EXPLORING THE ACTION OF COCOA COMPOUNDS ON DOPAMINERGIC PATHWAY IN HALOPERIDOL INDUCED PARKINSON

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Abstract

Parkinson's disease (PD) is a common neurodegenerative disorder, which is Characterized by slowly progressive loss of dopaminergic neurons associated with Substantial. The present study was aimed to explore the action of cocoa compound on Dopaminergic pathway in haloperidol induced Parkinson in rats. Thirty Albino Wister male Rats weighing 250-270 gm were randomly assigned to five groups, each group contain 6 animals. Group I Vehicle group Received normal saline orally for 14 days, Group II Negative control group- Received haloperidol 2.6mg/kg,i.p. Group III Standard group - Received Levadopa (4mg/kg/day), i.p. + haloperidol (2.6mg/kg/day)., Group IV treatment group Received haloperidol (2.6mg/kg/day) i.p. + ethanolic extract of theobroma cocoa of low of 100mg/kg and GROUP V received haloperidol (2.6mg/kg/day) i.p.+ ethanolic extract of Theobroma cocoa of high dose of 400mg/kg. Parkinson's disease (PD) was induced by intra peritoneal injection of haloperidol 2.6mg/kg, i.p daily for a period of 14 days. The ethanolic extract of theobroma cocoa was induced and evaluated against haloperidol by monitoring in vivo behavioral parameters like muscle coordination, cognitive performance, catalepsy activity, Biochemical estimation of SGPT, SGOT, ALP, Total biluribin, urea, creatinine and brain antioxidant levels, Acetylcholine, dopamine. Changes were confirmed by Histopathological studies. Treatment group shows significant increase the body weight, feed intake, Locomotion action, muscle coordination, cognitive performance and dopamine level, also decrease in muscle rigidity, oxidative stress and cholinergic over activity.The ethanolic extract of theobroma cocoa shows significantly anti Parkinson's activity against haloperidol induced PD rats with milder GI toxicity as compared to the standard treatment group.

INTRODUCTION

Neurodegenerative disorders (NDs) are primarily characterized by neuronal loss, with Alzheimer's and Parkinson's diseases (PD) being the most common. Although several approved drugs exist, most only manage associated symptoms. NDs affect millions worldwide, with aging as the major risk factor. However, genetic and environmental factors also contribute to ND development [1].

Neuroinflammation and neurodegeneration often result from aberrant deposition of aggregated host proteins (amyloid-β, α-synuclein, prion) that activate inflammasomes, which act as intracellular sensors of microbial and host-derived danger signals [1]. The pathological mechanisms of NDs have drawn increased attention due to the aging population, as immune

activation within the CNS is a hallmark of ischemia, neurodegeneration, immune-mediated disorders, infections, and trauma [2]. Parkinson's disease is a clinically recognizable, fast-growing neurodegenerative disorder with diverse causes and presentations. Its global rise resembles a pandemic, though non-infectious. About 3–5% of PD cases are monogenic, while 90 genetic risk variants collectively explain 16–36% of heritable non-monogenic PD risk [3]. PD significantly affects patients and caregivers through progressive mobility and muscle control loss. Motor symptoms (resting tremor, bradykinesia, rigidity) result from striatal dopaminergic neuron loss, while non-motor symptoms indicate neuronal loss in other regions. PD is primarily idiopathic but can also arise from secondary or drug-induced causes [4]. Conventional PD treatments include levodopa, dopamine agonists, anticholinergics, COMT inhibitors, MAO-B inhibitors, and amantadine. PD-related psychosis is managed with antipsychotics. Levodopa's action involves GI absorption, BBB crossing, neuronal uptake, enzymatic conversion to dopamine, and synaptic dopamine release [5]. Deep Brain Stimulation (DBS), involving electrode implantation in subcortical structures like the subthalamic nucleus and globus pallidus internus, improves dopamine-responsive motor symptoms when levodopa-induced side effects become problematic. However, dopamine-resistant symptoms (e.g., gait disturbance, postural instability) respond poorly [6]. All approved anti-PD drugs effectively reduce "off-time" in fluctuating PD but differ in adverse event profiles such as dyskinesia, hallucination, and orthostatic hypotension. Therefore, therapy must be individualized. While many RCTs assess anti-PD efficacy and safety, few compare them directly; Network Meta-Analysis (NMA) enables indirect comparisons across multiple treatments [7]. Cocoa's health benefits are mainly attributed to its high antioxidant content. However, its methylxanthine components, theobromine and caffeine, contribute to psychoactive effects—enhancing mood and alertness. Theobromine, present in higher amounts than caffeine, appears largely responsible for cocoa's positive effects [8].

DRUG PROFILE – HALOPERIDOL

Haloperidol (HLP) is a potent butyrophenone derivative antipsychotic commonly used in the treatment of schizophrenia and bipolar disorders. It is highly effective but associated with several adverse effects.

Chemical name: 4-[4-(p-chlorophenyl)-4-hydroxypiperidino]-4′-fluorobutyrophenone [9]

IUPAC name: [4-(4-chlorphenyl)-1-[4-(4-fluorophenyl)-4-oxybutyl]piperidin-4-yl

Molecular formula: $C_{21}H_{23}ClFNO_2$

Molecular weight: 375.9 g/mol

Description: White or almost white crystalline powder

Melting point: 151.5 °C

Solubility: Very slightly soluble in water; freely soluble in chloroform

Category: Antipsychotic drug

Mechanism of Action

Haloperidol, a first-generation (typical) neuroleptic drug, primarily blocks dopamine D_2 receptors in the brain. Optimal therapeutic efficacy occurs when approximately 72% of dopamine receptors are blocked. It also antagonizes noradrenergic, cholinergic, and histaminergic receptors, contributing to several side effects [10].

Chronic administration decreases endogenous dopamine release in the striatum and nucleus accumbens, as demonstrated by in vivo electrochemical studies [11].

Dose

Haloperidol is administered intraperitoneally at a dose of **2.6 mg/kg daily** for 14 days to all groups except the normal control, along with the test compound.

Pharmacokinetics

Absorption:

Well absorbed orally, but first-pass hepatic metabolism reduces bioavailability to 40–75%. Steady-state levels are reached after the third monthly injection, maintaining stable control of psychotic symptoms.

Distribution:

Approximately 89.6% plasma protein-bound; distributes rapidly between plasma and blood cells (cell/plasma ratio = 1.12). Uptake by blood cells is proportional to free drug concentration.

Metabolism:

Undergoes extensive hepatic metabolism, with only about 1% excreted unchanged. The main pathways include glucuronidation, reduction, and oxidation mediated by **CYP3A4**, producing HAL-1,2,3,6-tetrahydropyridine (HTP).

Excretion:

Eliminated slowly through urine (~30%) and feces (~20%) via biliary excretion. Only ~1% of the dose is excreted unchanged.

Pharmacodynamics

Haloperidol acts as a potent **dopamine D_2 receptor antagonist**, reducing dopaminergic transmission in the mesolimbic and mesocortical pathways—mechanisms responsible for its antipsychotic effects. It also has affinity for serotonin (5-HT), alpha-adrenergic, and histamine receptors, but its primary clinical effects are due to dopamine blockade.

Therapeutic Uses

Haloperidol is widely used in the treatment of psychotic disorders. The depot formulation, **haloperidol decanoate**, allows monthly administration to improve compliance and minimize side effects while maintaining clinical efficacy [12].

Adverse Effects

Common adverse effects include:

- Extrapyramidal symptoms (EPS)
- Tardive dyskinesia (TD)
- Neuroleptic malignant syndrome (NMS)
- Cardiovascular complications
- Sedation and cognitive impairment

Patients should be closely monitored, and dose adjustments or alternative medications may be required to minimize adverse outcomes.

