CNS Depressant Activity of *Chakhwi* - A prepared food ingredient of Tribal people of Tripura

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***Abstract*: A popular food ingredient *Chakhwi* was prepared from the ash of *Melocanna baccifera* and evaluated for CNS depressant activity. The test sample was given orally and standard drug diazepam was administered intraperitoneally. The basal activity score of mice was measured before and after treatment of test sample & standard drug diazepam using actophotometer. The results indicated that the sample has significant CNS depressant effects compared to control group.**

***Keywords*: CNS depressant activity, *Melocanna baccifera, Chakhwi,* Diazepam, actophotometer**

INTRODUCTION

Depression is considered as common mental sickness which characters are depressed temper, disinterestedness, decrease of force, loss of interest about neighbouring, disturbances in sleep & appetite, slowing down of thoughts, a lowered rate of breathing, decrease of physical movement with conscious, melancholi, desperation, cheerlessness, unhappiness & poor attention [1,2]. (Hoskeri *et al*., 2011 & Suman *et al*., 2016).

It is an extensive psychiatric disorder. Depression is already considered to account as the 2nd largest emergence of world encumbrance of disorder after cardiac disease in 2020 (Sultana *et al*., 2018)

Though there are available CNS depressant & antidepressant medicines, still depression is a serious matter at modern age. It was reported that, angiotensin-converting enzyme(ACE) inhibitor like perindopril & captopril possess antidepressant effect on experimental laboratory animals(Hoskeri *et al*., 2011 & Suman *et al*., 2016)[1,2].

It is reported that recently 121 million human beings are suffering from depression (Sultana *et al*., 2018)[3]. Oxidative stress is also responsible for the pathophysiology of depression. It occurs generally in early adulthood life in people who have lower neurotransmitter like monoamine neurotransmitters (Sultana *et al*., 2018)[3]. Inspite of development of a number of new elements for treatment of depression, unfortunately so many patients remain untreated & undiagnosed.

Use of herbal medicine for the treatment of different disorders is going on from the human civilization. Synthetic medicines are not cheaper (Farhana *et al*., 2014)[4]. Now worldwide different plant origin products are easily available. Different medicinal plants are using as herbal medicines with slight side effects for the treatment of human disorder both CNS anti-depressant & depressant effect[1] (Hoskeri *et al*., 2011). These plant origin drugs are using for centuries beyond any unwanted side effects. "It is therefore necessary that efforts should be made to introduce new medicinal plants to develop new medicines" (Farhana *et al*., 2014)[4]. Medicinal plant is believed to be a major source of novel herbal drug (Farhana *et al*., 2014). These plants are using in different countries for treatment of different disorders[4].

Medicinal plants are using for the treatment of various pathological disorders as well as depression over years. Plant based medicines are invariably of single plant extract or mixture of extracts or fractions of several plants (Anandarajagopal *et al*., 2011)[5]. Herbal drugs possess least side effects when compared to synthetical drugs. At present, traditional drugs are being reevaluated by widespread research on several plants & their bioactive compounds globally. The abundant property of natural kingdom may represent a unprecendented origin of modern elements with important medicinal activities. The important characteristic features of the plant based drug supposed to be its efficacy, minimum ambivalent effects & minimum cost (Sultana *et al*., 2018)[3].

In our present investigation, *Chakhwi* was prepared from burnt ash of *Melocanna baccifera*, also called *Muia* in Kokborok, local language, third official language (both young and matured) were screened to find out the CNS depressant effect using actophotometer.

In Tripura, one popular food ingredient of Tribal people of Tripura is *Chakhwi.* They prepare many more dishes mixing with *Chakhwi* along with other vegetables. Tribals consider this type of food as an easily digested food, which does not produce any disturbances in stomach. They also believe that this *Chakhwi* with *Muia* acts as an antihelmintic and as washing agent of bowel[6,7]. Tribal people of Tripura believe that *Muia* has certain medicinal importances. They are utilizing *Muia* for various medicinal purposes e.g. the Tabashir obtained from *Bambusa arundinacea* is largly used as cooling tonic. It is also useful in cough, asthma and paralytic complaints[6,7]. Antimicrobial activity of methanolic fruit extract of *Melocanna baccifera* was reported by Kuddus *et al*., 2013)[8]. Antimicrobial activity of *Chakhwi* of *Melocanna baccifera* was also reported[9]. The food ingredient also exhibited significant analgesic activity (Uma *et al*., 2015)[10]. Antidiabetic activity of this food ingredient was also reported in STZ-induced diabetic rats (Bhaumik *et al*., 2019)[11].

