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EVALUATION OF ANTI-PARKINSON'S ACTIVITY OF *CYAMOPSIS TETRAGONOLOBA* METHANOL PLANT EXTRACT WITH BEHAVIORAL AND BIOCHEMICAL ANALYSIS

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Keywords:

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ABSTRACT: In the present study, we evaluated the Anti-parkinson's activity of *Cyamopsis tetragonoloba* methanol plant pod extract by Haloperidol Induced Catalepsy and Tacrine induced vacuous jaw movements for behavioral parameters and *in-vivo* assays to estimate neurochemical parameters like (GSH, LPO, and SOD). The behavioral analysis performed using Haloperidol Induced Catalepsy produces Parkinson's disease (PD) like symptoms, has been shown to reduce the catalepsy significantly ($P<0.001$) with *Cyamopsis tetragonoloba* methanol plant pod extract (200 and 400 mg/kg) doses. Tacrine-induced jaw movements and bursts symptoms closely resembling the PD symptoms shown to reduce treatment with plant extract and helped to manage the uncontrolled movements. The biochemical parameters like Lipid peroxidation (LPO), Glutathione (GSH), and Superoxide dismutase (SOD) were assessed using control, standard, and 200 and 400 mg/kg *Cyamopsis tetragonoloba* extract, results revealed that rats treated with plant extracts had increased levels of GSH and SOD which was found to be lower in the patients of PD and further plant extract analysis found efficient in reducing the LPO activity which helps to decrease the overall effect of oxidative stress in brain tissues and relieves the symptoms of PD. The studies proved that *Cyamopsis tetragonoloba* methanol pod extract possesses significant Anti-parkinson's activity and helpful in treating motor dysfunction and reducing oxidative stress, which may be associated with its antioxidant potential.

INTRODUCTION: Parkinson's disease is a neurological disorder that affects mostly the elderly; this is a progressive disorder and found to be the second most common neurodegenerative disease. This disease was described for the very first time by James Parkinson; this disease is associated with loss of neurons in the substantia nigra region of the brain affecting the production of neurotransmitter dopamine [1].

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The primary symptoms are rigidity, postural instability, tremors (involuntary shaking), brady-kinesia (slowness in movement), akinesia (absence of movement), and secondary symptoms are dementia, sleep disorder, difficulty breathing, and depression.

The disease arises from various factors, including genetic, biological mechanism, and environmental factors [2]. Amongst various pathophysiological mechanisms causing PD, oxidative stress was observed as one of the significant ones, oxidative stress arises when the endogenous antioxidant system overwhelms by the free radical formation in the body, and the exogenous antioxidant can provide the body an extra boost to deal with the adverse effect of oxidative stress [3].

Oxidative stress studies of PD patients were observed and found that it caused the degeneration of neurons in the substantia nigra pars compacta region. Enzymes such as GSH glutathione were recorded in a very lower amount in the studies of PD patients, indicating the operation of oxidative stress mechanism stimulating the neuro-degeneration in the brain; however, there can be an age-related decline in GSH as well. Advanced PD patients show Lewy bodies, the decline in dopamine, high GSSG concentration, and MDA formation, reduction in SOD, Catalase activity, and severe loss of neuronal cells [4]. It was observed that plants possess secondary metabolites having phenolics, terpenes and nitrogen-containing groups. Many such polyphenols have been found to exhibit a property of crossing the blood-brain barrier (BBB), this is a barrier present inside the brain which permits selective absorption of therapeutics or chemicals. An alternative approach of plant's natural metabolites can give an alternative passage to treat PD patients; beneficial effects of polyphenols were observed in recent studies [5]. *Cyamopsis tetragonoloba* is an annual herb widely grown in India and commonly known as Guar (Fabaceae). Guar bean is known from ancient times as traditional medicine; the basis of such medicine was phytochemical and pharmacological properties.

