# Evaluation of Psychopharmacological activities of Buchanania cochinchinensis seeds

**Soumya Chaudhary[1*], Poonam Yadav[1], Purnima Tiwari [1]**

*[1]Department of Pharmacy, Rameshwaram Institute of Technology and Management, Lucknow, Uttar Pradesh 227202, India*

Corresponding Author: soumyachaudhary055@gmail.com

**Abstract**

Psychosis is a significant mental disorder characterized by a partial loss of reality awareness. Medicinal plants, such as Buchanania cochinchinensis seeds, have been used to treat various ailments in traditional medicine. The methanol extract of Buchanania cochinchinensis seeds was screened for phytoconstituents like steroids, terpenoids, flavonoids, tannins, and saponins compounds.The antipsychotic screening was conducted on adult male Swiss albino mice. A standard bar test was used to measure haloperidol-induced catalepsy. The study reveals that the test drug extract Buchanania has a significant reduction in the cataleptic score for catalepsy induced by haloperidol in mice. Histopathological changes were observed using the staining method.

## Introduction

Psychosis is a significant illness among the different CNS disorders. The term "psychosis" describes a group of mental disorders characterized by a partial loss of reality awareness (Fig 1.1). The pharmacological treatment of psychotic diseases is frequently ineffective, despite significant advancements in therapeutic choices over the past century. Some people look for a more holistic approach to treatment, others anticipate that alternative medicines have fewer or no side effects, and many people with chronic mental health issues are understandably disappointed by the seeming inefficiency of conventional treatment. Medicinal plants are used either in addition to or as an alternative to orthodox medicine. Consequently, a large number of researchers are researching natural plants to treat neuropsychiatric disorders [1]. Numerous traditional botanicals used to treat psychosis have been shown to be effective in treating laboratory animals' positive, negative, and cognitive deficits associated with schizophrenia [2].

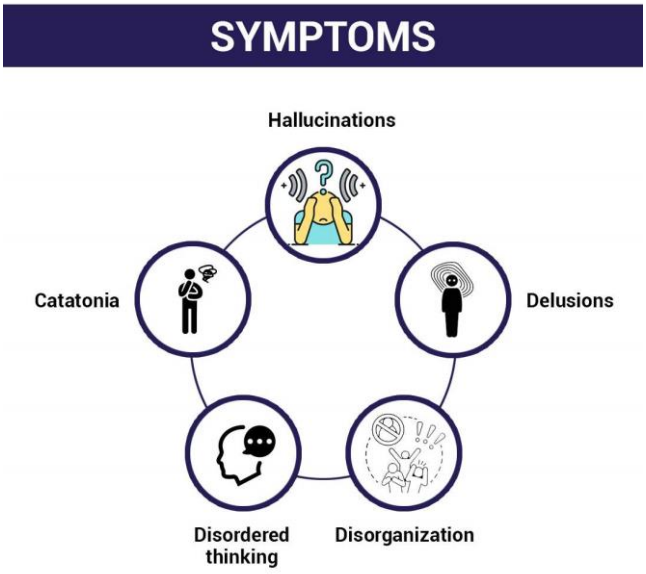


**Figure 1: Different indications of psychosis**

*Buchanania lanzan Spreng*, sometimes known as chironji, is a species of multifunctional tree. It is a member of the Anacardiaceae family and was born on the Indian subcontinent. It has long been used to treat a wide range of ailments in traditional medicine (Figure 2). According to traditional indigenous knowledge, practically every part of the plant—including the gum, seeds, leaves, fruits, and roots—has enormous therapeutic worth [3].

Seeds are frequently consumed and sold by the indigenous people as a means of subsistence and income. 3.0% moisture content, 59.0% lipid/fat, 19.0–21.6% protein, 12.1% starch/carbohydrate, 3.8% fibre, iron (8.50 mg), minerals (phosphorus, 528.0 mg, calcium, 279.0 mg), vitamins (thiamine, 0.690 mg, niacin, 1.500 mg, ascorbic acid/vitamin C, 5.00 mg, riboflavin, 0.530 mg), and 34–47% fatty oil are all present in the seeds [4].