METHODS & MATERIALS

The plants *Melocanna baccifera* were identified by Prof. B. K. Datta, Dept. of Botany, Tripura University. The animals were identified by Prof. Sukanta Banik, Dept. of Zoology, and Tripura University.

PREPARATION OF *CHAKHWI* (SAMPLE-2)[6, 7]:- To prepare *Chakhwi*, dry stem and shoots of selected bamboo(only from *Melocanna baccifera*) was allowed to burn. The burnt ash was taken in a basket which was hanged from a suitable support. A

container was kept below it to collect the extract of ashes. The water was poured slowly on ash and the ash extract trickled in the container, was known as *Chakhwi*.

Chakhwi was also allowed to evaporate under very low flame to obtain powder like substances from the bottom of the container which was treated as sample.

PHYTO-CHEMICAL STUDIES:- The physic-chemical properties such as colour observation on naked eyes, pH by pH meter and density by electric single pan balance were measured. Specific gravity of *Chakhwi* was calculated. The results obtained are furnished in Table-1.

Qualitative Phytochemical Tests:- Tests for organic components like alkaloid (by Dragendroff's test), fixed oil, tannin, saponin, carbohydrate (Molisch's test & Fehling's test**),** protein(Biuret test, Xanthoprotein test & Millon's test), fats (test for fats, Acrolein test), glycosides (Keller-Killiani test**)**, flavonoids and triterpinoids (Liebermann-Burchard's test) of the test sample was performed[12-15].

Qualitative Chemical Tests[16-18]:- Tests for inorganic components like chloride, calcium, phosphate, potassium, sodium, magnesium (Ammonium phosphate test), copper (Ammonia test, Ammonium hydroxide (NH_4OH test), tests for carbonate, bicarbonate and nitrate were also carried out. The results obtained are depicted in Table- 2.

ACUTE TOXICITY STUDY[19, 20]

The acute toxicity for *Chakhwi* was determined in Albino swiss mice, following the OECD Guideline[no. 423, Annexure 2d] method of Committee for the purpose of Control and Supervision of Experiments on Animals(CPCSEA)"(Veerarghavan P., 2001 & Deb *et al*., 2012). The mortality of treated animals has observed after oral administration of test samples at 2000mg/kgbw. The experiment was performed taking 3 animals.

The presence or absence of any signs of toxicity or mortality was monitored at 2000mg/kgbw in all cases. Usual aftereffect such as moderate diarrhoea, atrabilious and decrease of the weight of treated mice were checked within 7days watching[19, 20].

DOSE DEPENDENT STUDY

Gross Behavioural Study[21]:-The animals were observed for gross behaviours such as hyperactivity, piloerection, sedation, loss of traction, analgesia, abnormal secretion etc along with allergic reaction (skin rash, itching) for the next 24 hours in respect to normal animals for the sample with 50mg/kgbw, 100mg/kgbw & 150mg/kgbw dose taking 03 animals for each dose.

SCREENING OF CNS DEPRESSANT ACTIVIT [22,23]

Adult healthy albino swiss mice weighing between 18-30gm were selected for the experiment. These were fasted overnight. Animals were divided into 3 groups of six animals. The first group of four comprised the control and the remaining 2 groups were administered with standard and test drug. The test doses were prepared in sterile water to get the desired concentration of the extract. Each mouse was placed individually in the actophotometer for 10 minutes. The basal activity scores was obtained. Each mouse of control group was given vehicle (1ml water/kg bw). After 30 min the record was taken. Mice of group II were administered the standard drug Diazepam (4 mg/kg bw, i,p). Mice of group III were administered *Chakhwi* (150 mg/kg bw, p. o). After 30 min. the mice were placed again in the actophotometer for recording the activity score. The results found are presented in Table -3 as Mean±SEM.

RESULTS AND OBSERVATION

Acute Toxicity Study and Dose

Acute oral toxicity was carried out accordingly by following OECD guideline no. 423, Annexure – 2d) adopted by CPCSEA, Govt. of India. In this study, *Chakhwi* did not show any mortality or signs of toxicity at the dose level of 2000mg/kgbw. Therefore, 2000mg/kgbw dose was considered as ALD50 cut off the dose under GHS 5 (safe dose), described in OECD Guideline(2d).

