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Research Article

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Protective effect of *Eclipta alba* on haloperidol induced extrapyramidal movement disorders in albino rats

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ABSTRACT

Extrapyramidal movement disorders are major neurodegenerative disorders caused due to dopamine deficiency in the basal ganglia. It may be caused due to oxidative stress producing free radicals that destroys dopamine producing neurons. Catalepsy, Tardive dyskinesia etc are the symptoms of certain nervous disorders such as Parkinson's disease & epilepsy in animal models. Phytochemical analysis of Eclipta alba (Bhringaraj) shows that it contains urosolic acid having antioxidant properties. The present study was undertaken to evaluate the possible protective effect of Eclipta alba leaf extract (EALE) on oxidative stress & haloperidol induced extrapyramidal symptoms. Male wistar albino rats (150-200gms) were used for the experiments. Catalepsy was induced with haloperidol (1mg/kg IP) using block method & was measured by standard procedure of Zazpe etal 2006. Effect of EALE in a dose of 100,200,400mg/kg on haloperidol induced catalepsy &vacuous chewing movements (VCM) were measured and compared with the standard drug alpha tocopherol. The effect of EALE on oxidative stress was done by estimating the catalase & glutathione activity. Analysis was done by suitable statistical methods. The cataleptic score & VCM were significantly reduced with all the three doses of EALE. There was significant reduction of catalase activity whereas increase in glutathione activity with EALE. The antioxidant properties of Eclipta alba reduced the duration of catalepsy, number of VCM, decreased the elevated levels of lipid peroxidation in the haloperidol treatment groups & elevated the cellular defense mechanism such as glutathione that proves its anticataleptic activity.

Key words: Catalepsy, Eclipta alba, Haloperidol, Oxidative stress, VCM, Cataleptic score

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INTRODUCTION

Worldwide, researchers have come to know that catalepsy does not appear of its own accord; instead, that it often manifests as a one in a constellation of the symptoms which are caused by psychotic disorders. [1, 2]

Haloperidol is a typical antipsychotic drug which is used in the treatment of schizophrenia and other affective disorders. But, haloperidol causes movement disorders such as the neuroleptics malignant syndrome, dystonia and tardive dyskinesia. Oxidative stress, which is a culprit in many human diseases, has been implicated in haloperidol toxicity. Extra pyramidal symptoms (EPSs), including pseudo Parkinsonism, occur as distressing side effects in 20-75% of the patients who are administered typical anti-psychotics. The blockade on the dopamine receptor which is caused by haloperidol, increases the dopamine turnover in humans and rats. [3, 4, 5]

It has been proposed that haloperidol induced oxidative stress arises from the generation of free radical from catecholamine metabolism by monoamine oxidase (MAOs). The acute and chronic administration of haloperidol in animal models such as mice resulted in the generation of significant oxidative stress in their brain regions, as was evidenced by loss of the non protein thiol anti-oxidant glutathione (GSH) and by the increase of the lipid peroxidation product.[6,7].

Since haloperidol induced catalepsy has an underlying pathology of increased oxidative stress, the present study was designed to evaluate the effect of *Eclipta alba* (L.) (*E. alba*) on haloperidol induced catalepsy. Alfa-tocopherol was used as a standard drug.

Eclipta alba (L.) (*E. alba*) is commonly known as false daisy or Bhringaraja. It belongs to the Family Asteraceae and is widely distributed throughout India, China, Thailand and Brazil. The use of plant products for the treatment of human ailments has been a natural approach to health care since the beginning of civilization. It is reported to have antioxidant, antipyretic, anti-inflammatory, antihistaminic, hepatoprotective, expectorant properties. [8, 9]

EXPERIMENTAL SECTION

A pathogen free male albino rats weighing 150-200gms were used in our study. In order to eliminate sex-related difference in effects of test compounds, this study was carried out in male animals. Test (n=6) & control (n=6) groups were chosen randomly. The animals were housed in cages at room temperature with 12/12 hrs light and darkness and were fed with a normal chow diet and tap water.

PREPARATION OF ECLIPTA ALBA LEAF EXTRACT (EALE)

Leaves were collected and shade dried. They were crushed into coarse powder and extracted with 90% ethanol using soxhlet's apparatus for 24 hrs. The extract was concentrated under pressure and then dried in air. The concentrated ethanolic extract was suspended in Tween 80. Freshly prepared solution was used for each experiment.