The plant's various parts, such as pods, seeds, and leaves have been used for treating asthma, inflammation, diabetes as a weight loss agent and laxative since long [6]. Pharmacological properties show that plant is anti-diabetic, hypo-cholesterolemic, cytoprotective, anticholinergic, antimicrobial, antiasthmatic [7]. Guar bean is a rich source of protein and essential amino acids such as isoleucine, leucine, phenylalanine and tyrosine [8]. Legume seeds of Guar are rich in flavonoids, phenolic acid, vitamins, where polyphenols include gallic acid, gallotannins, myricetin-7-glucoside-3-glycoside, chlorogenic acid, stigmasterol, *etc.* [9] The legumes have known to provide with the symptomatic ease, as the family is known to possess L-DOPA or Levodopa in a varying amount which is a majorly used treatment to assess PD symptoms and the presence of secondary metabolites known to decrease oxidative stress. Beans like *Mucuna pruriens* (L-DOPA- 1%), *Vivia faba* (L-DOPA- 0.5%), *Phaseolus vulgaris* (L-DOPA- 0.25%) exhibit neuroprotective effect considered to be due to the presence of levodopa and various antioxidants [10]. In this study, we will be examining *Cyamopsis tetragonoloba* for its Anti-parkinson's activity through *in-vivo* biochemical and behavioral studies on albino rats.

MATERIAL AND METHODS:

Plant Selection and Extraction: *Cyamopsis tetragonoloba* pods were collected from the local market of Bhopal (M.P), in September and October. The plant was then verified by Dr. Zia-ul-Hasan HOD (Botany), Safia College, Bhopal. The Voucher Specimen number given was 448/Bot/ Saifia/17. Then for the extraction process, the plant pods were shade dried for 7-8 days and grinded to a coarse powder. The technique used for separation was maceration, where the plant extract was kept in a jar of solvents of various polarities and then separated for further studies, here we used the methanol extract.

Chemicals and Drugs: Haloperidol, Haloperidol + Carbidopa, Tacrine, Levodopa were provided by Pinnacle Biomedical Research Institute (PBRI), Bhopal. All chemicals were of analytical grade.

Animals: Albino Wistar rats of body weight 150-250 gm provided by the Pinnacle Biomedical Research Institute. The experiments were performed as per approval of the Institutional Animal Ethical Committee (IAEC) of PBRI, Bhopal (Reg No.1283/PO/c/09/CPCSEA) (Committee for Purpose of Control and Supervision of Experimentation on Animals) CPCSEA norms were followed during experiments. Protocol Approval Reference No. PBRI/IAEC/PN-17110.

Acute Oral Toxicity: Acute oral toxicity was tested according to OECD 423 guidelines, in which the toxicity of *Cyamopsis tetragonoloba* methanol extract was tested on 3 female rats of weights 100-200 gm.

Various dose levels such as 5, 50, 300, and 2000 mg/kg were tested and results were observed for the duration of 24 h, 48 h, and 14 days after administering the given doses and check for mortality. It was observed that LD_{50} value of plant extract was below 2000 mg/kg. The doses selected were 200 mg/kg and 400 mg/kg of *Cyamopsis tetragonoloba* [11].

Experimental Protocol: Protocol for dosing carried out for 5 days and on 5th-day haloperidol or tacrine was given in all groups except the control group in which it was given daily and vehicle, sacrifice took placed on the same day, and brain tissue homogenate was prepared.

Haloperidol Induced Catalepsy and *In-vivo* Antioxidant Assay (4 groups):

Group I: Control group (Haloperidol) 1mg/kg i.p

Group II: Haloperidol 1 mg/kg i.p + Standard drug (Levodopa + Carbidopa) 125 mg/kg

Group III: Haloperidol 1 mg/kg i.p + 200 mg/kg of *Cyamopsis tetragonoloba* methanol extract

Group IV: Haloperidol 1 mg/kg i.p + 400 mg/kg of *Cyamopsis tetragonoloba* methanol extract for

Tacrine Induced Vacuous Jaw Movements (5 Groups):

Group I: Vehicle group without any drug/extract

Group II: Control group (Tacrine drug) 2.5 mg/kg i.p.

Group III: Tacrine 2.5 mg/kg i.p. + Standard drug (Levodopa + Carbidopa) 125 mg/kg

Group IV: Tacrine 2.5 mg/kg i.p + 200 mg/kg of *Cyamopsis tetragonoloba* methanol extract

Group V: Tacrine 2.5 mg/kg i.p + 400 mg/kg of *Cyamopsis tetragonoloba* methanol extract

Behavioral Analysis:

Haloperidol-induced Catalepsy in Rats: Albino rats (100-250 gm) were divided into 4 groups (n=6). The rats were treated with *Cyamopsis tetragonoloba* extract (200 and 400 mg/kg) and levodopa (30 mg/kg i.p.) 30 min before giving them haloperidol (1 mg/kg i.p.), now a wooden bar elevated to 6 cm height is taken and forepaw of the rat was placed on it. The time duration of retention of the forepaw of a rat on the elevated bar was noted as 0, 30, 60, 90, and 120 min. The maximum run-off time was taken as 300 sec [12].

