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Original Article

Effects of *Torilis nodosa* [L.] Gaertn. Methanol Extract against Rotenone Induced Parkinson Diseases in Rats

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ABSTRACT

Background: *Torilis nodosa* [L.] Gaertn. a species of flowering plant in the family Apiaceae has conventionally been used for treatment of many human diseases. Currently we have designed to explore the pharmacological evidence of its in neurodegeneration. In the present study we have aimed to investigate the phytochemical screening, *in vitro* antioxidant activities and *in vivo* anti-Parkinson effects of *Torilis nodosa* methanol fraction against rotenone induced Parkinsonism in rats. **Materials and Methods:** Fully matured plant of *Torilis nodosa* was collected shed dried and extracted with methanol. The crude methanol fraction was further fractionated with various solvents (N-hexane, ethyl acetate, chloroform and butanol) with increasing polarity. Various free radicals were used to investigate antioxidant potential of various fractions. The most active methanol fraction was forwarded for *in vivo* analysis in rats against rotenone induced Parkinson disease in rats. **Results:** Findings of the current study revealed that various fractions of *Torilis nodosa* composed of flavonoids, alkaloids, terpenoid, saponins, tannins, anthraquinones. Various fractious of *Torilis nodosa* showed potent *in vitro* antioxidant activities however methanol fraction revealed high efficacy as compare to other fractions which may be due the presence of high amount of flavonoid and phenolic constituents. Correlation study presented that methanol fraction is markedly correlated with antioxidant activities. The *in vivo* ani-parkison effects of 150 mg/kg body weight and 300 mg/kg body weight methanol *Torilis nodosa* showed significant protective effects at behavioral, physiological, and biochemical levels. Brain tissue homogenate showed that *Torilis nodosa* amended the rotenone-induced aberrations, augmented antioxidant enzyme activity, and abridged lipid peroxidation. **Conclusion:** From the findings of the current research, it is inferred that *Torilis nodosa* (300 mg/kg) revealed more marked results than *Torilis nodosa* (150 mg/kg). These findings showed that *Torilis nodosa* may be utilized as precursor in the preparation of a promising drug in reducing the risk and progression of Parkinson's disease after further analysis.

Keywords: Nervous system, Parkinson's disease, Rotenone, Serum analysis, *Torilis nodosa*.

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INTRODUCTION

Neurodegenerative disease is a term applied to a variety of conditions which result from a chronic breakdown and deterioration of neurons, particularly, those of Central Nervous System [CNS]. Neurodegenerative Diseases [ND] such as Parkinson's Disease [PD] and Alzheimer's [AD] and Multiple Sclerosis [MS] are characterized by a gradual onset of progressive symptoms including loss of memory and tremor, difficulty in learning or retaining information, inability to handle complex tasks, impaired spatial orientation and abilities, language deficits and behavioral changes (Adewusi *et al*., 2010). The greatest risk factor for ND is aging, which carries mitochondrial dysfunction, chronic immune inflammatory response, and oxidative stress (Angelopoulou *et al*., 2020; Angelopoulou *et al*., 2019), the major causes of neuronal damage and death.

Parkinson's Disease [PD] is considered as the most common neuronal destructive disease after Alzheimer's Disease [AD] and is characterized by the dopaminergic neuronal loss in the Substantia Nigra pars compacta [SNpc], as well as the accumulation of Lewy bodies and Lewy neurites in the degenerating neurons, mainly consisting of α-synuclein. PD patients display motor [bradykinesia, rigidity, resting tremor] and nonmotor symptoms [depression, dementia, autonomic dysfunction, sleep disorders]. Overall, the disease may be characterized by mixed phenotypic characteristics (Abayomi *et al*., 2013). Although, its exact etiology remains unrevealed, a complex interplay between mitochondrial dysfunction, oxidative damage, autophagy impairment, excessive neuroinflammation, and dysregulation of apoptosis has been shown to contribute to its pathogenesis (Ahmed *et al*., 2018). In

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addition, there are several environmental factors [like pesticides and neurotoxicants] and genetic factors [including mutation in the α- synuclein [SCNA], leucine rich repeat kinase 2 [LRRK2], DJ1, Parkin, PTEN induced kinase 1 [PINK-1], GBA1 genes, etc.] that have been associated with the development of PD (Atala *et al*., 2009).

From thousands of years a remarkable number of modern drugs have been obtained from natural sources, particularly from the plants. Plant based medicines have played an important role in primary health care needs of human as well as animals (Aziz-ul-Ikram *et al*., 2015). Variety of plants exhibit antimicrobial, larvicidal, anti-inflammatory and antioxidant activities due to the presence of some active compounds like essential oils, flavonoids, terpenoids, tri-terpeniods, glycosides, alkaloids and other natural phenolic compounds play a dominant role in the maintenance of human health since ancient times (Baker *et al*., 1995). Natural products play on important role in drug development programmes in the pharmaceutical industry (Brahmi *et al*., 2013).

