per se* and Imipramine (15 mg/kg, *p.o.*) *per se* administered for 12 successive significantly ($p< 0.01$) decreased plasma nitrite/nitrate levels in treated mice as compared to normal control group. The efficacy of Boswellic acid was found to be comparable to Imipramine. (Fig. 8)

**Effect of Boswellic acid on Blood plasma corticosterone levels:-** Boswellic acid *per se* and Imipramine *per se* administered for 21 successive days significantly ($p< 0.01$) reduced the corticosterone levels of treated mice as compared to normal control group. (Fig. 9)

Table 2: Effect of combination of Boswellic acid with sulpiride, baclofen, prazosin and *p*-CPA on immobility period of mice in TST

| Group No. | Treatment for 12 days *i.p.* | Dose ($kg^{-1}$) | Immobility Period (sec) |
|---|---|---|---|
| I | Vehicle (0.5% CMC) | 10 ml/kg | 187.16 ± 9.28 |
| III | Boswellic acid *per se* | 25 mg/kg | 95.83 ± 6.05 |
| VII | Vehicle (0.5% CMC) + Sulpiride | 10 ml + 50 mg | 211.67 ± 7.1$^{*}$ |
| VIII | Boswellic acid + Sulpiride | 25 mg + 50 mg | 139.3 ± 6.6$^{\#\#}$ |
| IX | Vehicle (0.5% CMC) + Baclofen | 10 ml + 10 mg | 214 ± 5.7$^{*}$ |
| X | Boswellic acid + Baclofen | 25 mg + 10 mg | 166.16 ± 7.16$^{\#\#}$ |
| XI | Vehicle (0.5% CMC) + Prazosin | 10 ml + 62.5 µg | 231.16 ± 6$^{**}$ |

| | | | |
|---|---|---|---|
| XII | Boswellic acid + Prazosin | 25 mg + 62.5 µg | $156.3 \pm 5.5^{\#\#}$ |
| XIII | Vehicle (0.5% CMC) + *p*-CPA | 10 ml + 100 mg | $119.66 \pm 7.212^{*}$ |
| XIV | Boswellic acid + *p*-CPA | 25 mg + 100 mg | $217.5 \pm 6.44^{\#\#}$ |

Values are expressed as Mean ± SEM. N = 6 in each group.
Data was analyzed by one-way ANOVA followed by Dunnett's t-test.
$^{*}p < 0.05$ when compared with normal control group (I)
$^{**}p < 0.01$ when compared with normal control group (I)
$^{\#}p < 0.01$ when compared with Boswellic acid treated group (III)
$^{\#\#}p < 0.01$ when compared with Boswellic acid treated group (III)

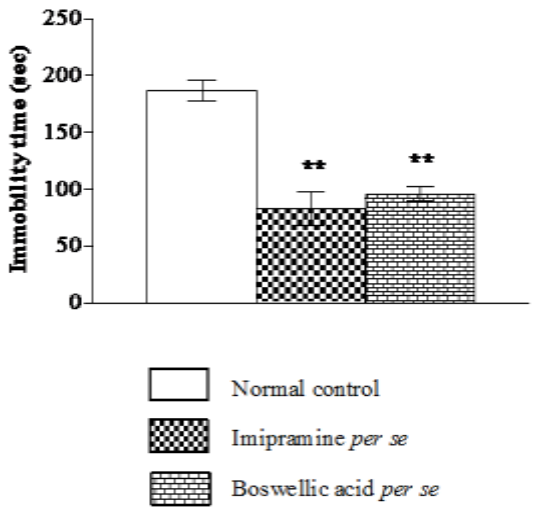


Figure 1: Effect of Boswellic acid on Imobility time of mice using Tail suspension test
Values are expressed as Mean ± SEM. n=6 in each group. Data was analyzed by One-way ANOVA followed by Dunnett's t-test. **p<0.01 when compared with normal group.

