Original Research Article

Neurobehavioral Evaluation Of Ethanolic extracts from *Newbouldia Laevis* leaf in 6-hydroxyldopamine- lesioned rat model of Parkinson’s disease treated with L-DOPA

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The regeneration of the neuronal system in the injured brain is necessary for the therapeutic treatment of neurodegenerative disorders. In this study we evaluated the effect of the ethanolic extract of *Newbouldia laevis* on the central nervous system of rats. The extract considerably ameliorated apomorphine-induced rotational behaviour. The lesioned rats displayed contralateral rotation after the administration of apomorphine. Furthermore, pretreatment with different doses of the extract reduced the rotations in a dose-dependent manner. In another experiment, the extract relieved the declined dopamine level in the 6-hydroxydopamine (6-OHDA) – injured striatum, while the levels of dopamine in rats without 6-OHDA lesions were not altered. Also, animals that received the extract and exposed to chronic stress in levodopa (L-DOPA) – treated - 6 – OHDA lesioned displayed further decrease in the level of dopamine. The extract was also found to cause considerable reduction of spontaneous motor activity, revealing the neuroprotective and neuro-rescue properties rested in the plant extract. This decrease in activity is closely related to sedation resulting from depression of the central nervous system. Similarly, the loss of tyrosin hydroxylase (TH)-immunopositive neuronal cell induced by the exposure of rats to chronic stress was restored by the administration of the extract 48.4%, 72.6% and 94.6% dose dependently. These findings offered insights into the understanding of the Parkinson’s disease process, its cause or origin. The extract has equally stepped down the prevailing neurodegeneration in Parkinson’s disease.

**Keywords:** *Newbouldia laevis* extract, apomorphine lesioned rats, chronic stress, 6-OHDA – injured striatum, L-DOPA, TH-immunopositive neuronal cell.

INTRODUCTION

Parkinson’s disease (PD) is a disorder of the central nervous system (CNS) that affects movement including tremors. Nerve cell damage in the brain causes dopamine (DA) levels to drop, leading to mortal symptoms of parkinson’s disease which often starts with a tremor in one hand, which may be followed by other symptoms such as slow movement (bradykinesia), stiffness and loss of balance. Others are rigid muscles, speech changes and impaired posture. These motor manifestations can be partnered by non- motor symptoms such as impaired odoriferous organs, sleep loss and other neurodegenerative disorders (Blesa and Serge 2012; Forno 1996; Braak et al. 2004; Chaturvedi et al. 2006).

The motor and non- motor symptoms of Parkinson’s
disease have been shown to be mediated by oxidative stress (OS) (Fahn and Sulzer 2004; Chaturvedi et al., 2006; Chaudhuri et al., 2006), whose deficiency triggers an increase in the level of reactive oxygen species (ROS) and pose a major constraint in L-DOPA therapy. The auto-oxidation of L-DOPA and endogenous dopamine (DA) leads to the formation of quinone and ROS, which may increase the progression of Parkinson’s disease (Chaturvedi et al., 2006; Zhu 2004; Fahn and Sulzer 2004; Foster and Hofter 2004).

Parkinson’s disease has been described as the second most common neurodegenerative disorder which is characterized by loss of a high percentage of the dopaminergic neurons (Blesa and Serge 2014; Lees et al., 2009).

Animal models of Parkinson’s disease have been discussed by various authors devising new pharmacological approaches to therapy such as the administration of reserpine or haloperidol to animals and administration of L-DOPA (Duty and Jenner 2011).

Various anti-oxidants supplements have also been proposed to have shown to play an important role in neuroprotection (Mattson et al., 2002). Unfortunately, none of these drugs therapies have been effective or abruptly step down the neurodegeneration in Parkinson’s disease. Hence, current researches are finding treatment, preferentially using natural products which could help in preventing the prevailing neurodegeneration in Parkinson’s disease (Dawson and Dawson 2002; Chaturvedi et al., 2006).

It is estimated that over 65% of Nigerian population rely on traditional medicine, which is based on curative plants (Odin and Okwute, 2000). The awareness of the value of natural plants products in the development of new drugs is on the increase (Cox, 1990).

*Newbouldia laevis* (Bignomiceae) is shrub or small trees growing in forest areas of West Africa. It has a wide range of medicinal uses (Irvine, 1961).