Contraindications

Haloperidol should not be used in individuals with known hypersensitivity to the drug or any of its components. It is contraindicated in severe CNS depression, as it may further suppress central nervous system activity and worsen symptoms.

Drug Interactions

- **Antiepileptics (carbamazepine, phenytoin):** Increase metabolism, reducing efficacy.
- **Valproate:** Inhibits metabolism, increasing plasma concentration and risk of side effects.
- **Antihypertensives:** May enhance hypotensive effects; monitor blood pressure.
- **CNS depressants (alcohol, benzodiazepines, opioids):** Additive sedation and respiratory depression risk.

PLANT PROFILE

Botanical name - Theobroma cacao

Synonyms - chocolate tree

Common name - cacao, kacao.

Taxonomical Classification

Kingdom -plantae

Class -Tracheophytes (Vascular plants)

Clade - Angiosperms (Flowering plants)

Order - Malvales

Family - Malvaceae (formerly Sterculiaceae)

Genus -Theobroma

Species -Theobroma cacao

MATERIALS AND METHODS

Extraction of Plant Material

Preparation of Ethanolic Extract of Theobroma cacao (Wild) Seeds by Soxhlet Method

Seeds of Theobroma cacao (wild) were collected, washed thoroughly to remove impurities, and shade-dried for 7 days at room temperature. The dried seeds were coarsely powdered using a mechanical grinder. The powdered material was subjected to Soxhlet extraction using 100% ethanol (70:30 ratio) for 3 days. After extraction, the filtrate was passed through Whatman No.1 filter paper and concentrated using a rotary evaporator. The final dried extract was stored in an airtight container for further studies.

Ethanolic extract of Theobroma cacao seed powder by Soxhlet method

Drugs and Chemicals - All chemicals used were of analytical grade.

Drugs used:

Haloperidol

Levodopa

Selection of Animals

Healthy adult male Wistar rats (270–350 g) and female Swiss albino mice (25–30 g) were used. Animals were housed in polypropylene cages under standard laboratory conditions (25 ± 2°C; 50 ± 15% humidity; 12 h light/dark cycle) with free access to standard pellet diet and water.

All experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC) of Swamy Vivekanandha College of Pharmacy and performed in accordance with CPCSEA guidelines.IAEC Approval No.: SVCP/IAEC/PG/2/05/2024

Animal Grouping

Group	Treatment	Animals
I	**Normal control – 0.5 ml normal saline orally for 14 days**	**6**
II	**Haloperidol (2.6 mg/kg, i.p.) for 14 days**	**6**
III	**Haloperidol (2.6 mg/kg, i.p.) + Levodopa (2–10 mg/kg, i.p.) for 14 days**	**6**
IV	**Haloperidol (2.6 mg/kg, i.p.) + Cocoa extract (100 mg/kg, p.o.) for 14 days**	**6**
V	**Haloperidol (2.6 mg/kg, i.p.) + Cocoa extract (400 mg/kg, p.o.) for 14 days**	**6**

Induction of Haloperidol-Induced Parkinsonism

Parkinsonism was induced by administering haloperidol (2.6 mg/kg, i.p.) daily for 14 days to all groups except the normal control, along with the respective test treatments.

Physical Evaluation

Body Weight: Measured weekly using a weighing balance, and changes were recorded.

Feed Intake: Daily feed consumption was recorded for each group using a standard balance.

Behavioural Assessments

Behavioural parameters were evaluated on the 0th, 7th, and 14th days, 30 minutes post drug administration.

Tests included:

a) Exploratory and locomotor activity (Actophotometer)

b) Muscle coordination (Rota Rod)

c) Cognitive performance (Morris Water Maze)

d) Catalepsy test

Actophotometer Test (Exploratory and Locomotor Activity)

Locomotor activity was assessed using an Actophotometer (Dews, 1953). Each rat was placed individually in the activity cage for 10 minutes, and interruptions in light beams were counted as activity scores on day 0, 7, and 14.

Rota Rod Test (Muscle Coordination)

Neuromuscular coordination was evaluated using the Rota Rod apparatus (Dunham and Miya, 1957). Rats trained to remain on a rotating rod (20–25 rpm) for 5 minutes were selected. Fall-off time was recorded 30 minutes post drug administration on day 0, 7, and 14.

Morris Water Maze Test (Cognitive Performance)

Spatial learning and memory were assessed using a black circular pool (160 cm × 80 cm × 40 cm) filled with opaque water (23 ± 2 °C). A hidden platform (10 cm diameter) was submerged 1.5 cm below the water surface. Rats were released from different start positions, and the latency to find the platform was recorded on day 0, 7, and 14.

Catalepsy Test

Catalepsy was evaluated on day 7 and day 14 using a scoring method:

Normal movement: 0

Failure to move when pushed: 0.5

Maintaining forepaws on 3 cm block for 10 s: 1

Maintaining forepaws on 9 cm block for 10 s: 2

Maximum total catalepsy score: 3.5

Biochemical Estimations

On day 14, animals were anesthetized using diethyl ether. Blood was collected via retro-orbital plexus, and serum was separated by centrifugation (3000 rpm, 10 min). Serum was analyzed for SGPT, SGOT, ALP, total bilirubin, urea, and creatinine using standard biochemical methods.

Estimation of Brain Acetylcholine (ACh) Levels

A 0.4 ml aliquot of brain homogenate was mixed with 2.6 ml phosphate buffer (0.1 M, pH 8) and 100 µl DTNB. Absorbance was measured at 412 nm, followed by addition of 5.2 ml acetylthiocholine substrate. Change in absorbance per minute was recorded for 10 minutes at 2 minute intervals.

Estimation of Dopamine Levels

A dopamine assay was performed by oxidation with iodine and stopped using sodium thiosulphate in NaOH, followed by acidification and heating (100°C, 6 min). Fluorescence intensity (excitation

330–375 nm) was measured using a spectrofluorimeter, and tissue dopamine concentration was calculated relative to internal standards.

Histopathological Evaluation

At the end of the study, animals were sacrificed, and the substantia nigra was dissected and fixed in 10% formal saline for 24 h. After dehydration in graded alcohols and clearing in xylene, tissues were embedded in paraffin. Sections (4 µm) were stained with hematoxylin and eosin (H&E) and examined under a light microscope for histopathological changes.

Statistical Analysis

Data were analyzed using SPSS (version 20.0). Results were expressed as mean ± SEM (n = 6). Statistical comparisons were made using three-way ANOVA followed by Scheffe's post hoc test. Differences with $p < 0.05$ were considered statistically significant.

RESULTS

Effect of ethanolic extract of theobroma cocoa on body weight against haloperidol induced Parkinson in rats.

Body weight of each rats in all groups were measured weekly till the end of the treatment using the weighing balance and changes are recorded. The body weight of animals treated with haloperidol group was significantly decreased where as levadopa 4mg/kg, EETC 100mg/kg, EETC 400mg/kg were significantly increased in body weights compared to th control group. The results are shown in

Effects of EETC on feed intake against haloperidol induced Parkinson in rats

In this study, feed intake group II was significantly decreased when compared to control group I. The levadopa group (group III) and the treatment groups (group IV & V) shows significantly increased in feed intake when compared to group II. The results are shown in

Effect of EETC on Exploratory and Locomotors Activity by Using Actophotometer in haloperidol Induced Parkinson in Rats

Haloperidol group showed significantly ($P<0.05$) decrease in locomotor activity when compared to the control group. Levadopa Group III shows significantly increases when compared to haloperidol Group II. In this no significant difference in treatment Group IV on the 7th day.

On the 14th day levadopa Group III shows significantly (P<0.05) increase when compared to control Group I. Treatment Groups IV & V exhibited (P<0.001) significantly increases when compared to haloperidol Group II and Levadopa Group III. The results shown in

Effect of EETC on Muscle Coordination Behavior by Using Rota Rod in haloperidol Induced Parkinson in Rats

The mean fall- off time of Group I animals from the rota rod was observation of the treatment. On the 7th day Group II showed significantly (P<0.05) decrease when compare to the control Group I. Treatment groups IV& V shows significantly increased (P<0.001) when compared to the haloperidol group II.