Tacrine Induced Vacuous Jaw Movements: Rats were divided into 5 groups of 6 rats each and treated with *Cyamopsis tetragonoloba* extract (200 and 400 mg/kg) and levodopa + Carbidopa daily for 5 days. On the 5th day, after 20 min of dosing tacrine (2.5 mg/kg i.p.) was given to rats, and tremulous jaw movements and orofacial bursts were measured for 60 min [13].

Biochemical Analysis:

***In-vivo* Antioxidant Assay:**

Lipid Peroxidation (LPO): Up to 5 ml of solution was prepared by using 0.2 ml of 10% w/v tissue homogenate from the brain, 0.2 ml 8.1% SDS, 1.5 ml 20% acetic acid, 1.5 ml 8% TBA, and add water to make up. Place the above solution in a water bath for 60 min. Add 5 ml of butanol: pyridine (15:1) vortex and centrifuge at 3000 rpm for 10 minutes. At 532 nm OD was taken by separating the organic layer keeping butanol: pyridine (15:1) as blank [14].

Glutathione (GSH): Take 0.2 ml tissue homogenate made by (0.1 M Phosphate buffer, 7.4 pH, 10%) add 20% TCA and 1mM EDTA incubate for 5 minute. This was centrifuged at 2000 rpm for 10 minutes and supernatant (200 µl) is collected and shifted to a tube. 1.8 ml of Ellman's reagent (0.1 mM) was made in 0.3 M phosphate buffer with pH 7 having 1% sodium citrate. Keep the mixture at 0-5 °C. Make total volume to 2 ml with water, at 412 nm OD was taken keeping water as blank [14].

Superoxide Dismutase (SOD): Take 0.2 ml of tissue homogenate 10% w/v prepared in 0.15 M tris or 0.1 M Phosphate buffer, centrifuge for about 15 min at 15000 rpm at 4 °C. 0.1 ml of supernatant is collected and mix with 1.2 ml sodium pyrophosphate buffer (8.3 pH, 0.052M), 0.1 ml phenazine methosulphate (185 µM), 0.3 ml nitrobluetetrazolium (300 µM), 0.2 ml NADH (750 µM). Mix 0.1 ml glacial acetic acid to the above mixture and finally added in 4 ml butanol solution, keeping it for 10 minutes. The whole mixture was centrifuged, and the butanol layer is separated, OD is taken at 560 nm with butanol as blank [15].

$$\% \text{ Inhibition} = (\Delta \text{ Absorbance}_{\text{control}} - \Delta \text{ Absorbance}_{\text{sample}}) \times 100 / \Delta \text{ Absorbance}_{\text{control}}$$

Control possess reaction mixture except for sample, taking buffer/water in place of sample (1.5 unit/assay of purified enzyme gives 80% inhibition)

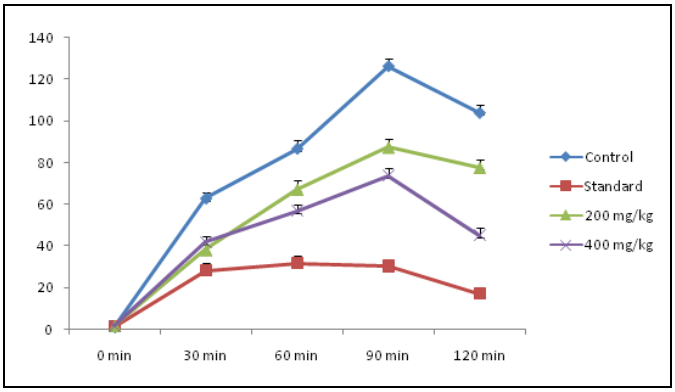
$$\text{U/ml} = \% \text{ Inhibition} \times 3.75$$
$$\text{U/gm tissue} = \% \text{ Inhibition} \times 3.75 \times (1 \text{ per gm tissue used})$$

Statistical Analysis: The data was illustrated as Mean ± SEM. Significance was estimated by one-way analysis of variance (ANOVA) followed by Multiple Comparison Procedures (Bonferroni t-test). Results were considered significant at ($P<0.001$).