In Southern Nigeria, the plant is known by the Edos as *Ikhigh* and the Ibibios call it *Itono* (Bukil 1985). The Igbo in Awka calls it *ogilisi*. It is known by the Hausa in Nigeria as *Aduraku*, while the Yorubas call it *Ikoko* (Bukil 1985). Application of the crushed leaves over the spleen cures splenic enlargement (Gbeasor et al., 1990).

In Nigeria and Ghana, the bark and the leaves are used for the treatment of breast tumors (Lorke 1983). The ethanolic extract of the leaf has been found to have anti-malarial activity against *Plasmodium falciparum* in-vitro.

*N. laevis* has been used extensively in the African traditional medicine. A decoction of the bark is given to children in Ivory Coast and Nigeria for epilepsy and convulsions, while in Guinea, the bark is used to treat snake bite. In Gabon the plant is used for headache and dysentery (Amos et al., 2002).

In this study, we evaluated the neurobehavioral effect of the ethanolic extract of *N. laevis* leaf in 6-hydroxydopamine-lesioned rat model of Parkinson’s disease. We reported the robust phytochemical constituents of the ethanolic fraction which showed the presence of flavonoids as the major constituents, revealing the robust anti-oxidant properties rested in the plant ethanolic fraction. Compounds with anti-oxidant properties have been reported to induce neuronal regeneration (Tohdo et al., 2005; Vaibhava et al., 2013). The anti-oxidant properties have equally been registered to mitigate behavioral impairment, damage, histological alterations and apoptosis in feral cerebral ischemia reperfusion and other neurological deficits (Kumar et al., 2013).

**MATERIALS AND METHODS**

**Chemicals and Drugs**

Ethanol, Hydrogen Chloride, Sodium hydroxide, Chloroform, Hexane, Ascobic acid, Saline solution, Para formaldehyde, Cryostat, Phosphate buffered saline and other chemicals used complied with international standards on health and safety as approved for commercial use by OECD- (Organization of Economic Cooperation and Development), UNEP-(United Nations Environmental Programmes). This provides the environmental credentials of the chemicals. All chemicals were obtained from different sources (Lavans, Aldrich, Nerck) and were used without further purification. In this study, the following drugs were used: Aldafaxan was from Roche Nigeria Ltd., while Desipramine, Pargyline, Apomorphine, 6-hydroxydopamine (6-OHDA), 6.4-hydroxy phenylalanine methyl ester hydrochloride) were obtained from Sigma Chemical Company, USA. All drugs were freshly prepared. Parallel control experiments were done in each case to correct possible effects caused by the vehicle alone.

**Sample Collection**

This was in the manner of previous works (Amos et al., 2002). Green leaves of *N. laevis* were collected in the month of August at Yangoji village, Abuja, Nigeria and were authenticated at the National Institute for Pharmaceutical Research and Development (NIPRD) Abuja. The sample deposited at the Herbarium have a voucher No. N-02AP-13.

The specimens were air dried on the laboratory bench. The dried leaves for phytochemical screening and acute toxicity studies were reduced to powder. The ethanolic extract was prepared for the respective text carried out in the manner of (Sofowora 1982; Emos et al., 2002). The powdered material (200g) was cold macerated with 2 liters of ethanol for 24h. The extract was filtered and evaporated in-vacuo using a rotary evaporator. The yield was 60g (30%) of greenish residue.

**Preliminary Phytochemical Test**

Phytochemical screening for the presence of flavonoid, alkaloid, saponins, antracenes, glycosides, tannins, balsans
were as previously reported by (Trease et al., 1983; Amos et al., 2002).

**Acute Toxicity Studies**

In order to minimize drug attrition rates within Pharmaceutical development as reported by Micheal et al. (2016); Kola and Landis (2004); Palmer (2012); Waring et al. (2015), over 10% of all marketed drugs between 1966 and 1999 were withdrawn as a result of their adverse effects on the Central Nervous System (CNS) (Michael et al., 2016; Fung et al., 2001; Hamdam et al., 2013). However there is the need to meet the needs and demand for novel pharmaceutical/ natural product therapies in order to adequately address the previously reported adverse effects of drugs on CNS. Acute toxicity (LD50) was determined following the method described earlier (Odin et al., 2019; Amos et al., 2002; Giridi et al., 2012; Lorké (1983). Animals were divided randomly into six groups of six mice each. The samples were administrated intraperitonealy (ip) in the range of doses 2.5, 5, 10, 25, 50, 75, 100, 250, 500 and 1000 mg/kg body weight. The animals were observed for 72h. At the end of the experiment, they were sacrificed, then autopsied and examined microscopically for any pathological changes.