On the 14th day haloperidol Group II showed significantly (P<0.001) decreased when compare to the control Group I. Levadopa group III showed significantly (P<0.001) increased when compare to the haloperidol Group II. Treatment Group IV & V showed significantly (P<0.001) increases when compare to the haloperidol Group II. The results are shown in

Effect of EETC on Cognitive Performance by Using Morris Water Maze in haloperidol Induced Parkinson in Rats

Antipsychotic related effects on swim speeds on the 7th day haloperidol Group II showed significantly (P<0.05) increased when compared to control Group I. Group III showed significant (P<0.05) decreased when compared to haloperidol Group II. Treatment Group IV & V showed significantly (P<0.001) increase when compared to the haloperidol Group II.

On the 14th day haloperidol Group II showed significantly (P<0.01) increased when compared to the control Group I. Levadopa Group III showed significantly (P<0.001) decreased when compared to the haloperidol Group II. Treatment Group IV& V showed significantly (P<0.001) decreased when compared to haloperidol Group II.

Effect of EETC on Catalepsy in haloperidol Induced Parkinson in Rats

On 7th day haloperidol Group II showed significantly (P<0.001) increased when compared to control Group I. standard Group III shows significantly (P<0.001) decreased when compared to haloperidol Group II. Treatment Group IV & V showed significantly (P<0.001) decreased when compared to haloperidol Group II.

On 14th day haloperidol Group II showed significantly (P<0.001) increased when compared to control Group I. standard Group III shows significantly (P<0.001) decreased when compared to haloperidol Group II. Treatment Group IV & V showed significantly (P<0.001) decreased when compared to haloperidol Group II.

Effect of EETC on biochemical parameter in haloperidol Induced Parkinson in Rats

In this study, serum glutamate pyruvate (SGPT), glutamate oxaloacetate transaminase (SGOT), Alkaline phosphates, Total bilirubin, was of haloperidol Group II showed significantly (P<0.001) increase in SGPT, SGOT, ALP, Total bilirubin level when compared to control Group I. In the 1, 2, 4-Triazin treatment group IV & V shows significantly low level when compared to the standard Group III.

Effect of EETC on Brain Ach Levels in haloperidol Induced Parkinson in Rats

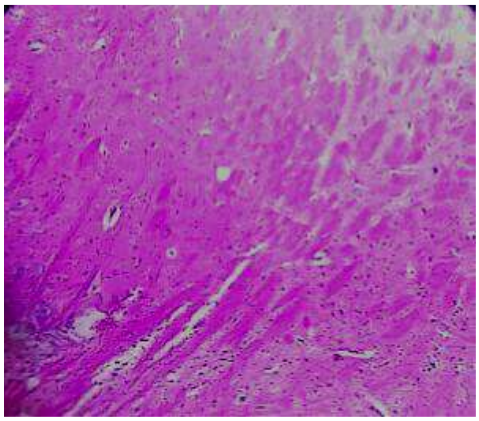
In this brain acetylcholine haloperidol group showed significantly (P<0.01) increase when compared to control group. Treatment group showed significantly (P<0.05) decrease when compare to haloperidol group. Standard group showed no significant difference when compared to control group.

Effect of EETC on Dopamine Assay in haloperidol Induced Parkinson in Rats

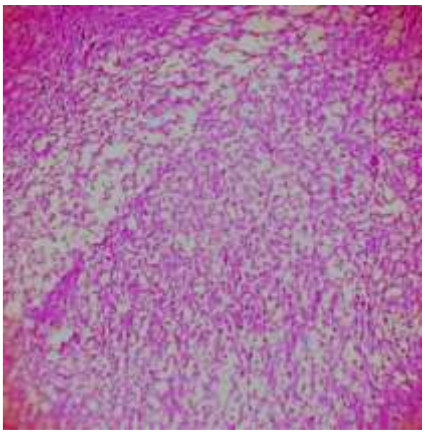
The dopamine level in chlorpromazine animals were reduced significantly (P<0.001) decrease when compared to control Group I. Treatment Group IV showed significantly (P<0.001) increase when compared to Diclofenac Group II. And then Treatment Group IV showed significantly (P<0.01) increase when compared to chlorpromazine Group III.

Histopathological evaluations

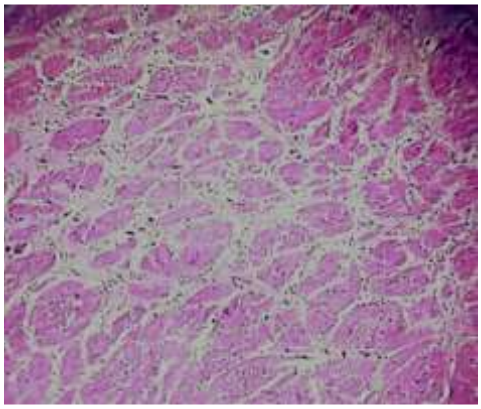
Histopathology of our samples showed that haloperidol caused degeneration in the mid brain region of the rats as neurons were under oxidative stress when compared with normal rats as can be seen from Presence of hyper chromatic nuclei with eosinophilic vacuolated cytoplasm in edematous. However sections examined from EETC treated group shows exhibits intact architecture with occasional hyper chromatic nuclei and mild vacuolization suggestive of mild degenerative changes in treated animals.



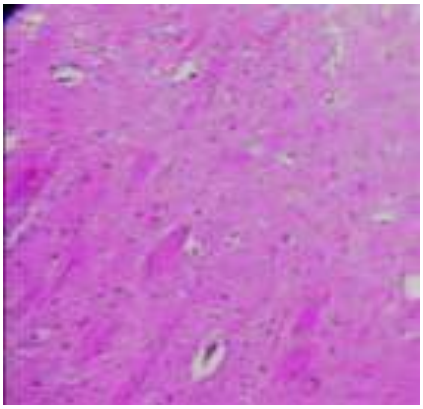
CONTROL GROUP



LEVODOPA GROUP



HALOPERIDOL GROUP



EETC GROUP

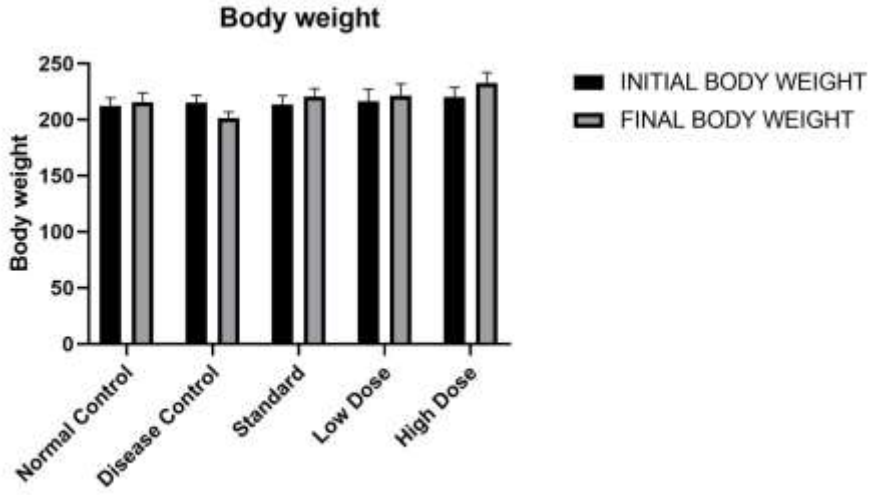
Effect of ethanolic extract of theobroma cocoa on change in body weight against haloperidol induced Parkinson in rats.

