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## ORIGINAL ARTICLE

# Effect of Aqueous Fruit Extract of *Emblca Officinalis* on Haloperidol Induced Catalepsy in Albino Mice and

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### ABSTRACT

Neuroleptic drugs used in the treatment of schizophrenia and other affective disorders are known to produce extrapyramidal side effects. Catalepsy was induced by these drugs in animals and these have been used as models for the extrapyramidal side effects associated with antipsychotic agents in human beings. In the present study, we have attempted to evaluate the protective effect of the aqueous extract of the fruits of *Emblca officinalis* (EO) on haloperidol (1.0mg/kg intraperitoneal administration) induced catalepsy in mice by employing the standard bar test. Mice were allocated to seven groups, each group containing six animals. The effects of the test drug EO (0.8, 2.0 and 4.0mg/kg doses) and the standard drugs scopolamine (1.0mg/kg) and ondansetron (0.5 and 1.0mg/kg doses) were assessed after single and repeated dose administration for seven days, 30 minutes prior to the haloperidol. Mice were sacrificed on the seventh day and super oxide dismutase (SOD) activity in the brain tissue was estimated by using the Beauchamp and Fridovich method. A significant ( $P<0.001$ ) reduction in the cataleptic scores was observed in all the test drug treated groups as compared to the control, with maximum reduction in the dose 4.0mg/kg group. Similarly, the maximum reduction in SOD activity ( $P<0.01$ ) was observed in the dose 4.0mg/kg group. Our study suggests that EO has significantly reduced oxidative stress and the cataleptic score induced by haloperidol. It could be used to prevent drug- induced extrapyramidal side effects.

**Key words:** *Emblca officinalis*, haloperidol , catalepsy

**Key message:** Neuroleptics (antipsychotic drugs) are usually associated with extrapyramidal side effects. The mice were treated by standard drugs like scopolamine or centrally acting anticholinergics; but these have many side effects like dryness of mouth, blurred vision etc. *Emblca officinalis* is a commonly available fruit in India. It was the best alternative to treat the extrapyramidal side effects induced by neuroleptics.

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## Introduction:

Neuroleptics that are commonly used in the treatment of schizophrenia and other affective disorders [1] are often associated with distressing extrapyramidal side effects [2,3]. The phenomenon of cataleptic immobility induced in rodents by typical neuroleptics (eg. haloperidol) is a robust behavioural model to study the nigrostriatal function and its modulation by cholinergic, 5-hydroxytryptamine (5-HT, serotonergic), nitrgic and other neurotransmitter systems [4,5]. Haloperidol induced catalepsy (HIC) occurs due to the blockage of dopamine (D2) receptors and reduced dopaminergic transmission [6]. Enhanced stimulation of the intrinsic central cholinergic system has also been implicated in haloperidol induced catalepsy, as it has been reported to be enhanced and antagonised by cholinergic agonists and the blocker, atropine respectively [7]. Evidence also suggests that the central serotonergic system modulates nigrostriatal dopaminergic transmission and 5-HT<sub>3</sub> antagonists are reported to alleviate neuroleptic- induced catalepsy [4]. Hence, scopolamine (a known anticholinergic agent) and ondansetron (a known 5-HT<sub>3</sub> antagonist) have been used as standard drugs in this study to compare the anticataleptic effect of the test compound, EO.

*Emblca officinalis* Gaertn. (*Phyllanthus emblica* L.) belongs to the family Euphorbiaceae, popularly known as Amla, which is a common household remedy that finds its use in the Indian indigenous system of medicine against several ailments. Its fruits have been reported to possess expectorant, purgative, spasmolytic, antibacterial, hypoglycaemic [8], hepatoprotective [9] and hypolipidaemic [10] activities. The aqueous extract of the fruits of EO has been reported to have a cytoprotective activity against radiation and heavy metal induced toxicities [11]. It also antagonizes serotonin and acetylcholine induced contractions of oestrogenised rat

uterus [12]. These anticholinergic and antiserotonergic properties are important to treat neuroleptic induced catalepsy. EO fruits mainly contain tannins and vitamin C like substances in abundance and their chemical constituents include gallic acid, ellagic acid, emblicanin A and B, punigluconin and some 10-12 flavanoids [13]. The aqueous extract of the EO fruits contain 30.0% tannins and 10.0% Gallic acid (estimation and purity of active principle was done by the Quality Control Laboratory, M/s. Natural Remedies, Bangalore, lab reference no.0505211, dt.31-05-2005). Recent studies on antioxidant property of EO fruits in rat brain; by increasing super oxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) in rat brain; frontal cortex and striatum, concomitant with reduction in lipid peroxidation (LPO) [14].