**Animals**

_Sprague- Dawley_ rats were used as animal models as a result of their close resemblance to the histological and hemodynamic features of human (Fortee et al., 2018; Okazaki and Murayame 1994). All experiments performed on laboratory animal in this study followed the principle of laboratory animal care (NIH publications no 85-23, revised 2015). 100 male _Rattus norregicus domestic_a, a specie of _Sprague Dowley_ rat weighing 150-250 grams were employed and maintained at the animal facility centre of Kogi State University at standard conditions and temperature (25±1 °C) and 12 h light and 12 h dark cycle. They were allowed free access to standard laboratory food and water. The animals were accommodated four per cage and were left for one week environmental habitation. During housing, the animals were monitored twice daily for health status. No adverse events were observed (Riz et al., 2019).

**Ethical review**

The study was conducted according to the guidelines for animal research (Guid for the care and use of laboratory animals). Approval by the Ethical and Research Committee of the University was not required in this study.

**OHDA Lesion procedure**

6- OHDA Neurotoxin was used as a tool to induce selective damage to DA Neurons.We employed and modified the method of Thiele et al. (2012). The rats were anesthetized intravenously (i.v) with Alfaxan (2 mg/kg) given slowly through the lateral ear. Desipramine (anti-depressants) and pergylne (a monoaminic oxide B inhibitor with antihypertensive properties) were administered to the rats prior to injection of 6 OHDA. This was given to enhance the selectivity and efficiency of 6- OHDA induced lesions.

In addition, the desipramine functions in the reduction of 6 OHDA induced noradrenaline and 5HTP depletion (Thiele et al., 2012; Gong et al., 2003). Noradrenaline imbalance has been linked to the obstruction of memory, while 5 HTP (5-Hydroxytryptamine) can affect sleep, temperature and pain sensation (Thiele et al., 2012; Harry et al., 1998).

**OHDA Lesion Surgery**

Prior to surgery, 6- OHDA solution was prepared by dissolving 6- OHDA.Br in 1% sterile solution containing 0.2% ascorbic acid as previously reported by Thiele et al. 2012. The weights of the animals were recorded 30 min before surgery. 20 mg/kg desipramine HCl and 15 mg/kg Pargyline HCl were administered to the rats. They also received 15 mg/kg sterile saline intraperitonealy (ip), using a Hamilton syringe, 30 minutes after the administration of desipramine HCl and pergylne HCl solution, and were anesthetized using sevoflurane inhalation to maintain anesthesia until proven that they were sufficiently anesthetized. The head of the rats were shaved and lidocaine skin cream was applied before surgery. Incision was made in the skull of the animals, while 20µg 6- OHDA in saline containing 0.1% Aoscic acid was applied unilaterally into substantia nigra using an injection needle. Upon completion of surgery, the animals were removed from the operational frame and were transferred into the recovery cage until consciousness was regained from the anesthesia (Thiele et al., 2012; Jian et al., 2013).

**Exposure to chronic stress.**

This was in the manner of previous report (Zang et al. 2019; Myang Koo Lee et al., 2012). Two weeks after the 6 OHDA lesions, rats were placed individually in an electric foot shock for 20s and 0.6mA intensity of every 10 min interval. The chronic stress exposure was at 16.00 hr everyday for 30 days.

**Drug therapy/ experimental design**

The animals were randomized into seven experimental groups of 10 rats each in the manner of (Vaibhav et al., 2013; Myang Koo lee et al., 2012; Chaturvedi et al., 2006; Napatr et al., 2012; Rongfei and Ming 2021; Silva et al., 2002 but with modifications.