TREATMENT	INITIAL BODY WEIGHT (g)	FINAL BODY WEIGHT (g)
GROUP I (normal control)	212.2 ± 7.33	215.5 ± 8.53
GROUP II (disease control)	251.1 ± 6.65	201.4 ± 5.67
GROUP III (standard)	213.4 ± 8.16	220.6 ± 7.12
GROUP IV (low dose)	216.6 ± 10.49	221.5 ± 10.40
GROUP V (high dose)	220.3 ± 8.64	232.7 ± 9.59

Values are expressed as mean ± SEM, n=6,

Comparisons were made between a-Group I VS II, III, IV, V b- Group II VS III, and IV ,V

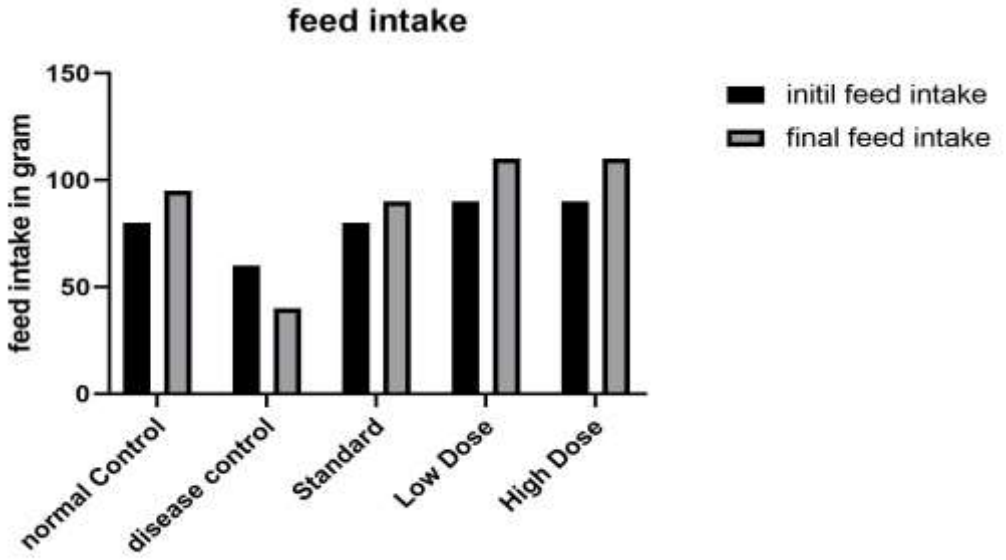
Symbols represent statistical: ***P<0.001, **-P<0.01, *-P<0.05



Effect of ethanolic extract of theobroma cocoa on change in body weight against haloperidol induced Parkinson in rats.

TREATMENT	INITIAL FEED INTAKE(g)	FINAL FEED INTAKE(g)
GROUP I (normal control)	80	95
GROUP II (disease control)	60	40
GROUP III (standard)	80	90
GROUP IV (low dose)	90	110
GROUP V (high dose)	90	110

Effects of EETC on feed intake against haloperidol induced Parkinson in rats



Effects of EETC on feed intake against haloperidol induced Parkinson in rats

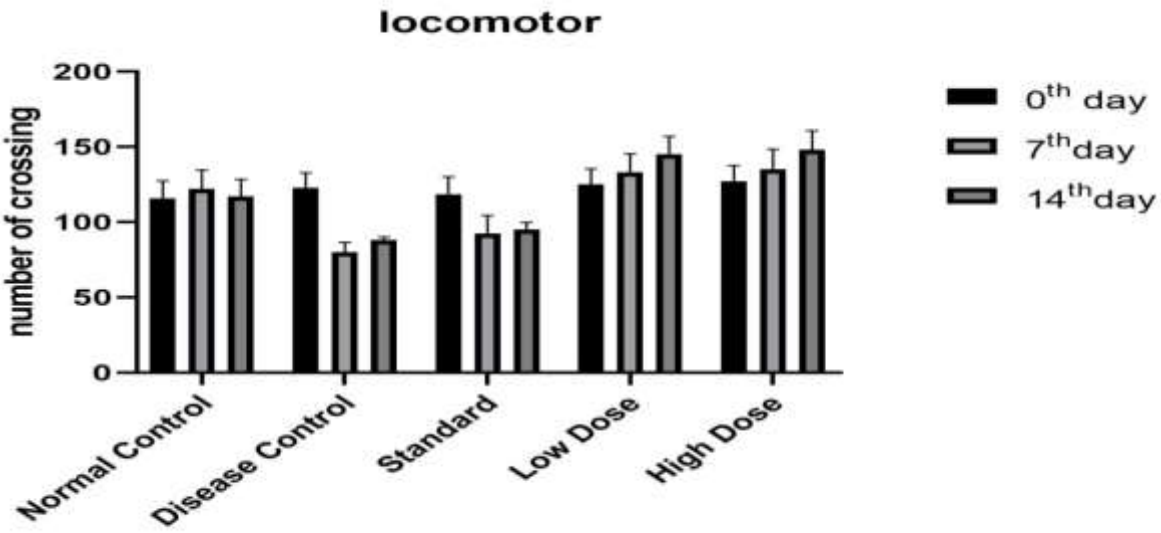
Effect of EETC on Exploratory and Locomotors Activity by Using Actophotometer in haloperidol Induced Parkinson in Rat

TREATMENT	0^{th} day	7^{th} day	14^{th} day
GROUP I (normal control)	115.7 ±11.79	122.3 ±12.21	117.0± 11.42
GROUP II (disease control)	122.7 ±10.13	80.17± 6.45	88.17 ±1.88
GROUP III(standard)	118.5± 11.53	92.50± 11.90	95.00 ±4.68
GROUP IV(low dose)	125.0 ±10.36	133.0 ±12.48	145.2 ±11.71
GROUP V(high dose)	127.3± 10.20	15.20 ±13.20	148.15 ±12.60

Values are expressed as mean ± SEM, n=6,

Comparisons were made between a-Group I VS II, III, IV, V b- Group II VS III, and IV ,V

Symbols represent statistical: ***P<0.001, **-P<0.01, *-P<0.05



Effect of EETC on Exploratory and Locomotors Activity by Using Actophotometer in haloperidol Induced Parkinson in Rat

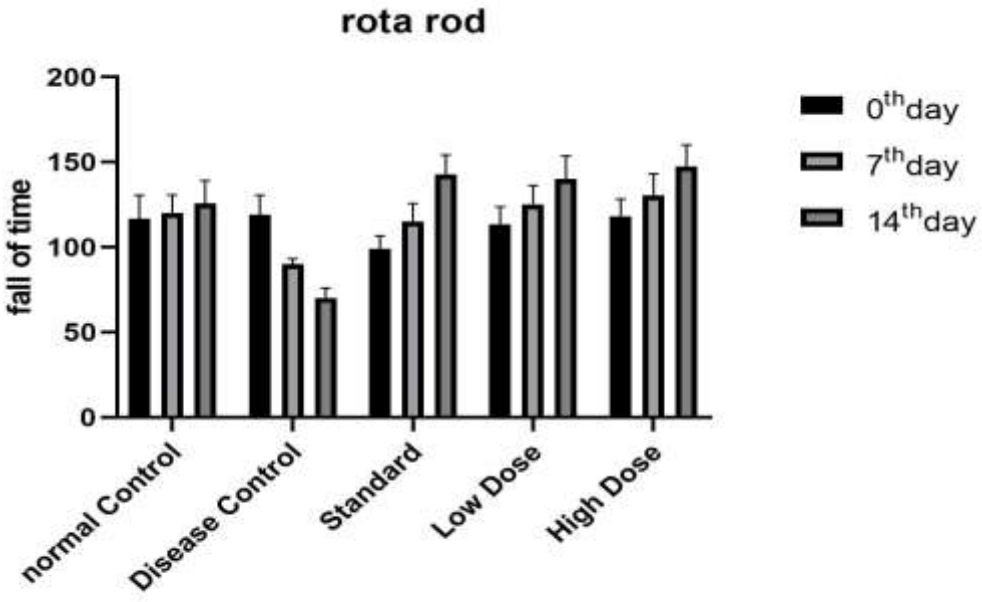
Effect of EETC on Muscle Coordination Behavior by Using Rota Rod in haloperidol Induced Parkinson in Rats