From our laboratory, we have reported the anticataleptic activity of NR-ANX-C (a polyherbal product) on haloperidol induced catalepsy in mice [15]. *Emblca officinalis* is one of the components of NR-ANX-C. The evidence of the anticholinergic, antiserotonergic and antioxidant properties of EO prompted us to evaluate for its anti-cataleptic activity.

## MATERIALS AND METHODS

### Animals:

Adult male albino mice (weighing 25-30gm), bred in the central animal house of Kasturba Medical College, Mangalore, were used for the study. The animals were housed under standard 12hr: 12hr light/dark cycles and were provided with food and water *ad libitum*. The animals were acclimatized to laboratory conditions before testing. Each animal was used once. Experiments were performed between 10.00 and 16.00hrs. The experimental protocol was approved by the Institutional Animal Ethics Committee and the study was conducted according to the

Indian National Science Academy Guidelines for the use and care of experimental animals.

### Drugs and Dosage:

**The test drug:** the aqueous extract of the fruits of *Emblca officinalis* (dry powder supplied by Natural Remedies Pvt. Ltd., Bangalore). The fresh fruits of EO were collected locally after proper identification, they were air dried, powdered and extracted with double distilled water. The extract thus obtained was vacuum evaporated in order to make it into powder form to be redissolved in double distilled water. It is a brown to very dark brown coloured powder with a characteristic odour and taste and was stored in air tight containers which are protected from light. Phytochemical analysis was done for total tannins by a spectrophotometer and Gallic acid was tested for by HPLC.

The standard drugs, scopolamine (German Remedies Ltd., Mumbai) and ondansetron (Cipla Ltd., Mumbai) were suspended/dissolved in 1% gum acacia solution while haloperidol (RPG Life Sciences Ltd., Mumbai) was dissolved in distilled water. The treatments received by each group (each group consists of six animals, n=6) are shown in Table 1 and 2. The group was received 1% gum acacia (10ml/kg) served as control. 1% gum acacia, scopolamine (1.0mg/kg), ondansetron (0.5 and 1.0mg/kg) and EO (0.8, 2.0 and 4.0mg/kg) were given orally, whereas haloperidol was given intraperitoneally.

### Experimental design

#### Haloperidol Induced Catalepsy

**(HIC):** Catalepsy was induced with haloperidol (1.0mg/kg i.p.) and was assessed at 30 minute intervals until 120 minutes and at the end of 240 minutes by means of a standard bar test [16,17]. Haloperidol (1mg/kg i.p.) was chosen so that it could elicit and thus enable the detection of either attenuation or potentiation of the

phenomenon [6]. Catalepsy was assessed in terms of the time for which the mouse maintained an imposed position with both front limbs extended and resting on a 4 cms high wooden bar (1.0cm diameter). The end point of catalepsy was considered to occur when both front paws were removed from the bar or if the animal moved its head in an exploratory manner. A cut-off time of 1100 seconds was applied. Between determinations, the animals were returned to their individual home cages. All observations were made between 10.00 and 16.00 hrs in a quiet room at 23-25° C.

### Scoring method

If the animal maintained the imposed posture for at least 20 seconds, it was considered to be cataleptic and was given one point. For every additional 20 seconds for which the cataleptic posture was maintained, one extra point would be given. The animals were tested twice at 30 minute time intervals and only the greater duration of immobility was considered [16].

In the acute study, EO, scopolamine and ondansetron were administered only once 30 min prior to the haloperidol administration. In the chronic study, these drugs were administered once daily, 30 min prior to the haloperidol administration for seven days. Catalepsy was determined 30 min after haloperidol administration on the first and on the seventh day of treatment. The animals were then sacrificed by cervical dislocation and the SOD activity of the whole brain tissue was estimated by using the Beauchamp and Fridovich method [18].