**GROUP 1:** Rats received 10 µL of saline containing 0.1% ascorbic acid by stereotactic injection in order to target brain areas in rats and served as control (Unlesioned)

**GROUP 2:** Rats were treated with 10 mg/kg BW L- DOPA for 28 days, two weeks after receiving 6- OHDA (10 µg/ml)
**GROUP 3:** For assessing the effect of L-DOPA, animals were treated with 15 mg/kg BW L-DOPA for 28 days, two weeks after receiving 6-OHDA (10 µg/ml)

**GROUP 4-6:** Rats were administered *Newbouldia laevis* at various doses ranging from 25, 50 and 100mg/kg BW once daily via oral route for 28 days, in L-DOPA (10 mg/kg), two weeks after receiving 6-OHDA (10 µg/ml)

**GROUP 7:** Rats were administered *N.Laevis* (25mg/kg (po) for 28 days in L-DOPA(10 mg/kg) two weeks after treatment with 6-OHDA and exposed to chronic stress.

### Neurobehavioral Assessment

Sixty days after drug treatment, assessment of neuroprotective and neurorescue prospects of *N.Laevis* was carried out using different neurobehavioral, neurochemical and immunohistochemical parameters (Charturvedi et al. 2006). Parkinson's Disease (PD) has been known to be a neurodegenerative disorder affecting dopaminergic neuronal systems with impaired motor functions. These neurodegenerative disorders including PD has remain incurable despite the great number of ongoing investigations (Vaihav et al, 2013).

However, Levo-DOPA (L-DOPA) remains the primary treatment choice. It can only relieve patient's inability to initiate voluntary movements (Vaibhav et al, 2013; Silva et al.,1997). Hence, the effect of the lesions on the neuroprotective and neurorescue prospects of *N.Laevis* was investigated.

### Rotational Behaviour

a. **Apomorphine-induced rotation in 6-OHDA lesioned rats:** Rats from different experimental groups (1-7) were subjected to the rotational behaviour. They were placed in glass cylinders and treated with subcutaneous injections of apomorphine dissolved in a 0.2 mg/ml ascorbic acid in 0.9% saline solution at doses of 0 mg/kg-15 mg/kg in the manner of (Bagga et al., 2015; Myung Koo lee et al., 2012; Jordi et al., 2015).

The number of 360° turns in the opposite direction to that of lesioned was recorded at 5 min intervals for 30 mins using a video camera. Rats having less than 150 rotations per 30 mins were removed and denied from further assessment. The greater the contralateral rotations, the more parkinsonian the rats.

b. **Apomorphine-induced rotation in non lesioned (control group) rats:**

Similarly, the non-lesioned rats were subjected to apomorphine induced rotational test at all doses (0 mg/kg-15 mg/kg) as reported by Bagga et al., 2015; Borkland and Dunnet (2019) but with modifications.

### Spontaneous motor Activity:

This was in the manner of previous reports by (Amos et al., 2012; Juan Li et al., 2017; Ling-Yun et al., 2008; Jian et al., 2013; Taesolikul et al., 1998). Rats from different experimental group were assessed for motor activity. They were singly placed in cages under a video camera. The rats were allowed to acclimatize the chambers for a period of 5 min. Their activity was recorded for 6 min at 30 min intervals for a period of 120 min using a Letica Activity Cage (LE886) connected to a multi-count (LE3806) which automatically counted the animal's movements across the bar on the cage floor.

### Tyrosine Hydroxylase Immunohistochemistry Staining.

In this study we employed and modified the method of ± Myang Koo lee et al., 2012; Akpita et al., 2015; Ling- Yun et al. 2008; Muzamil et al., 2005; Li xie et al., 2013.

Rats from different groups were anesthetized and perfused intracardially with 250ml phosphate buffered saline (P(B) 7.6). This was followed by 4% paraformaldehyde of the fixative solution (PB, P(B) 7.6). The rats were sacrificed and the brain removed from the skull and fixed overnight in the same paraformaldehyde fixative in order to preserve tissue. Cryostat was used to preserve the frozen brain, which was later sectioned using the instrument mounted inside the Cryostat (Crystals Microtome, MCM-AT Safire scientific company, Coimbators, India). The sectioned parts were washed in phosphate buffered saline (PBS) 4 times for 15 min each and incubated overnight at 4°C with primary anti-tyrosine Hydroxylase antibody [EPI533Y] (ab193083) rabbit monocloned and diluted in PBS containing 0.3% Triton x-100 a nonionic surfactant (1:300). Subsequently, the sections were rinsed in PBS and incubated for 1½ h with secondary antibody antimouse immunoglobulin G (IgG (Nexus Life care private Ltd, Naigouen East Mumbai, India) to protect against bacterial and viral infections. TH Immunoreactivity was observed with Lion Heart Fx Automated Microscope (BioTek Instruments. INC, Winooski, VT, USA).