Recently, a great number of natural medicinal plants have been tested for their therapeutic properties, showing that the raw extracts or isolated pure compounds from them have more effective properties than the whole plant as an alternative for the treatment of neurodegenerative diseases. These properties are due mainly to the presence of polyphenols, alkaloids, and terpenes, among others, that are micronutrients produced by plants as secondary metabolites (Calabrese *et al*., 2008).

Torilis nodosa is a species of flowering plant in the family Apiaceae known by the common names knotted hedgeparsley and short sock-destroyer It is an annual herb producing a hairy stem up to half a meter in maximum height. The alternately arranged leaves are each divided into several pairs of smooth-edged lance-shaped or linear leaflets and bipinnate. Peduncles sessile or very short, styles erect. Calyx teeth triangular, fruit ovoid and 2-4 mm long, distributed in Europe, Asia, North and South America (Breithaupt *et al*., 2002).

Traditionally *Torilis nodosa* is used for the treatment of gastrointestinal ailments, liver problems, respiratory tract infections like cold and cough. Literature revealed that there no such activities are reported. It is therefore selected to evaluate the Phytochemical screening and their role in prevention ND especially PD. Therefore, this project was designed to examine its antiparkinsonian properties.

MATERIALS AND METHODS

Plant collection

Torilis nodosa fully matured whole plant was collected from Goriwala areas of District Bannu. Khyber-Pakhtunkhwa, Pakistan after identification by Dr. Fizan Ullah, Chairman Department of Botany, UST-Bannu and confirmation with literature. The voucher specimens with Accession No. 140-Bot were submitted at herbarium in the Department of Botany UST Bannu. All the dust particles and impurities were swashed with running tap water. The plant was shed dried and processed into a fine mesh using grinding machine.

Extraction and Fractionation

Fine-grained powder of *Torilis nodosa* was sodden in 80% methanol and incubated for one-week at room temperature. After incubation the solution was filtered through Whatman filter paper No. 45. The filtrate was evaporated using a rotary evaporator and got crude methanolic extract.

The crud extract was first diluted in distilled water to create an aqueous extract, which was used to prepare different fractions based on polarity. Different solvents, such as n-hexane, ethyl acetate, chloroform, and butanol were used and various fractions were obtained through solvent-solvent partition technique. Different fractions were evaporated using a rotary vacuum evaporator and collected separately. The dried fractions were stored at 4°C for further investigations.

Phytochemical Analysis

Torilis nodosa plant *v*arious fractions were processed for phytochemical qualitative investigation using standard procedures. Various qualitative tests were carried out including tannins, saponins, flavonoids, terpenoids, alkaloids, phlobatannins, cardiac glycosides, coumarins, and anthraquinone for assessment of bioactive groups of phytoconstituents.

Determination of Total Phenolic Contents

Standard protocol of Atala *et al.* (2009) was adopted to find out the total phenolic constituents in various fractions of *Torilis nodosa* plant extract. Various fraction samples and gallic acid used as standard were mixed with 10 mL of the folin-Ciocalteau solution and incubated for 10 min. The mixture was added with 0.115 mg/mL Na_2CO_3 solution. Optical Density of the mixture was checked at 765 nm to measure the absorbance spectrum. The amount of total phenolic compounds was calculated as mg of gallic acid equivalents/g of dried material.

Test to Determine the Presence of Total Flavonoids

Standard procedures of Pompilio *et al.* (2022) were followed to investigate the total flavonoids constituents. According to this protocol 0.25 mL of each fraction and 15-250 g/mL rutin concentrations were used. Various fractions of *Torilis nodosa* and rutin used as standard were mixed with 1.5 mL of deionized water and 5% NH_4NO_3 and were gestated for 6 min. Then 0.2 mL $AlCl_3$ (w/v), 1.0 M Sodium Hydroxide (NaOH) were mixed and again incubated for 5 min. Optical Density of the mixture was find out at 510 nm. The dried fraction of rutin equivalent mg/g extract was used to calculate the total contents of flavonoids.

In vitro Antioxidant Tests

Evaluation of Plant Extracts for DPPH Radical Scavenging test

1,1-diphenyl di picrylhydrazine was used to assess the *in vitro* anti-oxidant activity of *Torilis nodosa* plant various fractions and ascorbic acid which were used as a standard (Khan *et al*., 2010). A stock solution of reagents was arranged by melting 0.006 mg of DPPH in 100 mL of methanol. 0.2 mL various plant fractions were assorted 2.8 mL DPPH solution and gestated for 30 min, using a spectrophotometer, the optical density was noted and considered at 517 nm. According to the formula below, the percentage of DPPH inhibition was premeditated as follows:

% DPPH inhibition=[(Absorbance of DPPH-Absorbance of sample) / (Absorbance of DPPH)] ×100