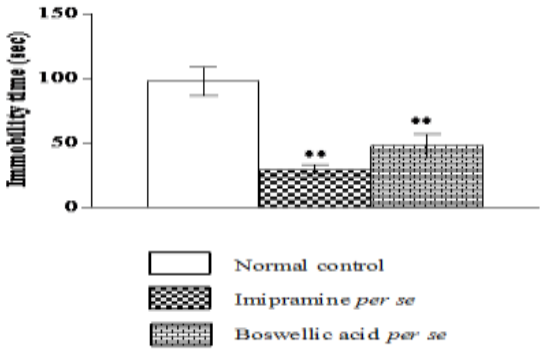


Figure 2: Effect of Boswellic acid on Immobility time of mice using Forced swim test
Values are expressed as Mean ± SEM. n=6 in each group. Data was analyzed by One-way ANOVA followed by Dunnett's t-test. **p<0.01 when compared with normal group.

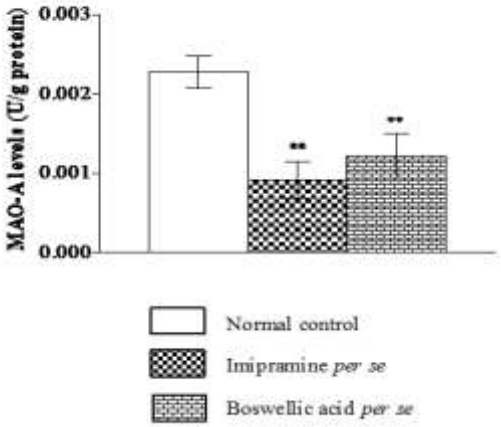


Figure 3: Effect of Boswellic acid on MAO-A levels in Brain of mice
Values are expressed as Mean ± SEM. n=6 in each group. Data was analyzed by One-way ANOVA followed by Dunnett's t-test. **p<0.01 when compared with normal group.

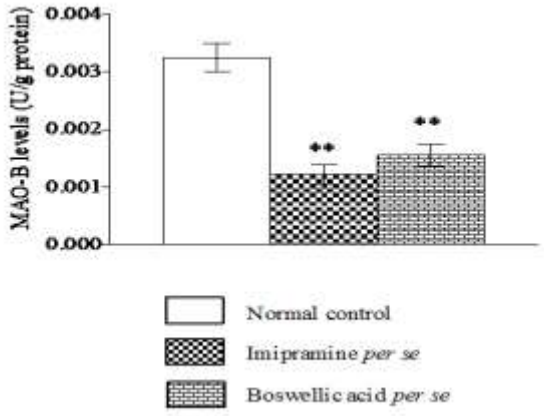


Figure 4: Effect of Boswellic acid on MAO-B levels in Brain of mice
Values are expressed as Mean ± SEM. n=6 in each group. Data was analyzed by One-way ANOVA followed by Dunnett's t-test. **p<0.01 when compared with normal group.