Furthermore, the seeds are employed as an expectorant and tonic. To cure skin issues and remove blemishes and defects from the face, oil extracted from the kernels is employed [5, 6]. Powdered kernels are used as an aphrodisiac and to treat fever and burning sensations when mixed with milk. Blood dysentery can benefit from the combination of bark powder and honey.[7]. *B. cochinchinensis* has been employed in pharmaceutical applications as emulsifiers, controlled release agents, and tablet binders. A grown child who has stopped receiving breast milk should be fed sugar candies, madhuka (Glycyrrhiza glabra) honey, dried paddy, and *B. Lanzan* kernels made into a sweet bolus [8].

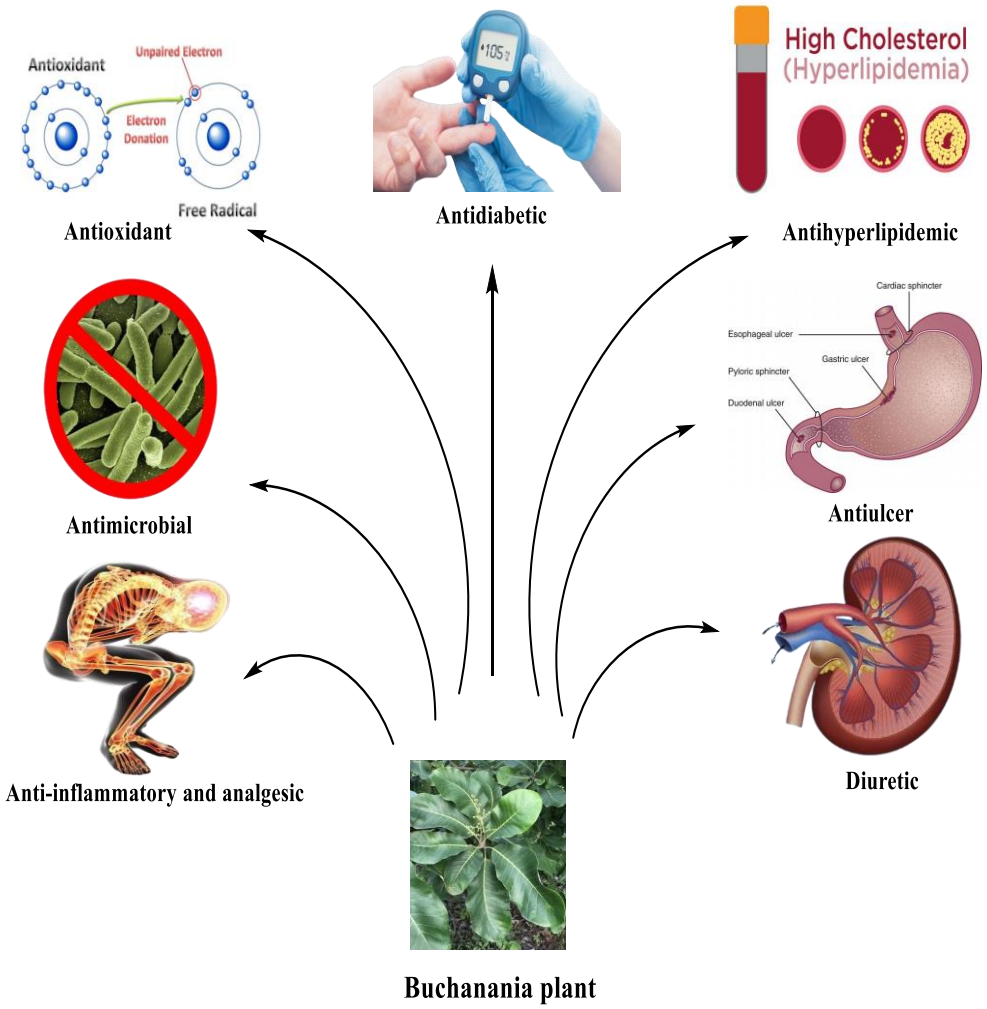


**Figure 2: An explanation of Buchanania's (Chironji) pharmacological implications**

## 2. Materials and Method

### 2.1 Collection of Drug and Chemical

Haloperidol (CAS Number: 52-86-8) and trihexyphenidyl (CAS Number: 52-49-3) drugs were obtained from Sigma Aldrich, India in powder forms.

### 2.2 Collection of Plant Material and Authentication

The Chief Scientist and Head of the Pharmacognosy Division of the CSIR-National Botanical Research Institute, Lucknow, Uttar Pradesh, Dr. Sharad Srivastava, validated the authenticity of the Buchanania plant seeds, which were gathered from the local market in Gorakhpur.