### Statistical Analysis:

Results Data are presented as mean ±SD and proportions. Comparing quantitative variables among groups were achieved using one-way ANOVA on an IBM-computer using SPSS 10.0 software. The X² (Chi Square) was used to determine the P-values to ascertain if test results are significant.

Significant differences between group means were achieved by Students T-test. A P value< 0.05 was considered to be statistically significant.

### RESULTS

**Newbouldia laevis Ameliorated Apomorphine induced rotation**

All the experimental rats were subjected to apomorphine
induced rotational behaviour. The non lesioned rats (Group 1) were also subjected to apomorphine induced rotation at all doses (0-15 mg/kg). This confirmed that the rotation was due to lesioning of the dopaminergic and not any other factor (Bagga et al.2015). They failed to exhibit rotational behavior upon apomorphine challenge (0.00±0.00).

The results in Table 1 indicated a significant effect of apomorphine in a dose dependant manner. There was minimal rotational response when the rats in group 2 were administered 1.5 mg/kg or 3.5 mg/kg apomorphine. However, rotations at 15 mg/kg apomorphine administration produced the highest number of rotations in group 3 rats, while the same 15 mg/kg administered to group 4-6 animals gave a negligible contralateral rotational response.

Comparisons were made between control and other groups. In the lesioned groups (2 and 3) without N. Laevis treatment, there was increased contralateral turning when compared to control group animals. However, the abnormal rotation was significantly reduced particularly in groups 5 and 6 rats treated with 50 and 100 mg/kg N. laevis respectively.

Similarly, in the groups 5 and 6 animals treated with 50 and 100 mg/kg N. Laevis in 10 mg/kg L-DOPA, the rotation at 30min were 7.51±0.80 and 3.89±0.1 respectively. These values were close to those of non-lesioned rats.

A significant increase in the number of apomorphine induced rotations were noticed in the lesioned groups (2 and 3) compared to control group animals. It was also identified that Newbouldia Laevis significantly reversed this abnormal behavior (25 mg/kg N.L+6 OHDA =14.83±1.1;

50mg/kg N.L+6 OHDA= 7.51±8.0, while 100mg/kg +6 OHDA=3.89±0.1)

These values were close to those of non lesioned rats.

Furthermore, the lesioned rats displayed contralateral rotations after the administration of apomorphine. However the pretreatment with different doses of Newbouldia laevis (25, 50, 100 mg/kg) reduced contralateral rotations in a dose dependant manner (Table 1).

**Newbouldia laevis** relieved the declined Dopamine level in the 6-OHDA injured striatum.

The levels of dopamine (DA) in rats without 6-OHDA lesions were not altered (Figure 1). However, dopamine levels were observed to increase slightly in group 2 rats treated with 10 mg/kg L-DOPA to 25% when compared with that of group 3 rats treated with 15mg/kg L-DOPA which had dopamine level of 20%.

Animals in group 7 that received 25mg/kg N.Laevis and were exposed to chronic stress in L-DOPA (10 mg/kg) treated 6-OHDA lesioned displaced further decrease in the levels of dopamine to 10%. This deficiency was recovered by 50 and 100mg/kg N. Laevis administration for 28days on groups 5 and 6 animals respectively which resulted in an improvement in the levels of dopamine in 10 mg/kg L-DOPA treated 6-OHDA lesioned to 60 and 80% respectively.