Drugs: From a pilot study it was indicated that Ethanolic extract of Eclipta alba was the most effective among all other extract. Haloperidol from Sigma alchid and Alfa-tocopherol from Vitabase were obtained in pure powder form.

Study protocol:

The study protocol design was approved by Institutional Animal Ethical Committee (Table no 1). The treatment was as follows Group I: Control group (1% v/v of tween 80), Group II: Haloperidol (1mg/kg)IP, Group III: Haloperidol (1mg / kg)IP+ EALE (Eclipta alba leaf extract) (100mg/kg) PO Group IV: Haloperidol (1mg / kg) IP+ EALE (200mg/kg) PO Group V: Haloperidol (1mg / kg)IP + EALE (400mg/kg) PO Group VI: Haloperidol (1mg / kg)IP + Alphatocopherol (10mg/kg)PO

Table 1: Study protocol design of the study

Group	Treatment	Remark
I	(1% v/v of tween 80)	Control
II	Haloperidol (1mg/kg)IP	Inducing group
III	Haloperidol (1mg / kg)IP+ EALE (100mg/kg)PO	TEST
IV	Haloperidol (1mg / kg) IP+ EALE (200mg/kg)PO	TEST
V	Haloperidol (1mg / kg)IP + EALE (400mg/kg)PO	TEST
VI	Haloperidol (1mg / kg)IP + Alphatocopherol (10mg/kg)PO	STANDARD

Acute toxicity study

Rats selected by a random sampling technique were used in the study. Acute oral toxicity was performed as per Organization for Economic Co-operation and Development (OECD)-423 guidelines. Three male Wistar rats weighing between150–200gms were used for each dose. The dose levels of 5mg, 50mg, 500mg, 1000mg, 2000mg, and 5000 mg/kg/body weight, per oral dose were selected. The lethal dose (LD)-50 value of the extract was determined. The drug was administered orally to rats, which were fasted overnight with water ad libitum before the administration of the drug. The body weight of the rat was noted before and after treatment. The animals were observed for toxic symptoms such as behavioral changes, locomotion, convulsions and mortality for 72 hours.

Experimental Design:
Group I: Control group received PO. 1% v/v aqueous solution of tween 80 between 9am to 10 am for 22days. Group II: Treated with haloperidol at a dose of 1mg/kg IP suspended in tween 80 between 9am to 10am for 22 days. Group III, IV, V were treated with haloperidol 1mg/kg IP daily for 21 days to induce adverse effects. On 22nd day EALE 100mg/kg, 200 mg/kg and 400 mg/kg, was suspended in 1% v/v solution of tween 80, was administered PO. After 30 minutes of administration of Eclipta alba leaf extract, haloperidol was injected IP 1mg/kg.. All the behavioral studies were performed at room temperature in a calm room without any external interference. After the 15 days, animals were sacrificed by cervical dislocation and the whole brain was immediately dissected out and washed in ice-cold saline to remove all traces of blood. The brains were weighed and a 10% tissue homogenate was prepared in 0.025 M Tris–HCl buffer at pH 7.5 and used to measure the activities of thiobarbituric acid reactive substances (TBARS). Enzyme activity was assayed in 10% brain homogenates prepared in 0.2 M phosphate buffer, pH 8.0.

Determination of catalepsy:
The catalepsy was measured by standard procedures (Figure 1). 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 cm high bar (1cm 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 5 minutes was applied. All observations were made between 10.00 to 16.00hrs in a quiet room at about 30º C.

Figure 1: Showing catalepsy model

The catalepsy was measured by standard procedures Stage I: Rats were moving normally when placed on table, scored as 0. Stage II: Rats were moving normally when touched/pushed, scored as 0.5. Stage III: when front paws of rat were placed on 3cm high block and if it fails to correct the posture in 10 seconds, score for each paw is 0.5 (total 1). Stage IV: If rat fails to correct the posture with in 10seconds, when placed on 9 cm high block, score for each paw is 1 (total 2). Thus for single rat, maximum cataleptic score is 3.5. [10]