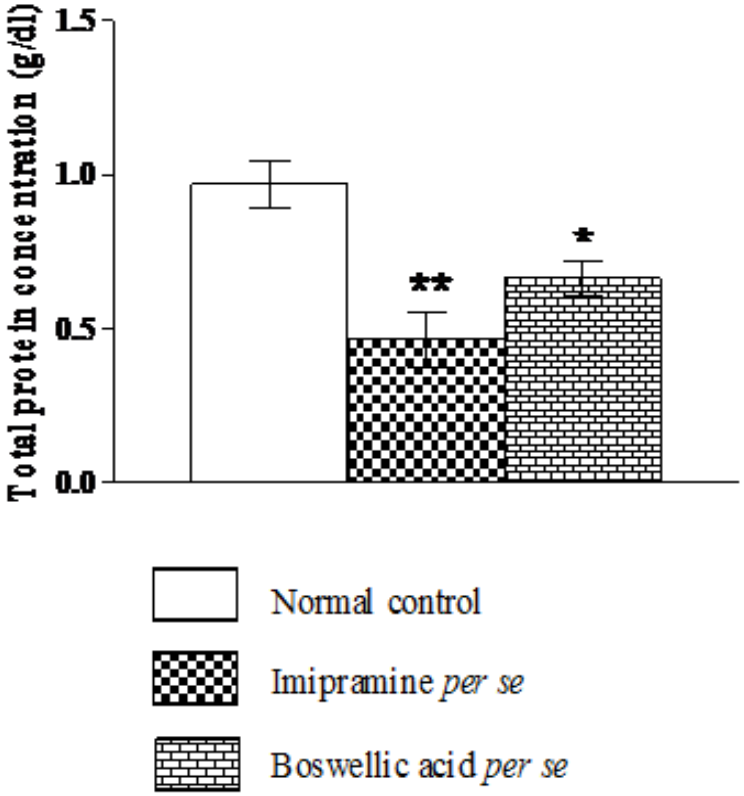


Figure 5: Effect of Boswellic acid on Protein concentration in Brain of mice
Values are expressed as Mean ± SEM. n=6 in each group.
Data was analyzed by One-way ANOVA followed by Dunnett's t-test.
*p<0.05 when compared with normal group.
**p<0.01 when compared with normal control

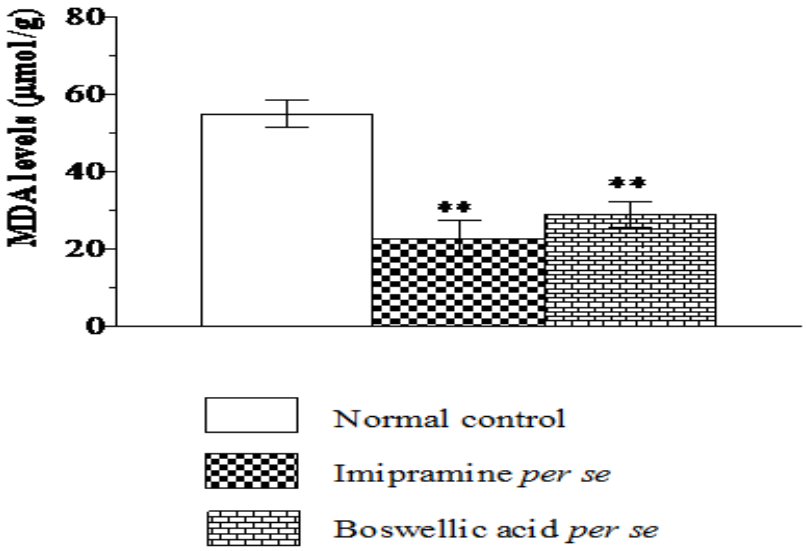


Figure 6: Effect of Boswellic acid on MDA levels in Brain of mice
Values are expressed as Mean ± SEM. n=6 in each group.
Data was analyzed by One-way ANOVA followed by Dunnett's t-test.
**p<0.01 when compared with normal control

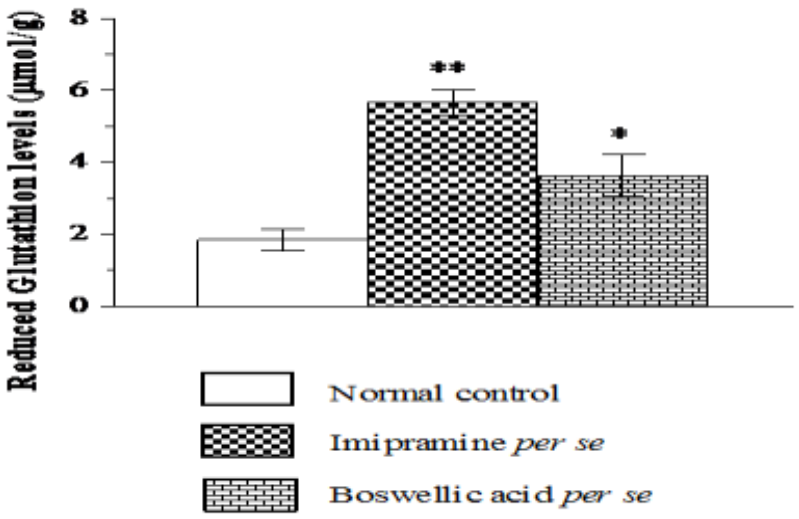


Figure 7: Effect of Boswellic acid on reduced Glutathione levels in Brain of mice
Values are expressed as Mean ± SEM. n=6 in each group.
Data was analyzed by One-way ANOVA followed by Dunnett's t-test.
*p<0.05 when compared with normal group.
**p<0.01 when compared with normal control