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**Table 1. Effects of Newbouldia Laevis Leaf on Apomorphine-Induced Rotational Behaviour**

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Unlesioned control + 0-15mg/kg APM</th>
<th>10mg/Kg L-DOPA + Lesioned</th>
<th>15mg/kg APM + 15mg/kg L-DOPA + Lesioned</th>
<th>25mg/kg apomorphine</th>
<th>50mg/kg</th>
<th>100mg/kg</th>
<th>25mg/kg L-DOPA + Chronic stress + Lesioned</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>1.5,3.5mg/Kg APM + 10mg/Kg L-DOPA + Lesioned</td>
<td>25mg/kg L-DOPA + Lesioned</td>
<td>100mg/kg</td>
<td>18.32±3.2</td>
<td>18.49±1.1</td>
<td>18.68±4.5</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>15mg/kg APM + Lesioned</td>
<td>14.20±6.0</td>
<td>14.51±6.0</td>
<td>14.83±1.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>15mg/kg APM + 10mg/kg L-DOPA + Lesioned</td>
<td>7.23±9.0</td>
<td>7.42±2.1</td>
<td>7.51±8.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>15mg/kg APM + 10mg/kg L-DOPA + Lesioned</td>
<td>3.71±8.2</td>
<td>3.80±5.0</td>
<td>3.89±0.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td>15mg/kg APM + 10mg/kg L-DOPA + Lesioned</td>
<td>18.32±3.2</td>
<td>18.49±1.1</td>
<td>18.68±4.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VII</td>
<td>15mg/kg APM + 10mg/kg L-DOPA + Chronic stress + Lesioned</td>
<td>18.32±3.2</td>
<td>18.49±1.1</td>
<td>18.68±4.5</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD. Significant differences between control and treated groups was One-way ANOVA. The X² (Chi square) test established P-values; P<0.05; N=10 in each group. Student's T-Test statistically analysed the differences between group means.
Figure 1: Effects of *N. Laevis* on the levels of dopamine

**KEY:**
- I = Unlesioned rats group 1 (control).
- II = Group 2 lesioned rats treated with 10mg/kg L-Dopa.
- III = Group 3 lesioned rats treated with 15mg/kg L-Dopa.
- IV = Group 4 lesioned rats administered 25mg/kg *N. Laevis* in 10mg/kg L-Dopa.
- V = Group 5 lesioned rats administered 50mg/kg *N. Laevis* in 10mg/kg L-Dopa.
- VI = Group 6 lesioned rats administered 100mg/kg *N. Laevis* in 10mg/kg L-Dopa.
- VII = Group 7 lesioned rats administered 25mg/kg *N. Laevis* in 10mg/kg L-Dopa, exposed to chronic stress.

Values are expressed as mean ±SD. Significant differences between control and treated groups was one-way ANOVA; The X² (Chi square) test established P-values. Student's T-test statistically analysed the differences between Group means*P<0.05, **P<0.01, ***<0.001. N=10 in each group.

Similarly, the rats in group 4 had 40% dopamine level in a dose dependent manner (25 mg/kg *N. Laevis*, when compared with groups 5 and 6 animals that received 50 and 100mg/kg *N. Laevis*).

Our experimental design showed that only the unlesioned group 1 animals were without L-DOPA treatment, while Group 2-7 rats received L-Dopamine treatment for 28 days, two weeks after lesioning. The levels of dopamine was noted to have been altered in the 6-OHDA lesioned rats groups treated with L-Dopamine, compared with those of group 1 rats (unlesioned). Dopamine levels in groups 3 animals decreased by 80% when the rats were treated with 15mg/kg body weight L-DOPA, while group 7 lesioned animals treated with 25mg/kg *N. Laevis* in 10mg/kg L-DOPA, and exposed to chronic stress decreased by 90% (Figure 1) compared to those of unstrressed groups.

**Effect of *Newbouldia laevis* on Spontaneous motor activity in rats**

This study was to assess the neuroprotective and neurorescue properties rested in *N. Laevis*. The ethanolic extract was found to significantly reduce the spontaneous motor activity in rats, which is an indication of the level of excitability of the Central Nervous System (Table 2). The decrease in activity exhibited by the extract is closely related to sedation resulting from depression of the central nervous system (Amos et al, 2002; Ozturk, et al. 1996).