RESULTS:

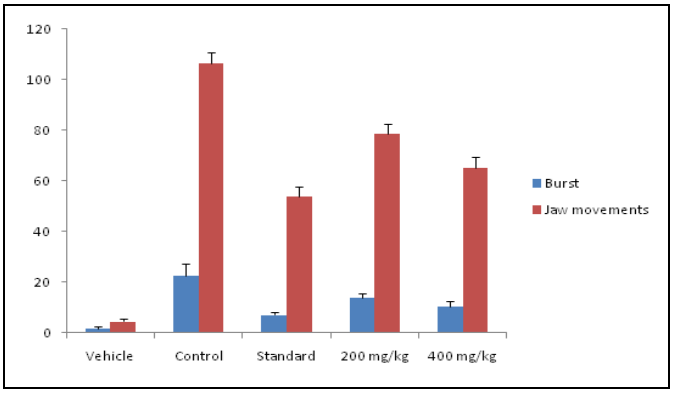
Evaluation of Behavioral Parameters: Haloperidol-induced Catalepsy in Rats: Haloperidol, a neuroleptic drug a dopamine D2 receptor antagonist, gives rise to catalepsy in rats, the symptoms related to akinesia of PD. Any drug which increases dopamine secretion or inhibits the haloperidol-induced catalepsy can be used for the treatment of PD [16]. The groups under study found to be significant ($P<0.001$) as shown by **Graph 1**.

There should be a gradual decrease between 30-120 minutes, having maximum catalepsy time at 90 minutes, and 120 being the lowest. Dosing took place for 5 days and showed that Group I (Control group) which was given Haloperidol (1 mg/kg i.p) showed the highest catalepsy score at 120 min. Group II was given a standard drug having the minimum catalepsy score of all. Group III and IV were administered with 200, and 400 mg/kg showed a significant reduction in the duration of catalepsy, in which 400 mg/kg value was found close to standard.



GRAPH 1: CATALEPSY SCORE WITH HALOPERIDOL TREATMENT IN *CYAMOPSIS TETRAGONOLOBA* Results were expressed as mean± standard error of mean (SEM), analyzed by one way Anova (ANOVA) n=6, followed by bonferroni test. *P<0.001 shows Statistical significance as compared to the Control group.

Tacrine Induced Vacuous Jaw Movements: Tacrine an anti-cholinesterase inhibitor produces motor functions resembling PD symptoms. Animals were divided into 5 groups of 6 animals and given tacrine except for vehicle group and jaw movements, and orofacial bursts were noted. The maximum results of movements were observed in the first 30-40 minutes after giving tacrine. The result revealed in **Graph 2** shows that Tacrine (control group) showed maximum value whereas the lowest movements were seen in standard (levodopa + carbidopa). Pre-treatment with 200 and 400 mg/kg *Cyamopsis tetragonoloba* extract showed a significant reduction in tacrine-induced Vacuous Jaw Movements and bursts. The results were taken using Anova (ANOVA) and represented as mean ± Standard error of mean (SEM) further bonferroni test applied. A value ($P<0.001$) is considered significant.

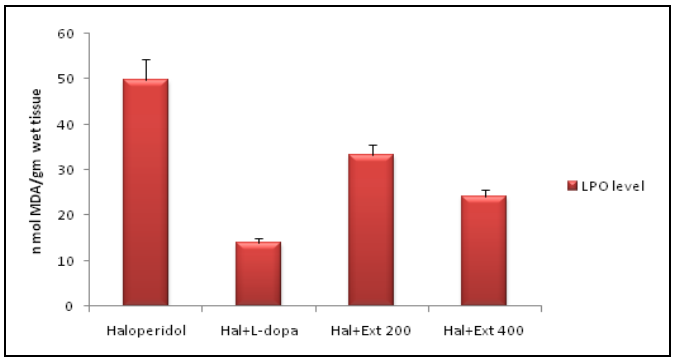


GRAPH 2: EFFECTS OF *CYAMOPSIS TETRAGONOLOBA* PLANT EXTRACT ON TACRINE INDUCED VACUOUS JAW MOVEMENT AND OROFACIAL BURSTS

Evaluation of Biochemical Parameters: In this analysis we estimate endogenous antioxidant enzymes present in the brain of rats; it is an important parameter that determines the antioxidant activity in Parkinson's disease. Brain tissue homogenate was prepared, and the following test were performed.