### 2.3 Preparation of Plant Extract

The *Buchanania cochinchinensis* seed would be gathered, shade-dried, and then ground into a powder in a grinder. After that, the material was run through #120 mesh to produce powder with a consistent size. The Soxhlet extractor would progressively extract the powdered plant material from the methanol. The powdered material and extract (residue) would be air dried below 50 °C prior to solvent extraction, and the extraction would be finished by removing a sample from the syphon tube on a TLC plate and placing it in an iodine chamber. The extract would be vacuum-concentrated, dried, and the solvent would be distilled out [9].

### 2.4 Preliminary Phytochemical Screening

The methanol extract of *Buchanania cochinchinensis* seeds was screened for the presence of various phytoconstituents like steroids, terpenoids, flavonoids, tannins and saponins compounds [10].

#### 2.4.1 Determination of tannins

A few drops of 10% ferric chloride solution (light yellow) were mixed with two millilitres (2 mL) of the extract's aqueous solution. Gallic tannins were suggested by the presence of a blackish blue hue, while catechol tannins were indicated by the presence of a greenish-blackish tint. When 0.5 ml of a 1% lead acetate solution is applied to 10 mg of extract, a precipitate is formed, which suggests the presence of tannins.

#### 2.4.2 Determination of saponins

In a test tube, 10 millilitres (mL) of distilled water were combined with three millilitres (3 mL) of the extract's aqueous solution. After tightly shaking the test tube for approximately five minutes, it was let to stand for thirty minutes, and the existence of honeycomb froth—a sign of saponins—was checked for.

#### 2.4.3 Determination of flavonoids

Ethanol (95%) was used to dissolve the extract test solution. Three to five drops of the concentrated HCl were added to this, along with a tiny bit of magnesium foil metal. The presence of flavonoids was suggested by the deep cherry red colour.

#### 2.4.4 Determination of alkaloids

Two millilitres of extract were subjected to a few drops of Hager's reagent in order to conduct the Hager test. The presence of alkaloids was shown by the formation of a yellow precipitate. To 2 ml of extract, add 2 drops of Wagner reagent, and well mix. The presence of alkaloids is indicated by a reddish colour. An orange-red precipitate was generated when 1 millilitre of Dragendorff's reagent was added to 2 millilitres of extract, signifying the presence of alkaloids.

#### 2.4.5 Determination of steroids

A test tube is filled with one millilitre of extract, the same volume of $H_2SO_4$, and thoroughly shaken. The mixture is left in the tube until it abruptly separates into two layers; the presence of steriods is indicated by the top layer being red and the bottom layer being green.

### 2.5 Antipsychotic Screening by Haloperidol-induced Catalepsy in Mice

Adult male Swiss albino mice, weighing between 20-25 grams, were used in this study and were obtained from the Animal House at Rameshwaram Institute of Technology and Management. Before the experiment, the mice were acclimatized to consistent husbandry conditions, maintained at 22 ± 3°C with a 12-hour light/dark cycle, for one week. They were provided with a pellet diet. All procedures were conducted following the guidelines for the care and use of laboratory animals, and the experimental protocol received approval from the Institutional Animal Ethics Committee (IAEC/M.PHARMA2022-24/15).

There would be six groups of animals (n=6). As the control group, group I was given 1% gum acacia solution (10 ml/kg). Group II was given haloperidol at a dose of 1 mg/kg to induce catalepsy. Group III was given the standard medication trihexyphenidyl at a dose of 10 mg/kg. Groups IV and V were given the test drug, which was a methanolic extract suspended in *Buchanania cochinchinensis* seeds at doses of 100 and 200 mg/kg, respectively. Group V did not undergo testing for antipsychotic action rather, it was tested for acute toxicity.

### 2.5.1 Procedure

All drug solutions would be given orally through a feeding tube, freshly made, suspended in 1% gum acacia solution. Haloperidol is delivered intraperitoneally at a dose of 1 mg/kg body weight thirty minutes after the medicines or vehicle have been provided. Thirty minutes before haloperidol was injected, in the acute study, the vehicle and/or medicines were provided. Standard and test medications were given once daily for Fourteen days as part of the chronic trial. The last dose was given on the fourteenth day, 30 minutes before haloperidol was administered (Figure 3).