Determination of Tardive dyskinesia (TD):
Tardive Dyskinesia is referred to as Vacuous Chewing Movements (VCMS). On the test day, i.e, 22nd day, rats were placed individually on a table for the assessment of oral dyskinesia. In the present study, VCMs were referred to as single mouth openings in the vertical plane not directed toward a physical material. The VCMs were measured and counted for a period of 5minutes. If VCMs occurred during a period of grooming, they were not taken into the account. [11]

Estimation of antioxidants:
Catalase (CAT) activity was assayed calorimetrically at 620 nm and was expressed as micromoles of H_2O_2 consumed per minute per mg of protein; using the method described by Sinha. The reaction mixture (1.5 ml, volume) contained 1.0 ml of 0.01 M pH 7 phosphate buffer, 0.1 ml of tissue homogenate and 0.4 ml of 2 M H_2O_2. The reaction was stopped by the addition of 2 ml of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid mixed in the ratio of 1:3). The assay for SOD was based on SOD mediated inhibition of the

reduction of nitro blue tetrazolium to blue formazan by superoxide anions as described by Beauchamp and Fridovich. The total protein present in the homogenate was estimated following the method described by Lowry . Units of SOD activity determined were expressed in terms of milligrams of total protein (TP). Reduced glutathione (GSH) was determined by the method of Ellman. One ml of supernatant was treated with 0.5 ml of Ellman's reagent and 3 ml of phosphate buffer (0.2 M, pH 8.0). The absorbance was read at 412 nm. The activity of GSH was expressed as nM GSH formed/g tissue.[12,13]

Statistical analysis:

Each group of rats assigned to a specific drug treatment each group consisted of 6 animals. All the values are expressed as mean ± standard error of mean (SEM). The data were analyzed by analysis of variance (ANOVA) followed by Dunnet's multiple comparison test.

RESULTS AND DISCUSSION

The observation indicated that there was no death in 5000mg/kg dose after 72hr.

A. HALOPERIDOL INDUCED CATALEPSY MODEL(Table 2 & Fig 2):

Cataleptic score:

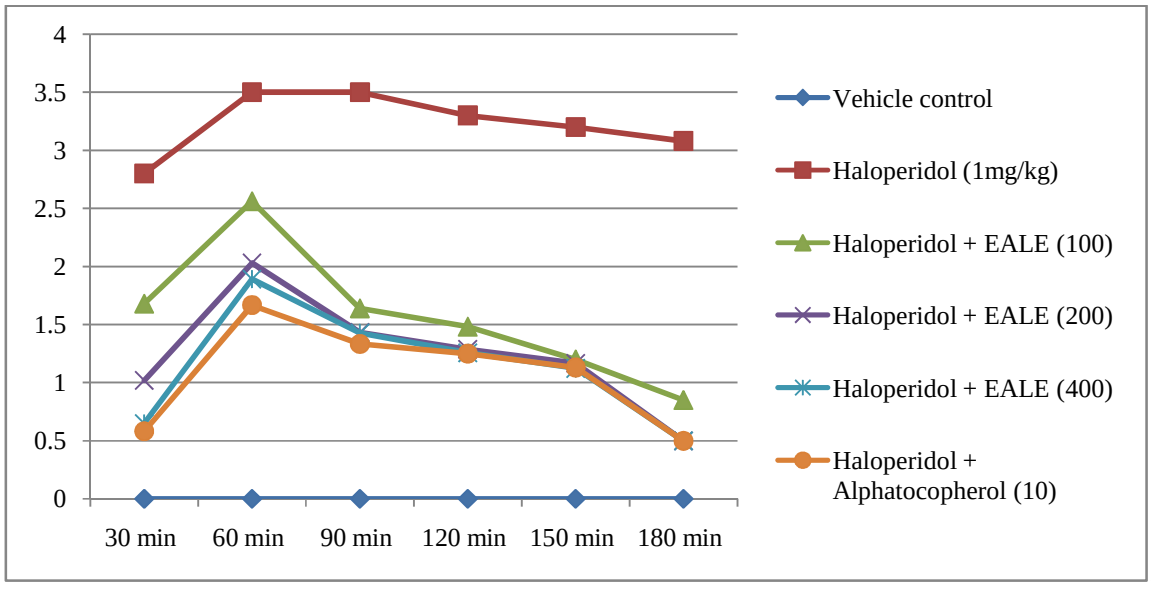
The cataleptic score was significantly reduced after 30 min, with both, the standard drug Alphatocopherol(10 mg/kg PO) and the test drug EALE in all the doses. The reduction in cataleptic scores with EALE was significant throughout the period of observations, till 180 min. The reduction in cataleptic score with the test drug was highly significant with 200 & 400mg/kg doses which was started from 120 min & continued up to 180min. The reduction of cataleptic score with Alphatocopherol(10 mg/kg PO) and EALE in all the dose groups were seen only after 30 min of observation.