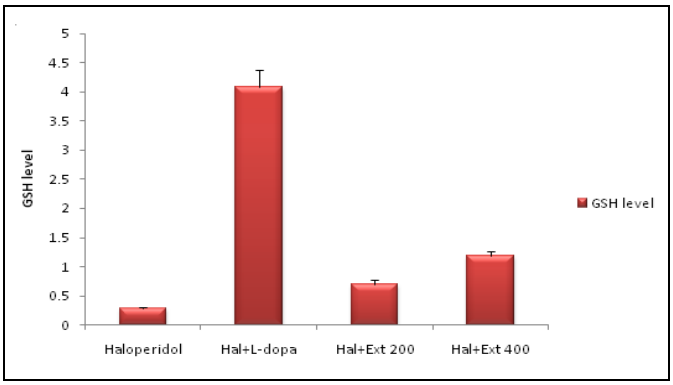
Lipid Peroxidation (LPO): Lipid peroxidation is a degradation of lipid membrane by free radicals, and generating a chain reaction, the end product of this reaction, malondialdehyde (MDA) is specifically harmful and damages biomolecule initiating oxidative stress [17]. In lipid peroxidation assay, MDA reacts with thiobarbituric acid (TBA) and produces TBARS, which is measured to determine the level of lipid peroxidation or MDA levels. In our studies of 4 groups, we used haloperidol in group I, which shows the highest MDA levels in the tissue homogenate of the brain; the standard group has the lowest value while the rest of the

groups showed a significant reduction in MDA levels when compared to the haloperidol group. The *Cyamopsis tetragonoloba* extract (200 mg/kg) had higher MDA as compared to (400 mg/kg) extract, as shown in **Graph 3**. The result suggests that methanol extract was found effective in reducing oxidative stress in brain tissue in **Table 1**.



GRAPH 3: LPO LEVELS OF *CYAMOPSIS TETRAGONOLOBA* PLANT EXTRACT

Glutathione (GSH): GSH is a very responsible enzyme and reduces the reactive oxygen species by converting to GSSG (oxidized); loss of Glutathione can cause oxidative stress and further neuronal loss inside the brain [18]. In the study on tissue homogenate shown in **Table 1**, it was found that haloperidol group has decreased GSH value as compared to the Standard group, which showed maximum value indicative of retention of the enzyme. At the dose of 200 mg/kg of *Cyamopsis tetragonoloba* extract, the GSH level has shown to increase and with an even higher dose of 400 mg/kg GSH levels increase further **Graph 4**. The results showed the effectiveness of guar extracts when compared to standard in a dose-dependent manner.



GRAPH 4: GSH LEVELS OF *CYAMOPSIS TETRAGONOLOBA* PLANT EXTRACT

Superoxide Dismutase (SOD): Superoxide radical is another parameter for oxidative stress and should be checked to determine oxidative stress in the brain and to control symptoms of PD.

Superoxide dismutase act as an antioxidant to revert the action of superoxide radical, Superoxide radical is generated by NADH (reduced b-nicotinamide adenine dinucleotide) acting as reductant, having PMS as a catalyzer. Nitro blue tetrazolium (NBT) reduces in the presence of superoxide radicals, so the % inhibition of NBT is taken as a parameter to evaluate SOD activity [19].