Dose Dependent Study(Gross Behaviour Study):- Change of gross behaviour like sedation and analgesia of mice were first observed at 150mg/kgbw dose for the sample. **Thus, 150mg/kgbw dose (minimum) was selected & applied in screening of the pharmacological property.** As the dose 2000mg/kgbw was well tolerated without producing any signs of toxicity & mortality, hence the dose may be selected as $1/10^{th}$ or $1/20^{th}$ of the maximum tolerated dose. (as per OECD guidelines 2d). Generally $1/10^{th}$ or $1/20^{th}$ of the cut of dose was used as therapeutic dose in any in vivo experiments. (Paliwal *et al*., 2017)[24]. We selected the dose as 150mg/kgbw.

Table-1: Physico-Chemical Data

Sample	Colour	pH	Density(gm/cm3)	Specific gravity
Chakhwi	colourless	8.23	1.1	1.103

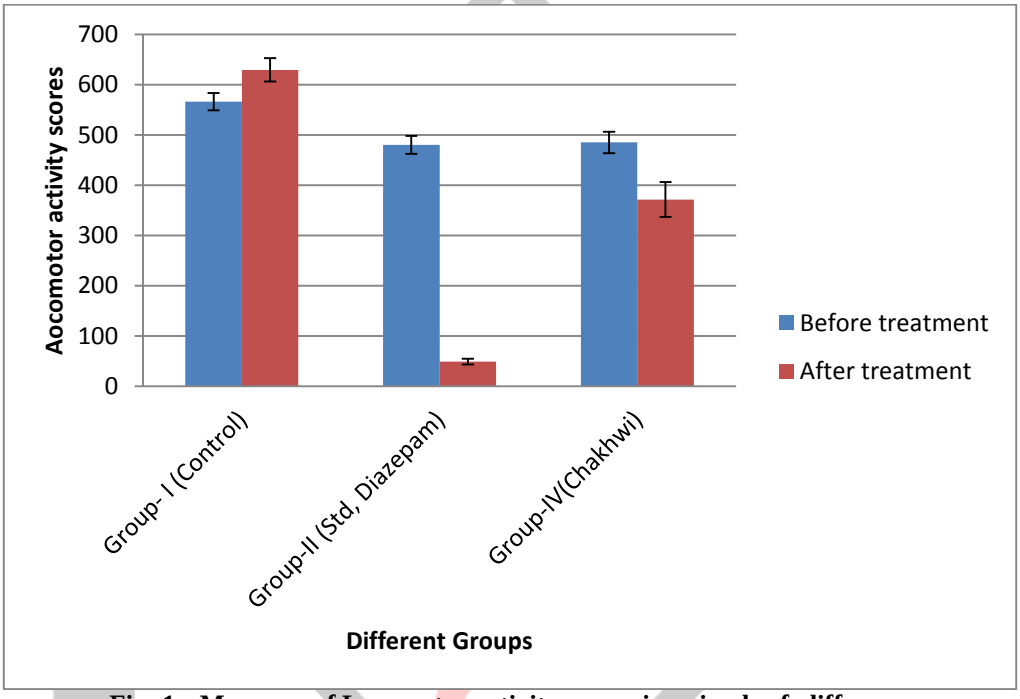
Table-2: The Results of Qualitative Phytochemical Tests

Samples	Organic Constituents present	Inorganic Constituents present
Chakhwi	Absence of mentioned organic constituents	Chloride, phosphate, calcium, phosphorus, magnesium, sodium & copper.

Table-3: Locomoor Activity Scores

Group & Treatment Compound	Dose	Locomotor Activity (Score) Before Treatment Mean±SEM	Locomotor Activity (Score) After Treatment (30min.) Mean±SEM
Group-I Normal Control(NC)	Vehicle 1ml/kgbw,p.o	566.17±16.964	629.33±23.156
Group-II Standard drug	Diazepam 4mg/kgbw,i.p	480.17±17.917	049.00±5.550***
Group-III *Chakhwi*	150mg/kgbw,p.o.	485.0±21.521	371.50±34.586***

Statistical analysis-[Averages values of raw data were expressed as a Mean ± Standard Error Mean (SEM), n=6, For numerical results, one-way analyses of variance (ANOVA) with Tukey-Compare all pairs of columns post tests were performed using GraphPad InStat Version 3 (GraphPad Software). The minimum value of $p<0.05$ was considered as significant. *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared to Group-1 (control) results.]