Table 2: Effect of ethanol extract of *Eclipta alba* on haloperidol induced catalepsy in rats

Drug treatment	30 min	60 min	90 min	120 min	150 min	180 min
Vehicle control	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
Haloperidol (1mg/kg)	2.8± 0.0$^{\#\#}$	3.5±0.532$^{\#\#}$	3.5± 0.146$^{\#\#}$	3.3 ± 0.04$^{\#\#}$	3.2 ± 0.12$^{\#\#}$	3.08 ± 0.026$^{\#\#}$
Haloperidol + EALE(100)	1.68 ± 0.3256*	2.562 ± 0.4862*	1.64 ± 0.5468*	1.482 ± 0.0562*	1.20 ± 0.3165*	0.85 ± 0.247**
Haloperidol + EALE(200)	1.02 ±0.5632*	2.03 ±0.2682*	1.432 ±0.2023*	1.287 ±0.4321**	1.168 ±0.1562**	0.500 ±0.562**
Haloperidol + EALE(400)	0.65 ± 0.6213*	1.89 ± 0.3625*	1.426 ± 0.4321**	1.257 ±0.0642**	1.123 ± 0.0564**	0.500 ± 0.224**
Haloperidol+Alphatocopherol (10)	0.5833 ± 0.833*	1.667 ± 0.117**	1.333 ± 0.105**	1.250 ± 0.112**	1.13 ± 0.042**	0.5 ± 0.0**

*$^{\#\#}p<0.001$, * $p<0.05$ **$p<0.01$*

Fig 2: Effect of ethanol extract of *Eclipta alba* on haloperidol induced catalepsy in rats by block method