TRAETMENT	0th day	7th day	14th day
GROUP I (normal control)	116.7± 13.80	120.0± 10.78	125.7± 13.40
GROUP II(disease control)	119.0 ±11.62	90.1± 3.23	70.33 ±5.83
GROUP III(standard)	99.33± 7.41	115.2± 10.53	142.7 ±11.50
GROUP IV (low dose)	113.5 ±10.29	125.3 ±11.00	140.3 ±13.35
GROUP V(high dose)	118.30 ±10.00	130.6± 12.5	147.6± 12.40

Values are expressed as mean ± SEM, n=6,

Comparisons were made between a-Group I VS II, III, IV, V b- Group II VS III, and IV ,V

Symbols represent statistical: ***P<0.001, **-P<0.01, *-P<0.05



Effect of EETC on Muscle Coordination Behavior by Using Rota Rod in haloperidol Induced Parkinson in Rats

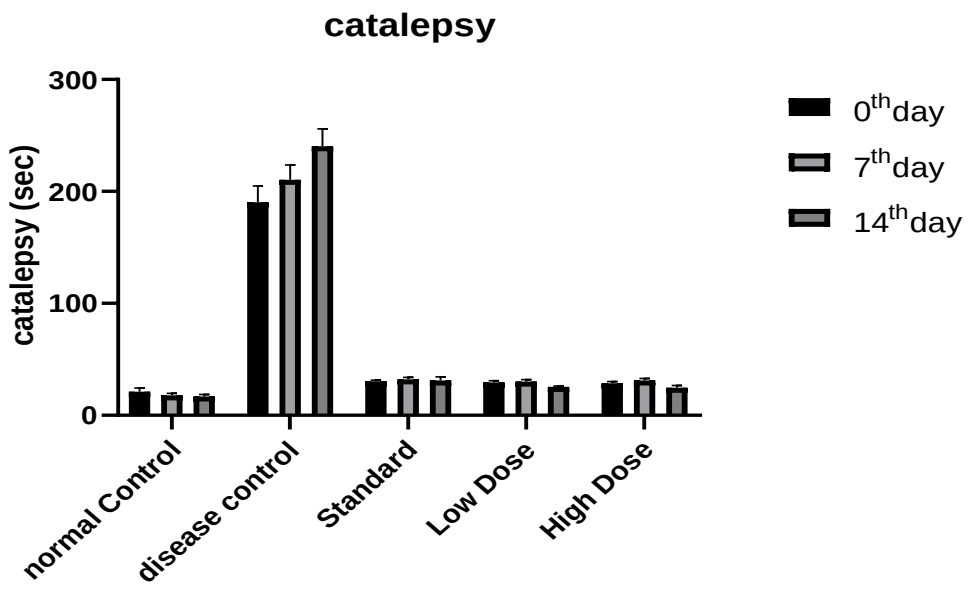
Effect of EETC on catalepsy in haloperidol induced Parkinson rats

TREATMENT	0^{th} day	7^{th} day	14^{th} day
GROUP I	21.17 ±3.08	17.83 ± 1.77	16.9 ±1.82
GROUP II	190.3 ± 14.49	210.3 ±13.29	240.4 ± 15.46
GROUP III	30.33 ±1.08	32.00 ±1.96	31.02 ± 3.28
GROUP IV	29.40 ±1.40	30.20 ± 1.67	25.30 ± 0.94
GROUP V	28.53 ± 1.60	31.10 ± 1.80	24.53 ± 2.20

Values are expressed as mean ± SEM, n=6,

Comparisons were made between a-Group I VS II, III, IV, V b- Group II VS III, and IV ,V

Symbols represent statistical: ***P<0.001, **-P<0.01, *-P<0.05



Effect of EETC on catalepsy in haloperidol induced Parkinson rats

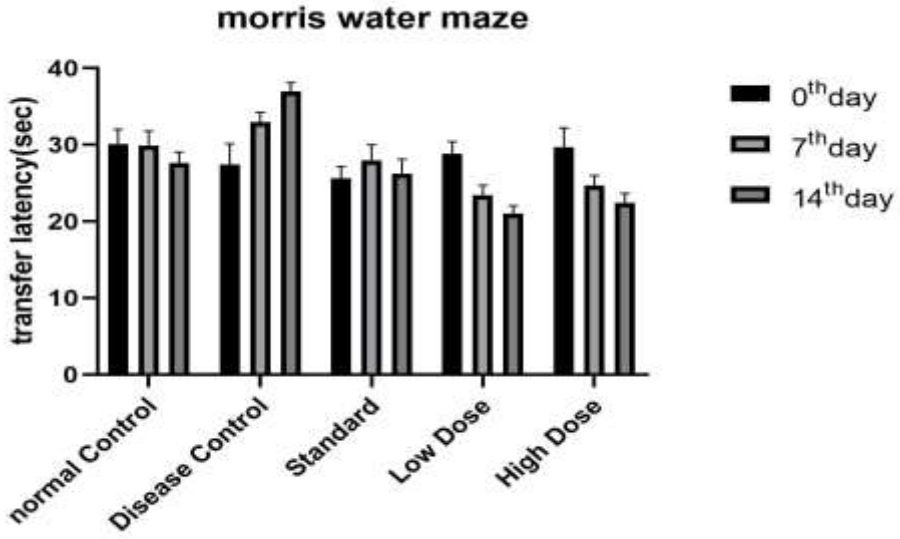
Effect of EETC on Cognitive Performance by Using Morris Water Maze in haloperidol Induced Parkinson in Rats

TREATMENT	0^{th} day	7^{th} day	14^{th} day
GROUP I(normal control)	30.1± 1.90	29.9 ±1.88	27.63 ±1.43
GROUP II(disease control)	27.43 ±2.71	32.95± 1.27	36.99± 1.13
GROUP III(standard)	25.65± 1.51	27.99 ±1.98	26.21 ±1.91
GROUP IV(low dose)	28.82± 1.60	23.45± 1.25	21.021± 1.03
GROUP V(high dose)	29.70± 2.50	24.67 ±1.30	22.45± 1.21

Values are expressed as mean ± SEM, n=6,

Comparisons were made between a-Group I VS II, III, IV, V b- Group II VS III, and IV ,V

Symbols represent statistical: ***P<0.001, **-P<0.01, *-P<0.05



Effect of EETC on Cognitive Performance by Using Morris Water Maze in haloperidol Induced Parkinson in Rats