### 2.5.2 Standard bar test

A standard bar test would be used to measure haloperidol-induced catalepsy at 30-minute intervals for 120 minutes, and then again after 240 minutes (Figure 4**)**. In order to cause a moderate level of catalepsy and allow for the identification of either attenuation or potentiation of the phenomena, haloperidol 1 mg/kg i.p. would be the recommended dose. The length of time the mouse kept an enforced stance, both front limbs extended, resting on a 4 cm high wooden bar with a diameter of 1 cm, would be used to measure catalepsy. When the animal removes both front paws from the bar or moves its head in an exploring manner, it is thought that the catalepsy has ended. The animal would be considered cataleptic and awarded one point if it kept the forced posture for at least twenty seconds. Each further twenty seconds that the animal remained in the cataleptic posture, one point would be awarded. The animals would undergo two tests, separated by thirty minutes, and only the longer period of immobility would be taken into account (11).

**(A) (B) (C)**

**Figure 3: (A) Feeding mice, (B) Treatment with Haloperidol, (C) Treatment with extract**

**Figure 4: Haloperidol-induced Catalepsy in Mice**

### 2.6 Histological Studies

Following death, animals were dissected and preserved for at least 24 hours at 10% neutral-buffered formalin saline. After a half-hour soak in tap water, each specimen was dehydrated using increasing alcohol concentrations, cleaned in xylene, and then embedded in paraffin. For histological analysis, 6μm thick serial slices were cut and stained with haematoxylin and eosin.

## 3. Results and Discussion

### 3.1 Preliminary Phytochemical Screening

The seeds of *Buchanania* contained alkaloids, steroids, flavonoids, tannins and saponins according to an initial phytochemical investigation (Table 1).

**Table 1. Phytochemical assay of *Buchanania* extract**

| **Category** | **Test** | **Buchanania extract** | |
|---|---|---|---|
| | | **Chloroform** | **alcohol** |
| Alkaloids | Hager's test | + | + |
| | Wagner's test | - | - |
| | Dragendorff's test | - | - |
| Flavonoids | Shinoda test | + | + |
| Steroids | Salkowski test | + | + |
| Tannins | Ferric chloride test | + | + |
| | Lead acetate test | - | - |
| Saponins | Foam test | NA | + |

*(-) indicates absent; (+) indicates present

### 3.2 Acute Toxicity Studies

The acute toxicity of total Methonolic extracts of *Buchanania cochinchinensis* seeds was determined as per OECD guideline no 423 (OECD, 2001). At a dosage of 200 mg/kg, the methanolic extract of *Buchanania cochinchinensis* seeds did not cause any alterations in behaviour or mortality.

### 3.3 Antipsychotic Screening by Haloperidol-induced Catalepsy in Mice:

The test drug extract's cataleptic score for catalepsy induced by haloperidol. Throughout the observation period, a substantial reduction ($P<0.001$) in the cataleptic score was found when comparing the standard medicine trihexyphenidyl 10 mg/kg and the test drug *Buchanania* at all tested dosages (100mg/kg and 200 mg/kg) to the vehicle plus haloperidol control (Table 2). *Buchanania*'s anticataleptic properties are similar to those of the common medication trihexyphenidyl (Figure 5).

A common model used to assess the extrapyramidal side effects of antipsychotic drugs is the cataleptic state, which is induced in rodents by typical neuroleptic drugs including reserpine, haloperidol, and chlorpromazine. The well-known neuroleptic haloperidol mainly functions in the mesolimbic-mesocortical pathway as a D2 receptor antagonist.

Its non-selective activity also results in the blockage of post-synaptic D2 receptors in the nigrostriatal pathway, which in animals causes catalepsy and extrapyramidal adverse effects in humans. A reliable behavioural technique for examining nigrostriatal function and how cholinergic, GABAergic, serotonergic, and nitrergic systems influence it is neuroleptic-induced catalepsy. Despite this data, a number of additional neurotransmitters, including opioids, acetylcholine, serotonin, angiotensin, and adenosine, have also been linked to the catalepsy brought on by neuroleptic drugs.