. Fig- 1:- Mean no. of Locomotor activity scores in animals of diff. group

CNS Depressant Activity:

The central nervous system (CNS) depressant property of test sample was studied using locomotor activity of mice in actophotometer. The locomotor behavioural score of individual animals were recorded for the period of 10 minutes. In the locomotor behavioural study showed that the treatment of *Chakhwi* (371.50 ± 34.586) at the dose of 150mg/kgbw have significant (***$p<0.001$) CNS depressant effects in the animals under investigation in comparison to vehicle treated (566.17 ± 16.964) normal control animals. The results of Diazepam (19.33± 2.171) treated (Standard drug) animals were also significant (***$p<0.001$) in comparison to vehicle treated normal control animals and Diazepam exhibited comparatively more CNS depressant effect than *Chakhwi* treated groups (Table-3).

DISCUSSION

On administration of the, *Chakhwi* and Diazepam it has been observed that the number of locomotor activity (scores) reduced in all cases. This activity is considered as an index of alertness (Sultana *et al.*, 2018)[3]. Decrease of locomotor activity is an indication of CNS depressant activity. In the CNS, GABA is a major inhibitory neurotransmitter. It is secreted by nerve endings (GABAergic neurones). GABA is synthesized from Glutamic acid by the enzyme called GAD(Glutamic acid decarboxylase) in the cerebral cortex, retina, spinal cord, cerebellum, corpus striatum, basal ganglia . The α-COOH group of glutamic acid is removed by GAD which bears PLP as prosthetic group. It is claimed that wavy shaped presynaptic terminals are inhibitory in nature. GABA binds to specific heterooligomeric glycoprotein called gamma amino butyric acid receptor on the membrane of post synaptic neurone. There are 5 subunits in the receptor-two α (alpha), two β (beta) & one γ (gamma) subunits and these subunits are arranged around the ligand gated chloride channel. Binding of gamma amino butyric acid to its receptor opens the chloride channel (D. Das, 2005 & C.C. Chatterjee, 1997)[25,26]. This neurotransmitter- receptor complex also opens ligand gated K^+-channel instead of Na^+-channel. As a result, K^+ comes out of the postsynaptic neurone to ECF & Cl^- enters in from ECF. The exit of K^+ & influx of Cl^- cause

more negativity inside, resulting hyperpolarisation. This hyperpolarised condition of the synapse inhibits neural transmission (P. Sembulingam, 2010)[27]. GABA is very much attached to physiological activities that are connected to psychologic & neurologic sickness such as hysteria, atrabilious, Parkinson disease & progressive disorder of brain, dementia. By the allosteric modifications of gamma-amino butyric acid receptor, GABA-system can be modified using several therapeutics at its synthesis level by initiating the inhibition of GABA mediated post synaptic membrane. It increases chloride conductance directly(Sultana *et al.*, 2018)[3]. Binding with its receptor, GABA regulates the excitability of nerve fiber. Its binding causes a conformational changes leading to opening of chloride channel. Diazepam binds to the allosteric site of $GABA_A$-receptor(at the interface between α(Alpha) & γ(Gamma) subunits of the receptor). This binding increases the affinity of GABA for its receptor. Thus, Benzodiazepines work as +ve allosteric modulator. Diazepam-receptor complex enhances the total chloride conductance(Nutt *et al*., 2001, Dhaliwal *et al*., 2020,Elisabet, 2018)[28,29,30].

In magnesium deficiency, the action of excitatory neurotransmitter increases & the actions of inhibitory neurotransmitter decreases (Seaman *et al*., 2003)[31].

Magnesium of ECF decreases the liberation of neurotransmitter by preventing the opening of calcium channel in presynaptic membrane. As a result, influx of calcium into presynaptic neurone stops. Thus, the locomotor activity reduces (Vink *et al*., 2011)[32].

So, it is predictable that the food ingredient *Chakhwi* ‘‘may act by potentiating GABAergic inhibition in the CNS via membrane hyperpolarisation leading to a reduction in the firing rate of critical neurones in the brain’’(Sultana *et al.*, 2018)[3]. Resulting reduction in locomotor activity. Standard drug Diazepam is a CNS depressing drug. It decreases the physical activity scores in compare to test groups and control. When compared the test group with control group, it has been observed that the locomotor activity decreases significantly.

CONCLUSION

In this experiment, it was observed that *Chakhwi* possesses significant CNS depressant affect, it may said that presence of magnesium might be responsible for CNS depressing action. Further extensive investigation will confirm their potency with mode of action.

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