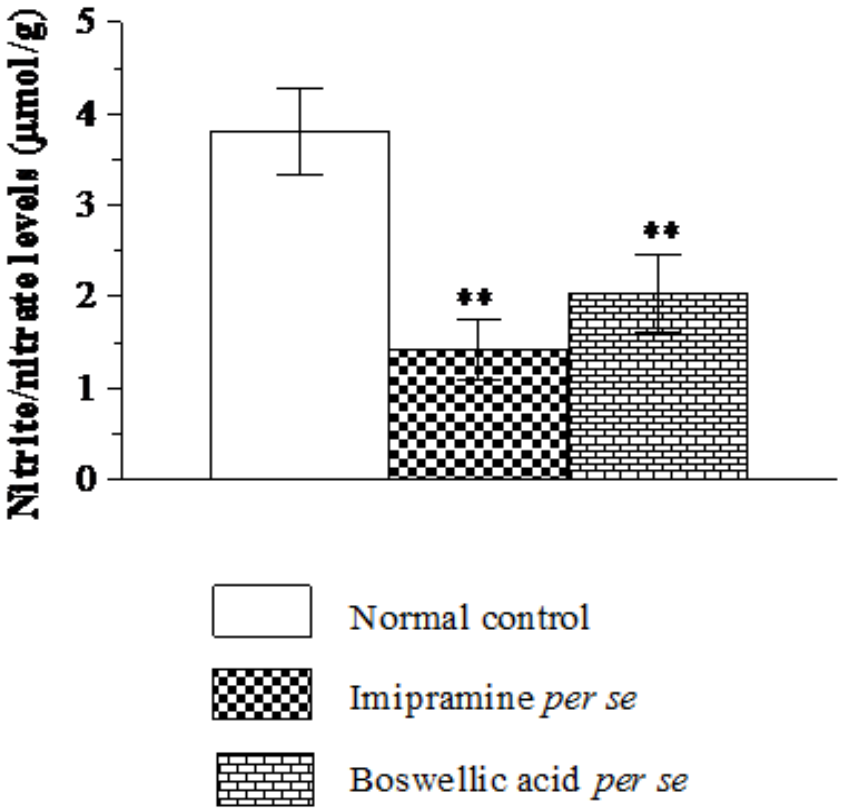


Figure 8: Effect of Boswellic acid on Nitrite/nitrate levels in Brain of mice
Values are expressed as Mean ± SEM. n=6 in each group.
Data was analyzed by One-way ANOVA followed by Dunnett's t-test.
**p<0.01 when compared with normal control