### Assay of SOD

The assay of SOD was carried out, based on the SOD mediated inhibition of the reduction of nitroblue tetrazolium to blue formation by super oxide anions, as described by Beauchamp and Fridovich. The total protein present in the homogenate was estimated following the method described by Lowry et al. [19]. The units of SOD activity determined, were expressed in terms of milligrams of total protein (TP). The

brains isolated from the individual groups of mice were homogenized (20% w/v) in 10 mM phosphate buffer pH 7.8. The homogenate was subjected to centrifugation. 0.1 ml aliquot of the homogenate (1:10 dilution) was used for the assay.

**Statistical analysis**

For each group, mean± SEM was calculated and the data was analyzed by one way ANOVA, followed by Dunnet’s Multiple Comparison test. P values <0.05 was considered to be statistically significant.

**Results**

**Acute study [Table/Fig 1]**

In the acute phase of the study, at the end of 30 min, there was no significant decrease in the cataleptic scores with both the standard drugs and the test drug. However, from 60 min onwards after haloperidol administration, scopolamine (1.0 mg/kg) and ondansetron (0.5 and 1.0 mg/kg) lowered cataleptic scores significantly (P<0.001) than the vehicle treated groups in mice. On the other hand, at 2.0 mg/kg and 4.0mg/kg doses, EO showed significantly (P<0.001) lowered cataleptic scores than the vehicle-treated group in a dose and time dependent manner. At doses 2.0 and 4.0mg/kg, EO was more protective against haloperidol induced catalepsy than standard drugs.

Table/Fig 1. Effect of acute administration of *Emblca officinalis* on haloperidol induced catalepsy.

Time after haloperidol (min)	Control vehicle (10 mg/kg)	Scopolamine (1.0mg/kg)	Ondansetron (mg/kg)		<i>Emblca officinalis</i> (mg/kg)			F	df
			0.5	1	0.8	0.2	0.4		
30	13.6±1.43	12.26±1.27	13.66±1.25	12.63±0.76	14.68±1.17	13.08±0.44	13.58±1.64	3.42	6,35
60	26.7±1.26	19.07±1.60**	20.20±0.85**	19.16±1.25**	24.58±0.84	23.83±1.24	19.33±2.62**	13.66	6,35
90	27.4±1.64	26.95±1.16***	27.03±0.95***	26.83±0.69***	29.33±0.95**	27.95±1.15**	24.58±2.36**	17.05	6,35
120	44.8±3.26	28.37±1.35***	30.83±0.70***	34.83±0.70***	35.66±1.82**	29.16±0.87***	26.68±4.79***	24.79	6,35
240	54.6±1.37	29.36±1.47***	36.64±1.81***	42.66±1.81***	40.58±0.87***	26.50±0.56***	25.18±3.79***	81.35	6,35

(All values are Mean±SEM, (n=6). Statistical analysis by One-way ANOVA followed by Dunnet’s Multiple Comparison Test. \*P<0.05 \*\*P<0.01 & \*\*\*P<0.001)

**Chronic study [Table/Fig 2]**

In the chronic phase of the study, administration of the standard drugs and all doses of the test drug 30 min after the last haloperidol dose on the seventh day, gave cataleptic scores similar (except ondansetran 1.0mg/kg and EO 4.0 mg/kg) to that of the vehicle-treated group. However, 60 min

after haloperidol administration, scopolamine (1.0 mg/kg), ondansetron (0.5 and 1.0 mg/kg), and EO (0.8, 2.0 and 4.0 mg/kg) showed significantly (P<0.001) lower cataleptic scores than the vehicle-treated group in a dose and time dependent manner.

Table/Fig 2. Effect of chronic administration of *Emblca officinalis* on haloperidol induced catalepsy.

Time after haloperidol	Control vehicle (10 mg/kg)	Scopolamine (1.0mg/kg)	Ondansetron (mg/kg)		<i>Emblca officinalis</i> (mg/kg)			F	df
			0.5	1.0	0.3	2.0	4.0		
30	15.13±1.83	15.46±0.57	15.59±0.68	12.29±0.43**	14.15±0.47	13.66±3.71	12.10±0.47*	5.59	6,35
60	20.34±1.56	17.33±1.26*	17.26±0.31*	15.57±0.16**	17.83±0.40*	16.33±3.49*	14.56±0.76**	8.54	6,35
90	29.33±0.84	28.34±0.70**	22.37±0.61**	21.63±0.76**	23.33±0.49**	21.80±2.57**	20.59±0.61**	22.32	6,35
120	37.95±1.76	21.95±0.59**	27.58±0.62**	22.08±0.33**	23.33±0.59**	20.33±3.69**	19.16±0.61**	98.1	6,35
240	49.26±1.56	23.54±0.55***	25.16±0.30**	24.00±0.63***	22.66±0.42**	21.60±3.42**	20.33±0.41***	207.68	6,35