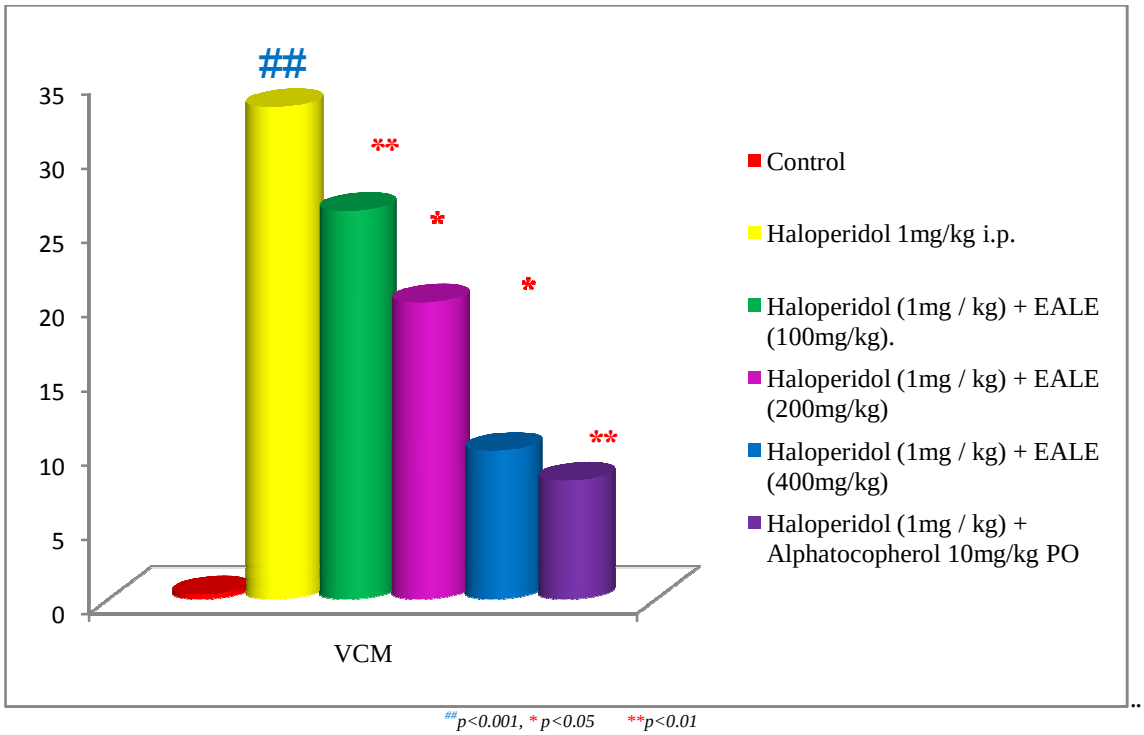
** $p<0.05$ **$p<0.01$ ***$p<0.001$*

B. HALOPERIDOL INDUCED DYSKINESIA

Assessment of orofacial dyskinesia (Fig-3):

Haloperidol (1mg/kg, ip.) treated animals shown increased frequencies of vacuous chewing movements and tongue protrusions compared with control. Treatment with Alphatocopherol(10 mg/kg PO) and EALE in all the dose groups of animals significantly reversed reserpine induced vacuous chewing movements and tongue protrusion in animals which was highly significant with the highest dose of the EALE (400mg/kg).

Fig 3: Effect of EALE on Haloperidol induced VCM in rats



*##p<0.001, * p<0.05 **p<0.01*

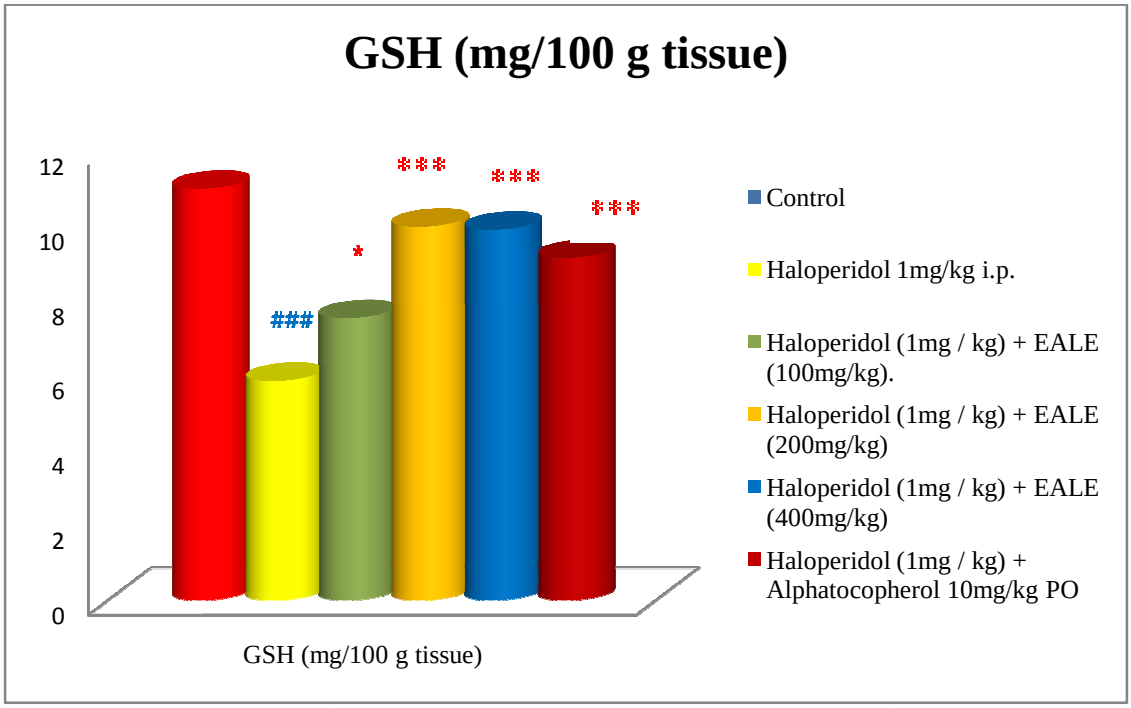
Fig 4: Effect of EALE on Haloperidol induced glutathione level in rats

C. Haloperidol induced neurodegeneration

Biochemical Estimation

(a). Measurement of Glutathione (GSH) level :(Fig: 4)

The Glutathione (GSH) level in rat brain is significantly ($p<0.001$) decreased by Haloperidol (1mg/kg, IP) in rats as compared to control. On administration of Alphatocopherol(10 mg/kg PO) there was increase in the level of glutathione which was highly significant. The level of glutathione was significantly increased in the all the doses of EALE. The increase in glutathione level was highly significant with the highest dose of EALE (400mg/kg) which was comparable with the standard drug Alphatocopherol.