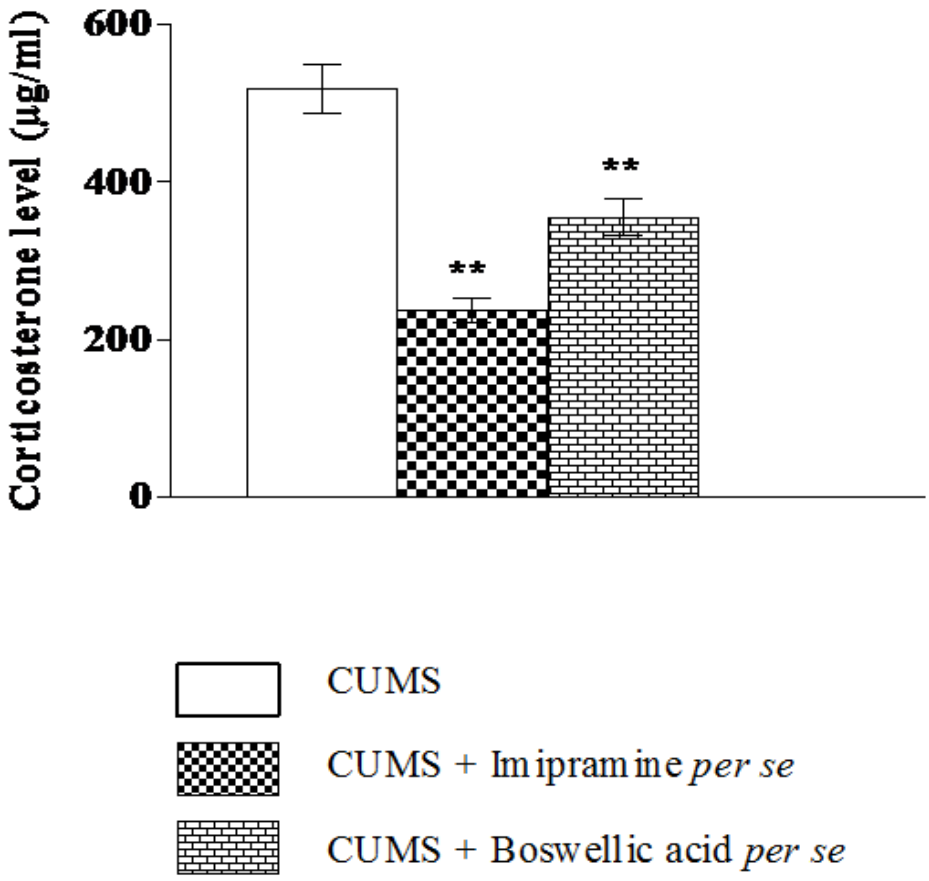


Figure 9: Effect of Boswellic acid on Blood plasma Corticosterone level of mice
Values are expressed as Mean ± SEM. n=6 in each group.
Data was analyzed by One-way ANOVA followed by Dunnett's t-test.
**p<0.001 when compared with normal group.

**5. 2. Discussion**

Antidepressant-like activity of Boswellic acid, one of the major chemical constituent present in *Boswellia serrata* was evaluated in mice employing Forced swim test (FST), Tail suspension test (TST) and Sucrose preference test (SPT) followed by Chronic unpredictable mild stress (CUMS). These models are widely employed in rodents to predict antidepressant potential by decrease of immobility period produced by several different classes of antidepressant drugs [13, 46-48,]. Earlier study carried out on *Boswellia serrata* suggests its anti-depressant activity [32]. In the present study Boswellic acid (25 mg/kg, *p.o.*) *per se* administered for 12 successive days to mice produced significant antidepressant-like effect in TST and FST, when compared to normal control group. Boswellic acid administered for 24 successive days significantly increased the sucrose preference in the animals, which were previously subjected to chronic unpredictable stress for 21 days. The efficacy of Boswellic acid *per se* was found to be comparable with Imipramine (15mg/kg, *p.o.*). Boswellic acid didn't show any significant change in locomotor functions of mice as compared to normal control, so it did not produce any over motor effects, there by Boswellic acid was selected to investigate the

possible underlying mechanisms of antidepressant-like action using sulpiride (selective D2-receptor antagonist), baclofen ($GABA_B$ agonist), prazosin (α1- adrenoceptor antagonist),and *p*-CPA (a serotonin synthesis inhibitor). Levels of monoamines like Dopamine, norepinephrine and serotonin are decreased in depression. The anti-depressant drugs enhance the levels of these monoamines [49, 50]. $GABA_B$ receptor antagonism may serve as a basis or the generation of anti-depressant [51].

In the present study sulpiride (selective D2-receptor antagonist) showed elevated immobility time possibly by blocking the $D_2$ receptor and thus the release of Dopamine. Prazosin (α1- adrenoceptor antagonist) produced increased immobility period in mice probably through decreased nor adrenergic activity as due to α1- adrenoceptor receptor blockage. Similarly *p*-CPA (a serotonin synthesis inhibitor) increased the duration of immobility time probably through inhibition of serotonin synthesis. Further, baclofen being $GABA_B$ agonist showed increase in immobility time as it acts on $GABA_B$ receptors and increase the levels of $GABA_B$ in brain of mice. Boswellic acid per se significantly effects immobility period of animals pretreated with sulpiride, baclofen, prazosin and *p*-CPA, results obtained indicated that Boswellic acid produce antidepressant-like effects by interaction with $D_2$ receptors, $GABA_B$ receptors, α1-adrenoceptors, and serotonergic hence increasing the levels of dopamine (DA), norepinephrine (NE), and serotonin (5-HT) and decreasing the levels of $GABA_B$ in mice brain.