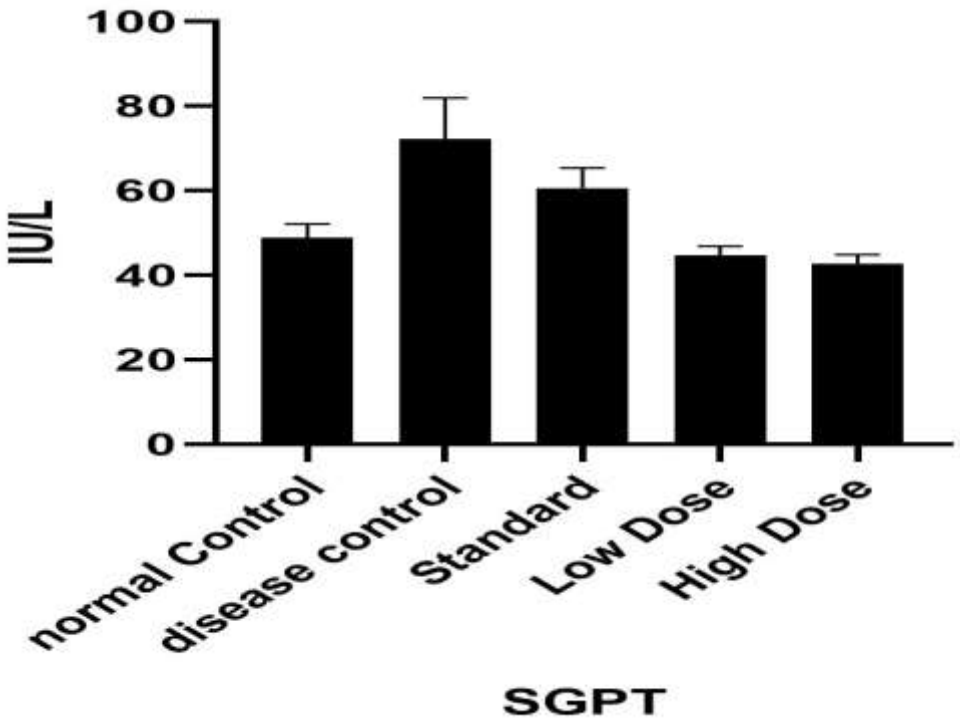
Effect of EETC on biochemical parameter in haloperidol induced Parkinson in rats.

TREATMENT	SGPT(IU/L)	SGOT(IU/L)	ALP(IU/L)	BILIRUBIN(mg/dl)	UREA(mg/dl)	CREATININE
GROUP I(normal control)	48.89 ±3.27	18.98 ±2.92	79.25 ±8.04	0.157 ±00.1	13.68± 1.91	0.5115 ±0.06
GROUP II(disease control)	72.17 ±9.79	37.23 ±8.47	140.7 ±4.39	0.661± 0.08	21.94 ±1.29	0.9118 ±0.02
GROUP III(standard)	60.64 ±4.77	33.9± 4.05	90.32 ±2.82	0.1965± 0.02	20.25 ±1.05	0.806± 0.07
GROUP IV(low dose)	44.80 ±2.04	17.50 ±2.82	60.73±6.96	0.1315± 0.01	12.75± 1.70	0.321± 0.06
GROUP V(high dose)	42.79 ±2.10	18.00± 2.90	65.70 ±4.30	0.1320± 0.03	13.00± 1.60	0.4201 ±0.08

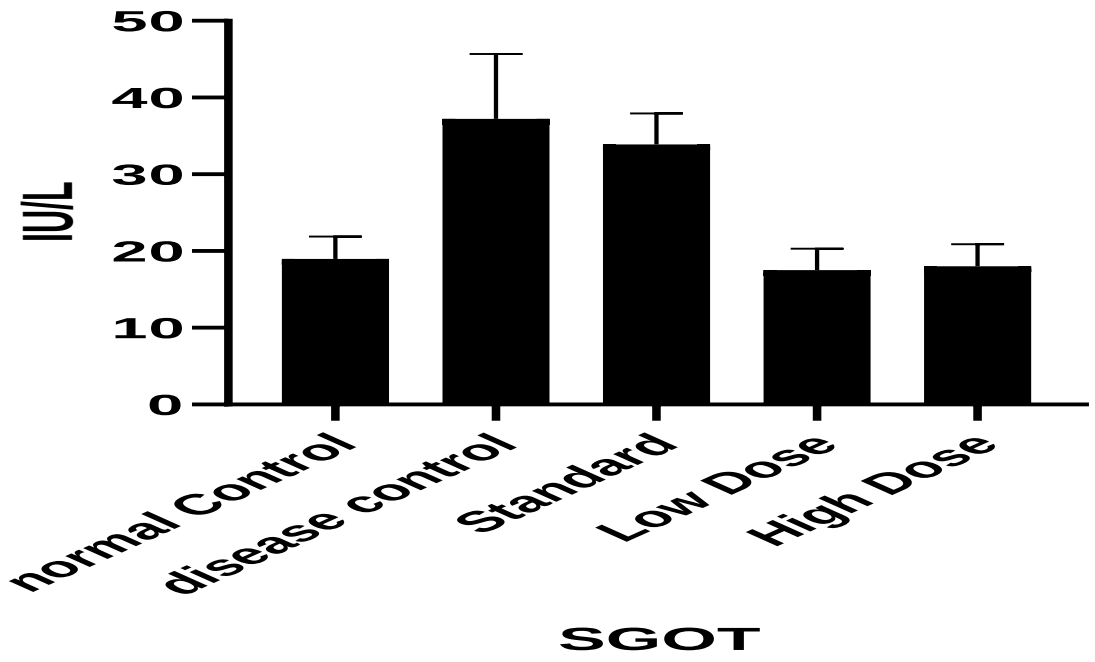
Values are expressed as mean ± SEM, n=6,

Comparisons were made between a-Group I VS II, III, IV, V b- Group II VS III, and IV ,V

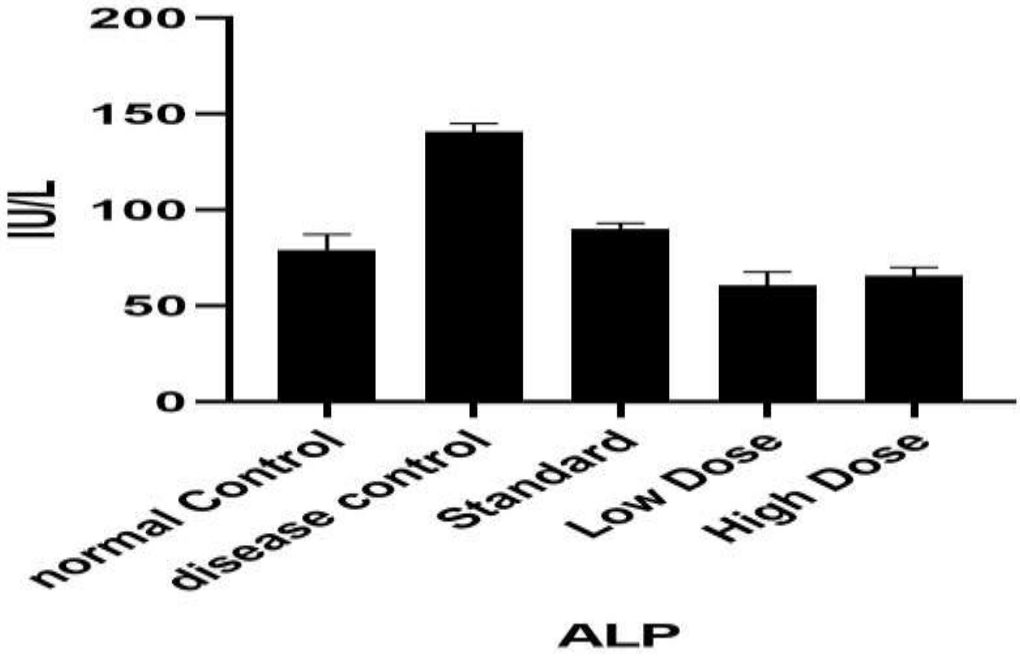
Symbols represent statistical: ***P<0.001, **-P<0.01, *-P<0.05