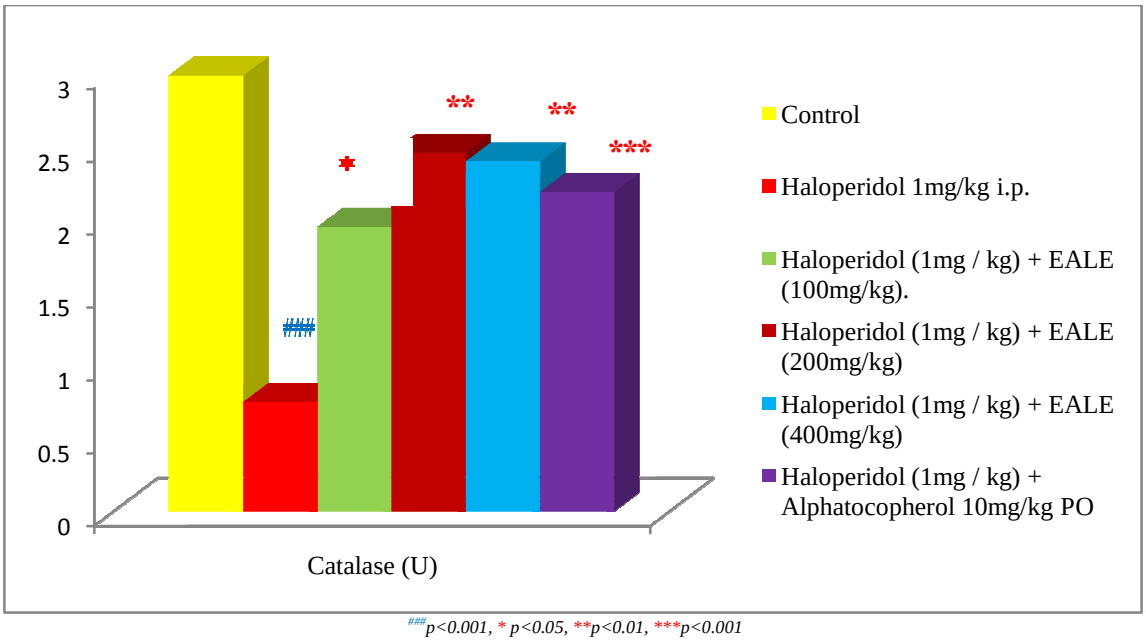


*###p<0.001, * p<0.05, ***p<0.001*

(b). Measurement of catalase level: (Fig: 5)

The Catalase level in rat brain is significantly (p<0.001) decreased by Haloperidol (1mg/kg, IP.) in rats as compared to control. On administration of Alphatocopherol(10 mg/kg PO) there was increase in the level of glutathione which was highly significant. The level of glutathione was significantly increased in the all the doses of EALE.

Fig 5: Effect of EALE on Haloperidol induced catalase level in rats:

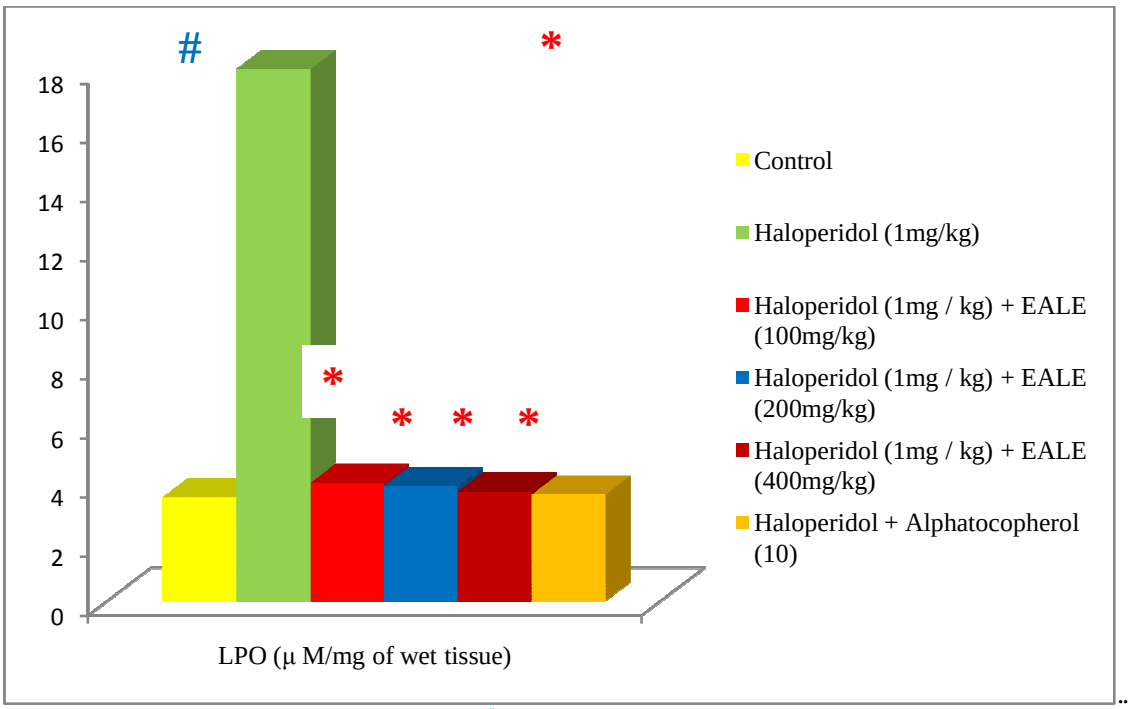


*###p<0.001, * p<0.05, **p<0.01, ***p<0.001*

(c). Measurement of lipid peroxidation :(Fig: 6)

There is a significant increase in lipid peroxidation in Haloperidol (1mg/kg, IP) administered rats as compared to control. On administration of Alphatocopherol(10 mg/kg PO) there was highly significant decrease in the level of lipid peroxidation product and the level of lipid peroxidation was significantly decreased in the all the doses of EALE.

Fig 6: Effect of EALE on Haloperidol induced lipid peroxidation level in rats



*#p<0.001, *p< 0.001*

Neuroleptics used commonly in the treatment of schizophrenia [14] are often associated with extrapyramidal side effects.[15, 16] For example, chronic treatment with haloperidol produced serious side effects such as Tardive dyskinesia (TD), catalepsy, acute dystonia. This occurs in 20-40% of the patient population. Haloperidol is the commonly used neuroleptic which has pro oxidant properties. Haloperidol is found to increase oxidative stress mediated neuronal damage in animals.[17] This neuronal damage by pro-oxidant actions of haloperidol suggests that TD is a result of haloperidol induced oxidative injury. Existing evidence indicates that an excessive production of free radicals is associated with chronic haloperidol use and might contribute to onset of TD.

Haloperidol act by blocking dopamine receptors,[4] this blockade increases catecholamine turnover, which leads to excessive production of free radicals, especially in catecholamine rich areas such as basal ganglia. Because of high oxidative metabolism in these regions, neurons are particularly vulnerable to membrane lipid peroxidation and cell death.

In the present study, Ethanolic extract of *Eclipta alba* protected the rats from catalepsy induced by haloperidol as effectively as the standard drug, Alphatocopherol. The compound identified from the leaves of *Eclipta alba*[18] contain ursolic acid, oleanolic acid which has antioxidant properties and gives remarkable protection against lipid peroxidation.

CONCLUSION

Since haloperidol induced catalepsy had an underlying pathology of increased oxidative stress and as *Eclipta alba* has anti oxidant property in the present study, the effect of *Eclipta alba* was evaluated in haloperidol induced cataleptic rat. The anti-oxidative properties of *Eclipta alba* shows neuro – protective effect by reducing the duration of the catalepsy, decreased the elevated levels of lipid peroxidation in the haloperidol treated animals and elevated

the cellular defense mechanisms such as glutathione, further suggesting the role of free radicals in the pathophysiology of the haloperidol induced extra-pyramidal syndrome. However, further studies would be necessary to evaluate the contribution of other substances for the activity showed as it still remains to be determined which components were exactly responsible for these effects.

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