Serotonin/5-HT metabolize to 5-hydroxyindoleacetic acid (5-HIAA) in the presence of monoaminooxidase enzyme (MAO). So drugs like tricyclic antidepressants, monoamine oxidase inhibitors, Selective serotonin reuptake inhibitors (SSRI) which enhance the levels of monoamines have been used for treatment of depression [49]. Within CNS, MAO-A is expressed predominantly in noradrenergic neurons, while MAO-B is expressed in serotonergic and histaminergic neurons. MAO regulates the metabolic degradation of catecholamines, serotonin and other endogenous amines in CNS. Inhibition of this enzyme causes a reduction in metabolism and subsequent increase in the concentration of biogenic amines. MAO-A preferentially metabolize adrenaline, nor-adrenaline and serotonin. MAO-B metabolizes phenylethylamines. Dopamine is metabolized by both MAO-A and MAO-B. Experimentally, selective MAO-A inhibitors (clorgyline, moclobemide) are found to more effective in treating major depression than MAO-B inhibitors like selegiline [52]. Presently groups administered with Boswellic acid reduced MAO-A and MAO-B levels in the brain of mice and thus inhibits the metabolism of monoamines, particularly serotonin and noradrenaline. Further, its MAO-A and MAO-B inhibition activity was comparable to standard drug Imipramine.

Free radicals like hydroxyl radicals, superoxide anion, hydrogen peroxide, peroxynitrite and, produced during normal cellular metabolic functions, produce oxidative damages in brain [53]. The microsomal lipid peroxidation of polyunsaturated fatty acids (PUFA) produced malondialdehyde (MDA), lipid hydroperoxides. The restraint stress enhances the level of lipid peroxidation, hence increased Malondialdehyde, nitrite and significantly decreased reduced glutathione levels [54]. Several studies reveal Boswellic acid as anti-oxidant [20, 30, 55]. Proving its antioxidant effects, Boswellic acid potentially reduces oxidative stress, by altering malondialdehyde

(MDA), nitrite/nitrate and reduced glutathione (GSH) levels, when compared to normal control.

Chronic stress induced hyperactivity of HPA axis causes increased serum corticosterone level, which is supported by observations from other studies [56, 57]. Studies on stress mice have suggested that there is a close correlation between a stable remission of the clinical symptoms and a normalization of HPA regulation [58]. In present results Boswellic acid might show anti-depressant effects by reducing Chronic unpredictable mild stress induced hyperactivity of HPA axis and thus plasma corticosterone level in mice.

Thus, Boswellic acid a main chemical constituent of *Boswellia serrata* showed potential therapeutic values for the management of depressive disorders. Therefore Boswellic acid can be look forward for anti-depressant in clinical management of depression.

The present study concluded Boswellic acid as anti-depressant as suggested by the results obtained. Boswellic acid administration reduces immobility period in TST and FST models reverse Sucrose preference in CUMS group, when compared to normal control. Further, Boswellic acid effects were comparable to Imipramine, a standard anti-depressant drug. Anti- depressant activity shown by Boswellic acid was due to possible involvement of serotonergic, dopaminergic and adrenergic system, which were revealed by its action against prazosin, sulpiride, baclofen and *p*-CPA. There was significant decrease in brain MAO-A and B activity after the administration of Boswellic acid. Further, Boswellic acid decreased MDA levels and increased GSH levels in the brain of mice. Thus, Boswellic acid showed significant antidepressant-like activity in mice probably through positive modulation of monoaminergic system; decrease in GABAminergic activity and through its antioxidant potential.

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