From Table 2, the activity was typically decreased in the 6-OHDA lesioned rats compared with unlesioned group that serve as control. However, the activity was significantly reduced by 25, 50 and 100mg/kg) administration of *N. Laevis*. The L-DOPA treated groups 2 and 3 (10 and 15 mg/kg) respectively without *N. Laevis* also exhibited improvement in the motor activity dose and time dependantly. An increase in the motor activity was noticed in the group 7 rats treated with 25mg/kg *N. Laevis* but exposed to chronic stress when compared with group 4 animals that received equal amount of 25mg/kg *N. Laevis* but without exposure to chronic stress. The general observation indicated that the effect of the *N. Laevis* extracts was dose and time dependant. The decrease in activity was registered at 30min and continued to decline with increase in time. These data indicated that *N. laevis* significantly improved the impaired spontaneous motor activity in
Parkinson disease rats.

*Newbouldia laevis* Attenuated the Degeneration of TH-immunopositive Neuronal Cell.

Immunohistochemistry (IHC) has been proven to be one of the most important techniques in the characterization of neoplastic diseases in human (Arpita et al. 2015). These diseases are conditions that cause tumor growth. In our study, TH-immunopositive neuronal cell death by 6-OHDA lesions in the substantia nigra was attenuated by the administration of 25, 50 and 100 mg/kg of *Newbouldia laevis* (po) to rats for 28 days suffciently decreased the enhanced dopamine level in the unlesioned rats were not altered. However the lesioned animals treated with *Newbouldia laevis* administration to group 4-6 rats (25, 50 and 100mg/kg respectively) for 28 days protected against loss of TH-immunopositive neuronal cells in L-DOPA treated 6-OHDA lesioned rats without exposure to stress.

### DISCUSSION

The 6-OHDA lesion rat models remain the most reliable tools for the study of the consequences resulting from nigrostriatal degeneration in rats and mice (Borklomala and Dunnett, 2019). The results of our study showed the protective effect of *N. laevis* on animal model of Parkinson's disease (PD) induced by 6-OHDA. It was established that treatment with *N. laevis* at various doses once daily via oral administration to 6-OHDA- injured striatum, significantly reduced the spontaneous motor activity and attenuated the TH-immunopositive neuronal cell death by 6-OHDA lesions in the substantia nigra.

In addition, *N. laevis* ethanolic extract has been reported to possess anti-inflammatory (Amos et al. 2002), Antistress properties against chronic stress (Myung Koo Lee et al. 2012; Choi et al. 2008). In our study, contralateral turning was significantly reduced in the rats treated with *N. laevis* dose dependantly. However, the lesioned rats displayed contralateral rotations after the administration of Apomorphine. Furthermore, *N. laevis* administration 25 mg/kg for 28 days significantly decreased the enhanced neurotoxic effects induced by the exposure to chronic stress in 6-OHDA lesioned rats.

The declined dopamine level in the 6-OHDA injured striatum was relieved by *N. laevis* ethanolic fraction, while the levels of dopamine in the lesioned rats were not altered. However the lesioned animals treated with *Newbouldia laevis* administration to group 4-6 rats displayed further decrease in the levels of dopamine. This
deficiency was recovered by the administration of *N. Laevis* for 28 days, resulting in an improvement in the levels of Dopamine in L-DOPA treated 6-OHDA lesioned rats.

The ethanolic extract was noticed to significantly reduce the spontaneous motor activity in rats, indicating the level of excitability of the CNS. However, an increase in motor activity was noticed in rats treated with *N. Laevis* and exposed to chronic stress, indicating that *N. Laevis* was responsible for the improvement of the impaired motor activity in Parkinson disease animals. Besides, TH-immunopositive neuronal cell death by 6-OHDA lesions was blocked by the administration of *N. Laevis* in dose dependant manner. The lesioned rats exhibited significant reductions in TH-immunopositive neuronal cells when compared to that of the control unlesioned rats. However, the loss of TH-immunopositive neuronal cell induced by the exposure to chronic stress was restored by the administration of *N. Laevis* dose dependently. Additionally, the administration of *N. Laevis* protected against loss of TH-immunopositive neuronal cells in L-DOPA treated 6-OHDA lesioned rats that were not exposed to chronic stress.

In conclusion, our study revealed that *N. Laevis* may be useful in restoring the loss of dopaminergic neuronal cells, also in reducing the apomorphine induced contralateral turning, while the declined Dopamine level in the 6-OHDA-injured striatum was normalized.

**REFERENCES**


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