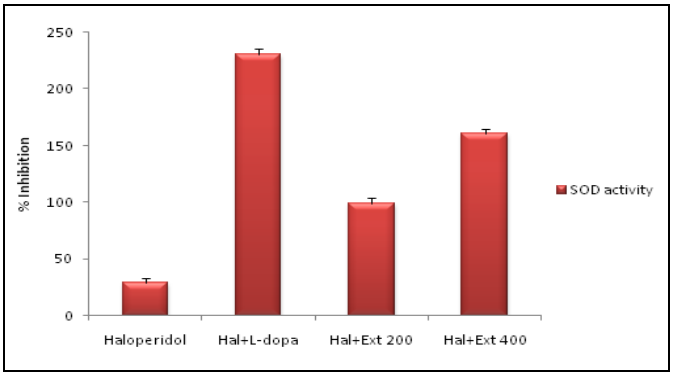
TABLE 1: VARIOUS GROUPS SHOWING BIOCHEMICAL ANALYSIS ON BRAIN TISSUE HOMOGENATE

Groups	Doses/kg	LPO	SOD	GSH
1. Haloperidol group	1 mg/kg	49.73±4.582	28.777±4.622	0.277±0.031
2. Haloperidol + L-dopa & carbidopa	1 mg/kg + 125 mg/kg	13.866±1.230*	230.61±5.18*	4.078±0.290*
3. Haloperidol + Methanol extract 200	1 mg/kg + 200 mg/kg	33.266±2.282*	99.18±5.16*	0.698±0.078*
4. Haloperidol + Methanol extract 400	1 mg/kg + 400 mg/kg	24.133±1.552*	160.99±3.87*	1.1758±0.080*

The superoxide dismutase activity was shown by the group I was not very high as the animal was diseased, the standard group have an increased level of SOD activity as compared to diseased.

The SOD levels were increased for plant extracts in dose-dependent manners, as 200 mg/kg of *Cyamopsis tetragonoloba* showed less SOD value as compared to 400 mg/kg **Graph 5**.

This showed in **Table 1** that with increased concentration of extract, the SOD activity increases.



GRAPH 5: SOD ACTIVITY OF *CYAMOPSIS TETRAGONOLOBA* PLANT EXTRACT

DISCUSSION: Parkinson's disease has been characterized by many motor and non-motor dysfunctions; by addressing some motor dysfunctions, the life quality of PD patients can be improved greatly. The plant pod extracts of *Cyamopsis tetragonoloba* that belongs to the legume family have been tested before and found that they are rich in antioxidant potential. This family is even known for the accumulation of levodopa which is considered as the gold standard for the symptomatic treatment of PD. The pod extract of guar was tested for two famous PD models, haloperidol-induced catalepsy and tacrine-induced vacuous jaw movements, haloperidol, and tacrine drugs that produce symptoms that resemble the symptoms of PD.

Haloperidol catalepsy was observed in rats and found that pre-treatment by *Cyamopsis tetragonoloba* pod extract for 5 days orally has shown significant ($P<0.001$) reduction in the catalepsy score of rats when compared with the control group. On the other hand, tacrine produces uncontrolled jaw movements and bursts resembling motor symptoms of PD. The results showed that the jaw movements and bursts decreased when the time increases, and data were collected between a one-hour time duration, and the extract showed effectiveness with decreased movements. The plant was proved to exhibit Anti-parkinson's activity in terms of behavioral analysis.

While dealing with neurological disorder oxidative stress cannot subside, oxidative stress is an important and underlying mechanism in Parkinson's disease. Studies revealed the absence of various important antioxidant enzymes in the brain of PD patients, such as Glutathione and Superoxide dismutase. Further increased in lipid peroxidation was found to produces a chain reaction and leads to oxidative stress. Plant extracts possess natural antioxidant which can be used to reduce oxidative stress. Animals were pre-treated with *Cyamopsis tetragonoloba* pod extract orally and induce PD on the last day and antioxidant activity was measured using *in-vivo* antioxidant enzymes assay. In several pre-clinical stages of PD patient's levels of glutathione were observed, and in the advanced PD stage, there was found a decrease in glutathione level in substantia nigra region [5]. To see the glutathione levels, we administered rats with 200 and 400 mg/kg extract doses of plant, and later tissue homogenate of the brain were examined and showed that the levels of glutathione had increased significantly compared to the one with diseased. Similarly, SOD activity was tested, and the improvement in SOD levels in the brain was observed, which caused a decrease in oxidative stress in the plant extract-treated group. Lipid peroxidation produces harmful MDA as the end product reduces effectively after treated with plant extract. In our studies, the higher concentration of the extract was found very effective in reducing the symptoms of PD in rats. The present study proves that *Cyamopsis tetragonoloba* pod extract is effective in reducing oxidative stress and shows Anti-parkinson's activity.

CONCLUSION: The given studies proved that *Cyamopsis tetragonoloba* plant pod extract exhibits Anti-parkinson's activity owing to the plant's natural antioxidant potential. The extract was able to control the behavioral symptoms associated with PD whereas effectively reducing oxidative stress, which can help to improve the condition of PD patients. The studies can further be conducted to isolate and separate the components responsible for the action, and research needs to be done to understand the detailed mechanism.

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CONFLICTS OF INTEREST: The authors declare that they have no conflict of interest

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