(All values are Mean±SEM, (n=6). Statistical analysis by One-way ANOVA followed by Dunnet’s Multiple Comparison Test. \*P<0.05 \*\*P<0.01 & \*\*\*P<0.001)

**SOD activity:**

The SOD activity (Table.3) in brain tissue was also found to be elevated from the normal values (5.51) in the vehicle treated group (9.45). Maximum reduction in SOD values was seen in EO at 4.0mg/kg (6.27) (P<0.01) than the standard drug scopolamine 6.58 (P<0.01) and moderate reduction was seen at EO 0.8mg/kg (P<0.05) as compared to normal SOD values. The SOD activity in the brain of the mice treated with haloperidol and the test drugs are shown in Table 3. The SOD activity in the brain was found to be elevated in the haloperidol treated group and the observed increase was about 80% above the normal values.

Table/Fig 3: Effect of EO on SOD activity in brain of albino mice

GROUPS	SOD Activity (Units / mg protein)
I - Normal	5.51± 0.48
II - Control	9.45 ± 0.48
III - Scopolamine 1mg/kg	6.58 ± 0.8 ***
IV - EO 0.8mg/kg	7.89 ± 0.31 *
V - EO 2.0mg/kg	7.76± 0.79 *
VI - EO 4.0mg/kg	6.27 ± 0.91 ***

(Values are given as mean± S.E.M. (n=6), \*P<0.05, \*\*P<0.01 & \*\*\*P<0.001 as compared to normal; df =6, 35)

## Discussion:

Typical neuroleptic agents like chlorpromazine, haloperidol and reserpine induce a cataleptic state in rodents and these are being used as models to test the extrapyramidal side effects involved with it. Neuroleptic induced catalepsy has been linked to a blockade of postsynaptic striatal dopamine D1 and D2 receptors [20]. Despite this evidence, several other neurotransmitters such as acetylcholine, serotonin, angiotensin, adenosine, or opioids have also been implicated [21]. In addition to implications of various neurotransmitters in catalepsy, many preclinical and clinical studies have proposed reactive oxygen species in haloperidol induced toxicity [22]. Evidence indicates that drugs which potentiate or attenuate neuroleptic catalepsy in rodents might aggravate or reduce the extrapyramidal signs respectively, in human beings [23].

In the present study, EO was found to effectively reduce the HIC and it is comparable to that produced by the standard drugs, scopolamine and ondansetron. The protective effect of EO against HIC was consistent with our earlier report on the anticataleptic effects of a polyherbal product NR-ANX-C [15], in which EO is one of the components. EO has been found to significantly decrease the SOD levels in rat brain; this suggested that it shows significant antioxidant properties in rat brain. Earlier neuropharmacological studies with EO in rodents have suggested that it has anticholinergic and sedative properties [7,24]. Recent investigations have shown that the tannoid principles of EO comprising emblicanin A and B, punigluconin and pedunculagin, have significant peroxidase antioxidant effects in rat brain frontal cortex and striatum [25]. The super oxide dismutase enzyme (SOD) is a major factor in oxygen toxicity and the SOD enzyme constitutes an essential defence against it. In the presence of a free radical quenching agent, the induction of the antioxidant enzyme is minimised. It is well established that the administration of haloperidol leads to an increase in the oxidative stress in the

brain tissue. The increase in SOD observed in the present study supports the above concept. This study reveals that the EO treated groups significantly reduce ( $P < 0.001$ ) both oxidative stress and the catalepsy score induced by haloperidol.

EO fruits are a rich source of vitamin C which was held responsible for its major biological actions including antioxidant properties. However, more recent investigations have indicated that vitamin C alone was not responsible for its antioxidant potential [26]. The anticataleptic effects of EO might be due to both its anticholinergic and antioxidant properties. Based on our results, we suggest the EO can be used as an alternative/adjuvant drug in preventing and treating the extrapyramidal side effects of antipsychotic agents in clinical practice. It was shown that in addition to vitamin C, other polyphenols like tannins and gallic acid may contribute to its effectiveness to reduce oxidative stress and catalepsy. Further studies may help to elucidate the possible mechanisms of action of *Emblca officinalis*.

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