In addition to the potential functions of different neurotransmitters in catalepsy, reactive oxygen species have also been suggested to be involved in the toxicity caused by haloperidol. *Buchanania* has been shown in a number of previous behavioural tests to exhibit dopamine facilitator action. It has also been shown to have antioxidant qualities and to provide exceptional protection against lipid peroxidation. Given that haloperidol-induced toxicity has been linked to reactive oxygen species, it is reasonable to presume that *Buchanania*'s antioxidant properties may also play a role in its anticataleptic activity. Therefore, more research is needed to clarify *Buchanania*'s targets of action and potential mechanism of action utilising more experimental paradigms and neurochemical analysis.

Similar to the common medication trihexyphenidyl, the present study shows that *Buchanania* has a preventive effect against haloperidol-induced catalepsy. According to our research, *Buchanania* may be utilised in clinical practice as an adjuvant or alternative medication to prevent and treat the extrapyramidal adverse effects of antipsychotic medications. To prove it, though, more preclinical and clinical research is needed.

**Table 2. Antipsychotic activity of drug and extract by Haloperidol-induced Catalepsy**

| **Groups** | **Dose(mg/kg)** | **Cataleptic scores at different time schedule (min)** | | | | |
|---|---|---|---|---|---|---|
| | | **30** | **60** | **90** | **120** | **240** |
| II (Vehicle+Haloperidol) | 10ml+1mg | 14.0±0.56 | 14.9±0.16 | 14.0±0.67 | 13.3±0.31 | 11.2±0.11 |
| III (Trihexyphenidyl+Haloperidol) | 10mg+1mg | 11.52±0.32 | 9.01±0.22 | 7.62±0.52 | 5.07±0.10 | 4.01±0.07 |
| IV (Extract+Haloperidol) | 100mg+1mg | 14.02±0.55 | 13.80±0.54 | 13.23±0.41 | 12.63±0.28 | 12.08±0.72 |
| V (Extract+Haloperidol) | 200mg+1mg | 13.09±0.70 | 12.71±0.37 | 11.99±0.44 | 10.10±0.60 | 9.99±0.36 |