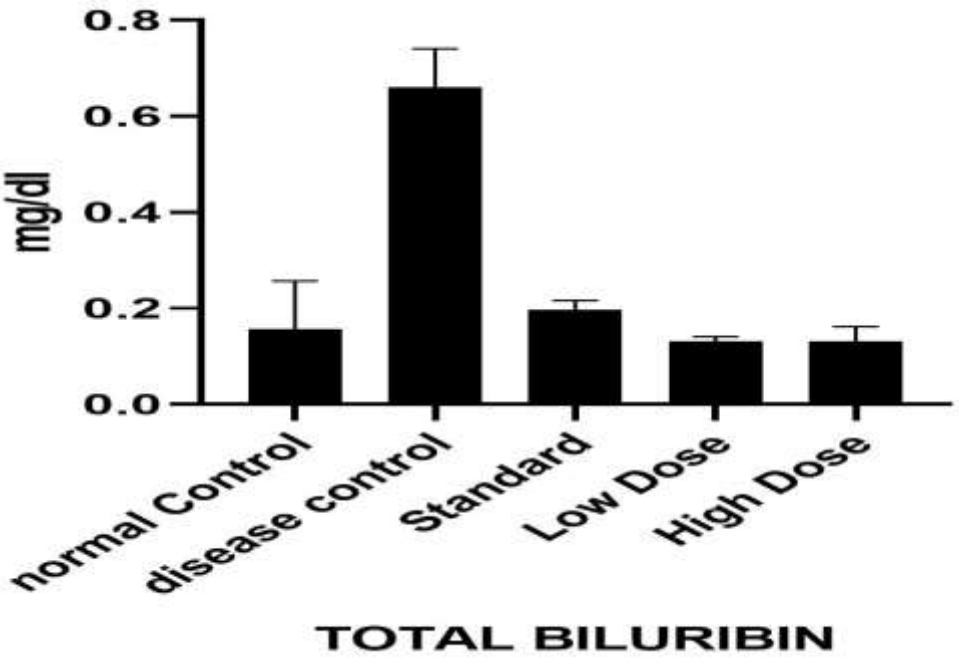
Effect of EETC on serum glutamate pyruvate (SGPT) in haloperidol induced Parkinson in rats



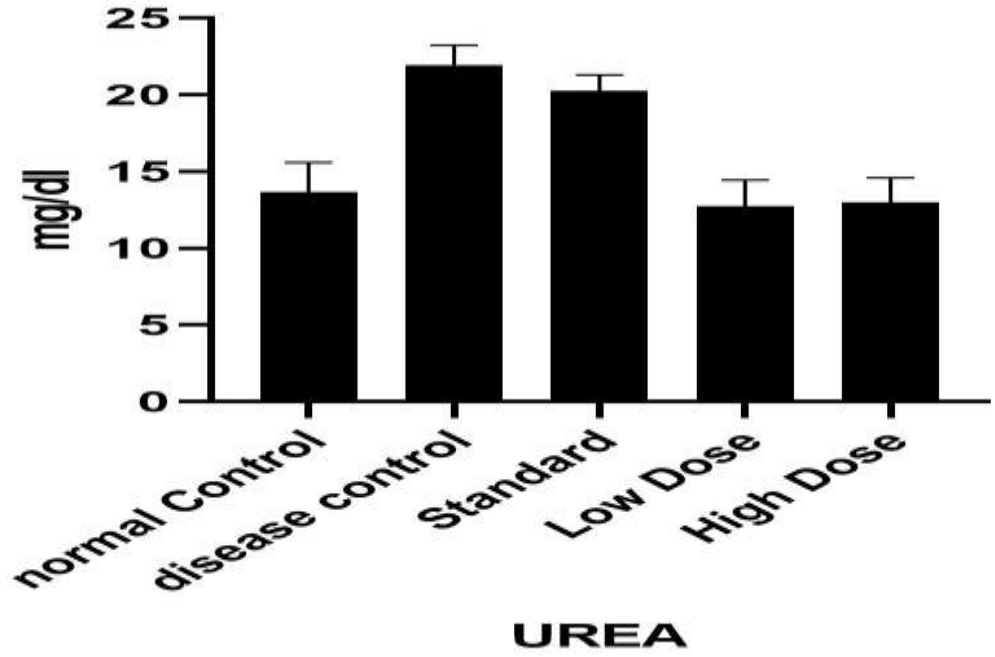
Effect of EETC on Glutamate Oxaloacetate Transaminase (SGOT) in haloperidol induced Parkinson in rats



Effect of EETC on haloperidol induced Parkinson in rats



Effect of EETC on total bilirubin in haloperidol induced Parkinson in rats



Effect of EETC on urea in haloperidol induced Parkinson in rats

Effect of EETC on Creatinine in haloperidol induced Parkinson in rats

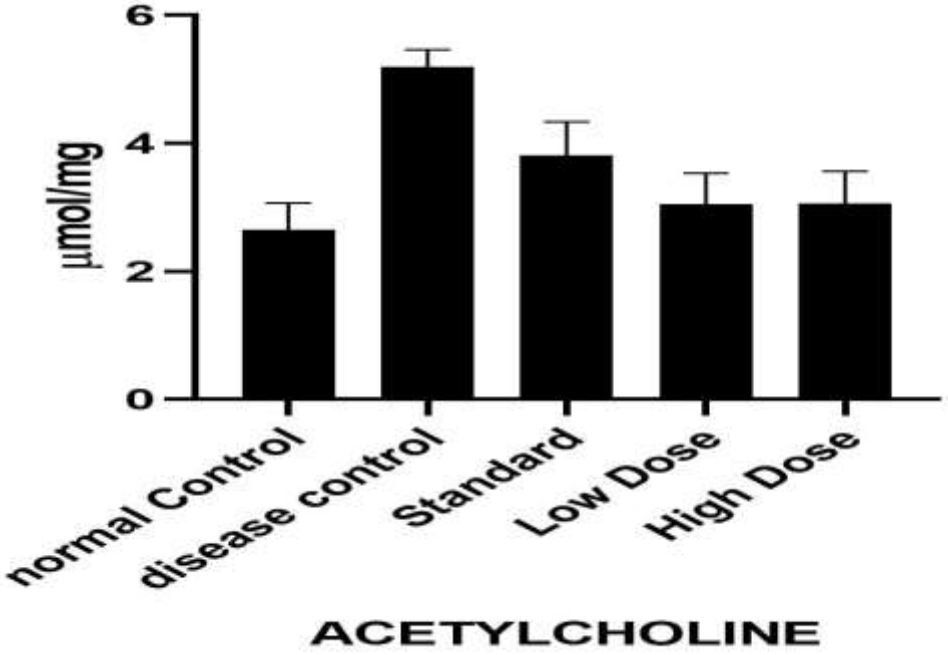
Effect of EETC on brain ACH level in rat brain

TREATMENT	ACETYLCHOLINE	DOPAMINE
GROUP I	2.648± 0.42	26.34± 2.20
GROUP II	5.190± 0.27	20.04± 2.26
GROUP III	3.807± 0.53	21.36± 1.89
GROUP IV	3.053± 0.48	31.10± 1.19
GROUP V	3.060± 0.50	33.11± 1.30