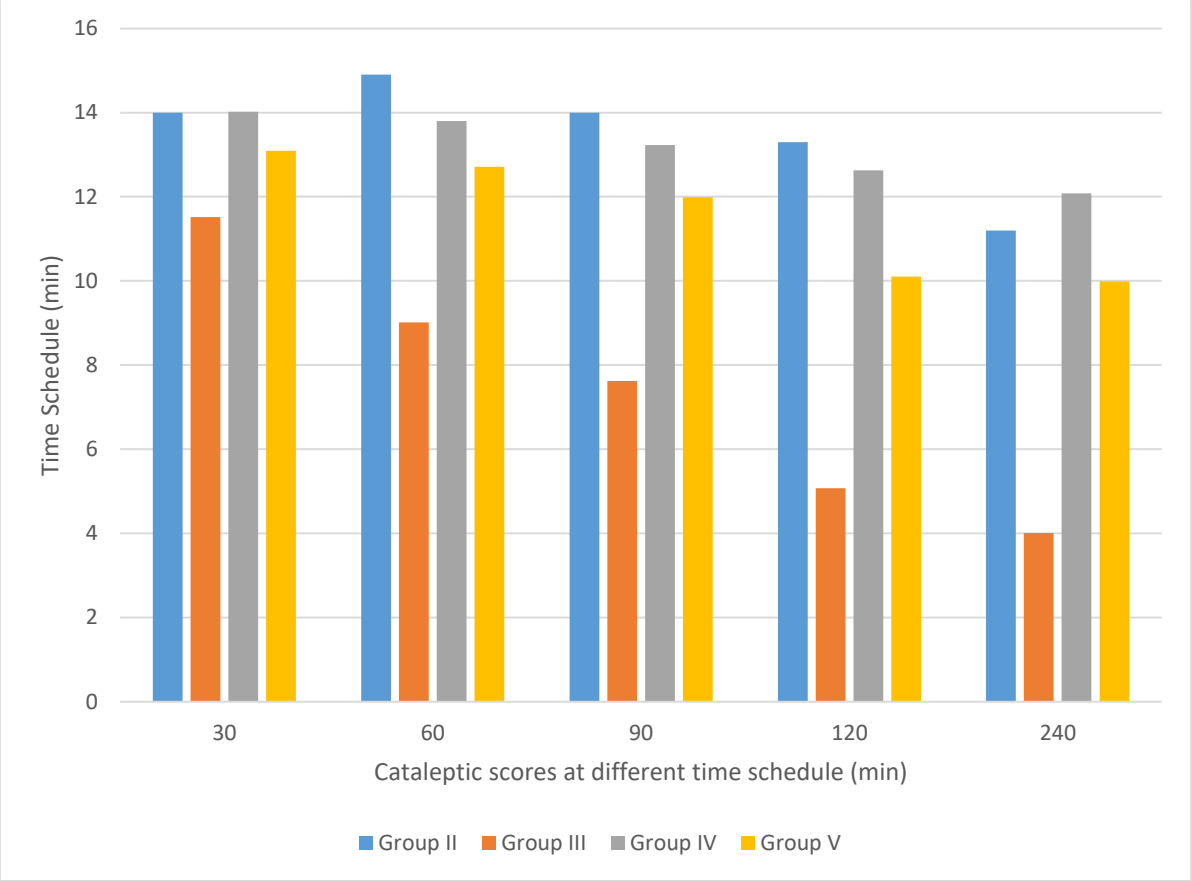


**Figure 5: Antipsychotic activity of drug and extract on mice cataleptic scores at different time schedule (min)**

### 3.4 Haematological Analysis

**Effect of *Buchanania cochinchensis* haematological parameter in mice**

When comparing treated mice with doses of 100 mg/kg and 200 mg/kg, there were no discernible and toxic effects on any of the haematological parameters in the cortex, including WBC(m/mm³), RBC(m/mm³), HGB(g/dl), Platelet(%), HCT(%), TLC(%), Neutrophils, Basophils, Monocytes(%), MCHC(g/dl), and Neutrophil counts (Table 3).

**Table 3. Effect of methanolic extract of *Buchanania cochinchensis* on haematological parameters in mice**

| S. No | Parameters | Control | *Buchanania cochinchensis* Treated group | |
|---|---|---|---|---|
| | | | Test Sample 1 | Test Sample 2 |
| 1 | HGB(g/dl) | 12.6 | 13.4 | 12.5 |
| 2 | Platelet Count(%) | 726 | 979 | 710 |
| 3 | Neutrophils (%) | 55 | 65 | 70 |
| 4 | Monocytes (%) | 6 | 3 | 3 |
| 5 | HCT (%) | 39.5 | 40 | 33.9 |
| 6 | TLC (%) | 13.5 | 11.2 | 14.7 |
| 7 | Basophils | 0.00 | 0.00 | 0.00 |

| | | | | |
|---|---|---|---|---|
| **8** | MCHC (g/dl) | 31.9 | 33.5 | 36.9 |
| **9** | Neutrophils Count | 7.42 | 7.28 | 10.29 |
| **10** | MCV (fl) | 50.1 | 49.0 | 50.2 |
| **11** | Eosinophils (%) | 4 | 2 | 2 |
| **12** | Eosinophils count | 0.54 | 0.22 | 0.29 |

### 3.5 Histopathology Analysis

The histology results are displayed in Figure 6. The histological results of the current investigation showed that the *Buchanania*'s extract protected against neuronal necrosis, neuronal density, intracellular spaces, and neutrophil infiltration in brain. As a result of the *Buchanania*'s extraction therapy's success in reversing the brain changes and bringing them back to favourable or almost normal levels.

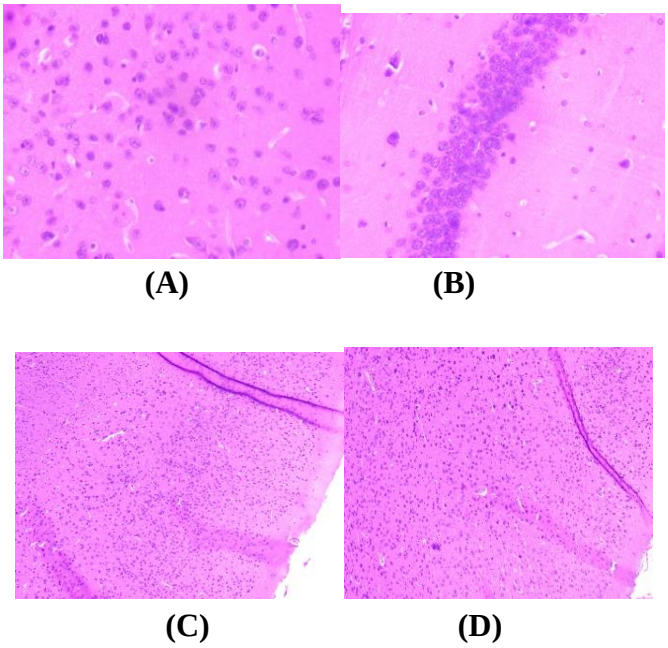


**Figure 6: Effect of *Buchanania* extract on histopathology of brain tissue in mice (at 40×). (A) normal control (B) disease control (Hippocampus) (C) Extract 100 mg/kg (D) Extract 200 mg/kg**

### 4. Conclusion

A superb tree for agroforestry, chironji, also known as Charoli, is a member of the Anacardiaceae. Its numerous applications and ability to endure harsh weather conditions make it extremely significant. It contains flavanoids, which are used to treat a variety of central nervous system conditions. Therefore the *Buchanania* extract antipsychotic activity was investigated by Haloperidol-induced Catalepsy in Mice. Numerous preceding behavioural tests have demonstrated that *Buchanania* exhibits dopamine facilitator effect. Additionally, it has demonstrated antioxidant properties and outstanding protection against lipid peroxidation.

Since reactive oxygen species have been connected to haloperidol-induced toxicity, it makes sense to assume that *Buchanania*'s antioxidant qualities contribute to its anticataleptic action. Therefore, more study is required to elucidate *Buchanania*'s targets of action and possible mechanisms of action through the use of more neurochemical studies and experimental paradigms.

Similar to the well-known drug trihexyphenidyl, the current investigation shows that *Buchanania* has a preventive effect against haloperidol-induced catalepsy. According to our research, *Buchanania* may be utilized in clinical settings as an adjuvant or replacement medication to treat and avoid the extrapyramidal side effects of antipsychotic medications. To support it, nevertheless, more preclinical and clinical research is needed.

**Conflict of Interest**

The authors have no conflict of interest regarding this investigation.

**References:**

1. Salem AA, El Shahawy NA. 2020, Hippocrates's Advice and Nutritional Secrets. Open J Nutr Food Sci. 2(1): 1010.
2. Oritsetimenyin Otimenyin S, Doosuur Ior L. Medicinal Plants Used in the Management of Psychosis. Complementary Therapies. IntechOpen; 2022.
3. Neeraj, Vinita Bisht, Shalini Purwar. 2020. Chironji (Buchanania lanzan) Wonder Tree: Nutritional and Therapeutic Values. Int.J.Curr.Microbiol.App.Sci. 9(02): 3033-3042.
4. Singh J, Patra AK, Nandeshwar DL, Meshram PB, Negi KS. Effect of growth regulators on the rooting of root cuttings of Chironji (Buchanania lanzan Spreng). Proceedings of National Workshop on Conservation of Medicinal Plants; 2002. p. 128.
5. Choubey A, Prasad R, Choubey OP, Pant NC et al., 1997, Some aspects of germination studies in Buchanania lanzan Spreng. seeds. J Tropical Forestry. 13(11):65-73.
6. Siddiqui MZ, Chowdhury AR, Prasad N, Thoma, M. 2014, Buchanania lanzan: a species of enormous potentials. World J. Pharm. Sci., 2(4): 374-379.
7. Warokar AS, Ghante MH, Duragkar NJ, Bhusari KP. Anti-inflammatory and antioxidant activities of methanolic extract of Buchanania lanzan Kernel. Indian J Pharm Educ Res 2010;44(4):363-8.
8. Gaikwad N, Kulkarni G, Gowthamarajan K, Kumar E. 2013. Development of controlled release spheroids using Buchanania cochinchinesis Gum. J Excipients Food Chem 4 (1): 4-11.
9. Nagulwar VP, Deshpande SA. 2020, Phytochemical Screening and Evaluation of Pharmacological Activities Of Buchanania Lanzan Spreng Leaves. IJPSR, 2020; Vol. 11(1): 156-162.
10. Mehta, Shalini Kapoor, Swarupananda Mukherjee and B. Jaiprakash. “Preliminary Phytochemical Investigation on Leaves of Buchanania Lanzan (Chironji).” (2010).
11. Nishchal BS, Rai S, Prabhu MN, Ullal SD, Rajeswari S, Gopalakrishna HN. 2014, Effect of Tribulus terrestris on Haloperidol-induced Catalepsy in Mice. Indian J Pharm Sci. 76(6):564-7.