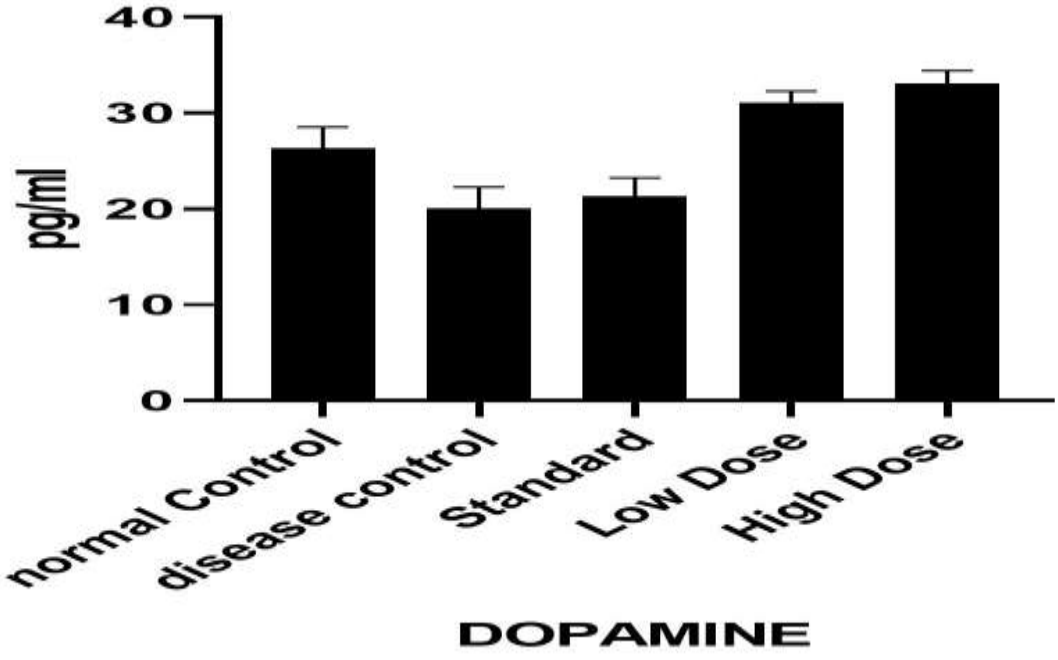
Values are expressed as mean ± SEM, n=6,

Comparisons were made between a-Group I VS II, III, IV, V b- Group II VS III, and IV ,V

Symbols represent statistical: ***P<0.001, **-P<0.01, *-P<0.05



Effect of EETC on brain ACH level in rat brain



Effect of EETC on dopamine assay in haloperidol induced Parkinson in rat

DISCUSSION

Parkinson's disease (PD) is a progressive neurodegenerative brain disorder. It is characterized by diminished facial expression, stooped posture, slowness of voluntary movement, festinating gait (progressively short- ended, accelerated steps), rigidity, and a "pill- rolling|" tremor but also of many other central and peripheral neuronal systems. The etiology of PD is unknown but several mechanisms have been proposed including environmental toxins, oxidative stress and neuro inflammation. The involvement of such dopamine and non-dopaminergic systems is responsible for the occurrence of the motor and non-motor Parkinson's symptoms.

Haloperidol is the first line drug used as a antipsychotic agent. In this research, *i.p.* injection of haloperidol induced neuroinflammation oxidative stress and progressive decline in cognition are considered as most appropriate animal model for sporadic PD. The oxidative damage and neuroinflammation caused by haloperidol is in line with earlier findings. Intraperitoneal injection of haloperidol induced memory impairment which was evaluated by the spatial memory, locomotor activity, initial latency and step through latency which is considered as the widely accepted model in cognition. The neuropsychological assessment was done by using Elevated plus Maze test.

Plants have been a good source of therapeutic agents for thousands of years; an impressive number of modern drugs used for treating human diseases are derived from natural sources. Cocoa and cocoa-derived products contain several substances, such as polyphenols and flavanols such as epicatechin and catechin, widely known for their excellent antioxidant activity .These substance are mainly involved in the neuronal protection. Generally, The neurodegenerative disorders like parkinson and Alzheimer are mainly caused by imbalance between acetylcholine and dopamine in brain. Here we discussed about the *theobroma cocoa,* having the ability to increase the level of dopamine in brain which helps in protection of Parkinson disease induced by haloperidol in rats.

Haloperidol not produced any toxic symptoms or mortality when given orally in mice and rats. Hence, the drugs were considered safe for further pharmacological screening. Up to 14 days Additional observations will be necessary if the animals continue to display signs of toxicity. Observations should include changes in skin; fur, eyes and mucous membranes, and also cardio vascular system, central nervous systems, autonomic nervous and gastro intestinal tract in the animals, during the period test compound do not produce any major toxicity.

In PD condition reduction in the body weight during the study period development may be due to hypothalamic regulation, energy expenditure, or dopaminergic signaling mechanism. In treatment with extraction of theobroma cocoa shows significantly increase in body weight as compare to levadopa group and haloperidol group.

In this study PD condition caused decreased in feed intake and BMI variation which may reflect a dysregulation of dopaminergic control of eating behavior rather than modification of energy metabolism. The standard groups shows significantly increased, feed intake when compared to haloperidol group and the EETC group are showed significantly increased feed intake when compared to standard group.

Actophotometer was used to evaluate the effect of locomotors activity improvement properties of rat. Haloperidol induced group were exhibited CNS depressant effect due to impaired cholinergic transmission, oxidative / nitrergic stress, neuroinflammation, and dyslipidemia. In this study levadopa treated group were showed no significant impact on locomotion when compared to the PD control. The EETC treated group shows significantly improved spontaneous locomotor activity hence indicating its CNS stimulant activity in Parkinson's rats when compared to PD control group.

Muscle coordination activity of haloperidol induced rat was significantly decreased because in the Sensory fibers detect lengthening of the muscles and cause motor neurons in the spinal cord to contract the same muscles. In this study the treatment group levadopa and EETC showed significantly increased in muscle coordination when compared to the PD control.

In this study, Morris water maze test on the manifested itself due to immersion stress, fatigue and sensory-motor deficits showed in haloperidol Group shows significantly decreased. Cognitive function was significantly increased in both the treatment groups when compared to haloperidol group. In comparison between the treatment group EETC shows more increased in cognitive function when compare to standard Group.

In PD condition, Catalepsy has as inability to correct an imposed abnormal posture while maintaining the righting reflex due to its non-selective action, it also produces blockade of post-synaptic D2 receptors in the nigro striatal pathway leading to the development of extra pyramidal side effects. In EETC treatment Group shows significantly increased in posture correction when compared to standard group.

In this study, serum SGPT, SGOT, ALP, Total bilirubin, was raised in Parkinson's disease rat due to liver cells damage. In the standard and EETC treatment group shows significantly low level of liver enzymes in serum. It reveals that the non toxic nature of levadopa and extracted compound on the liver when compared to the haloperidol treated Group.

In this study, brain acetylcholine increase significantly in haloperidol alone treated group when compared to control group. EETC treated group shows significantly decreased in acetylcholine level when compare to haloperidol group. Standard group showed no significant difference when compared to control group.

The dopamine level in haloperidol induced rats was reduced significantly when compared to control group. Treatment groups have high dopamine level indicate recovering neurodegeneration. Histopathology of mid brain portion of brain section of parkinson's rat showed hyper chromatic nuclei with eosinophilic vacuolated cytoplasm in edematous. In treatment group shows hyper chromatic nuclei and mild vacuolization suggestive of mild degenerative changes in treated animals. It conform that, the levadopa and EETC treatment group shows greater effect on neuronal inflammation.

CONCLUSION

Parkinson is a chronic, progressive neurodegenerative disease characterized by both motor and non motor features. The disease has a significant clinical impact on patients, families, and caregivers through its progressive degenerative effects on mobility and muscle control. The motor symptoms of PD are attributed to the loss of striatal dopaminergic neurons, although the presence of non motor symptoms supports neuronal loss in non dopaminergic areas as well.

The term *Parkinsonism* is a symptom complex used to describe the motor features of PD, which include resting tremor, bradykinesia, and muscular rigidity. PD is the most common cause of Parkinsonism, although a number of secondary causes also exist, including diseases that mimic PD and drug-induced causes.

In this study the ethanolic extraction of *Theobroma cocoa* were administered orally against the haloperidol induced Parkinson in rats. Haloperidol confirms the decreased locomotors function, muscle coordination, cognitive function and deflects of dopamine, also increased in muscle rigidity, oxidative stress and cholinergic activity in the levadopa alone administered group.

Levadopa and EETC treated groups significantly revert all this complication of PD. This are due to the presence of flavanols in the cocoa extraction which posses more potent anti-parkinson activity.

In conclusion, the cocoa compound explores the activity of anti-parkinson against haloperidol induced in rat by increase the level of dopamine in the brain with less toxicity.

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