ABTS Cation Radical Test

The standard protocol of Brahmi *et al.* (2013) was used to assess the scavenging capability of ABTS free cation radicals with a minor amendment. According to this protocol we have mixed 7 mM of ABTS reagent with 2.45 mM potassium per sulphate reagent and incubated in the dark room for 8 hr. Then 50% methanol solution was used to dilute the sample after proper incubation and optical density was recorded at 745 nm of the mixture. A diluted reagent having 2.8 mL volume was mixed with various fraction and standard ascorbic acid separately and incubated for 6 min at room temperature. Optical density of each mixture was checked at 745 nm using spectrophotometer and percentage inhibition was calculated using the formula:

Scavenging impact (%)=(Control Ab-Sample Ab) / (Control Ab) × 100

Superoxide Radical Scavenging Test

In this procedure each fraction of *Torilis nodosa* and ascorbic acid used as standard was mixed with Nicotinamide Adenine Dinucleotide Reduced (NADH) reagent and 0.5 mL of Nitro Blue Trizol. PMS was added, and then incubated for 15 min at 25°C. Calculations of the absorbance spectrum was carried out at 530 nm (Kakkar *et al*., 1984; Gyamfi *et al*., 1999).

Superoxide Scavenged%=[Control Absorbance - Standard Absorbance/ Control Absorbance] x 100

Assay for Detection of H_2O_2 Radicals Reducing Effect

Standard procedure was used to determine the H_2O_2 free radical activities of ascorbic acid and various fractions of plant extract Gutteridge and Halliwell (2000). Samples prepared from various fractions and standard ascorbic acid was mixed with 0.4 mL H_2O_2 (50 mM phosphate buffer, pH 7.4), to test the reduction of the H_2O_2 free radical. Phosphate buffer was used as a blank to measure the absorbance of mixtures at 230 nm. The experiment was repeated in replicates to determine the hydrogen per oxide scavenging ability.

Hydrogen peroxide scavenging percentage=[Control absorbance - sample absorbance] /Control absorbance] *100

Total Antioxidant Activity Assessment of Various Fractions

Total antioxidant activity of various fractions and ascorbic used a standard was calculated using the standard protocol of Umamaheswari and Chatterjee (2008). 4 mM ammonium molybdate, 28 mM sodium phosphate and 0.6 M sulfuric acid was combined to form the reaction mixture. From this mixture, 0.1 mL of the reagent solution take off and mixed with 0.1 mL of the aliquot solution. The mixture was placed in a test tube lined with silver foil and incubated at 90°C for 90 min in a water bath. The optical density spectrum was noted at 765 nm using spectrophotometer. The following formula was used to compute the compound's antioxidant capacity:

% Inhibition of Samples and Ascorbic acid=[(Control OD-sample OD) / (Control OD)] * 100

Determination of Hydroxyl Radical Inhibition

The protocol of Gutteridge and Halliwell, (2000) was followed to check hydroxyl radical scavenging efficacy. Various reagents including 500 mL of 2-deoxyribose (2.8 mM), 100 mL of 300 mM ascorbate and 100 mL ferric chloride (100 mM) was combined to form the reaction mixture. To catalyze the process 200 mL of sodium phosphate buffered water (pH 7.4) was used. The whole mixture was placed for 1h at 37°C. 1 mL of an aqueous solution containing 2.8% (w/v) TCA, NaOH, and TBA was heated for 15 min. After cooling, the OD was measured at 532 nm, and the percent inhibition was used to determine the formula below:

Hydroxyl radical scavenging (%)=[(OD of control - OD of sample) / (OD of control)] ×100

In vivo anti-parkinson animal model

Approval of Ethical Recommendations

The research work was carried out in accordance with the international guideline for animal care and handling. The ethical guidelines were approved by ethical committee of Faculty of Biological Sciences, University of Science and Technology Bannu with written permission letter no. ustb/ethic/1231.

Experimental design

30 adults male Wistar rats were used for *in vivo* anti-Parkinson activities. Rats were obtained from National Institute of Health (NIH) Islamabad. Standard protocols for induction of Parkinson disease were used for the treatment of animal. Rats were randomly divided into 5 groups and rationale of the doses were selected on the basis previous published reports.

- **Group I (Control):** Received 0.5% N/saline p.o. every day.

- **Group II (Disease control group):** Rotenone in 1% DMSO (1.5 mg/kg per body weight s.c.) after every two days.
- **Group III (Standard drug control group):** Stalevo (5 mg/kg b.w.) after 24 hr.
- **Group IV (Low dose extract group):** *Torilis nodosa* methanol extract (150 mg/kg b.w.,) after 24 hr.
- **Group V (High dose extract group):** *Torilis nodosa* methanol extract (300 mg/kg b.w.,) after 24 hr.

The experiment was conducted for 21 days. During these experiment various parameters were checked to assess the behavioral effects of various groups of rats. At the end of experiment the rats were sacrificed by an overdose of xylazine and ketamine anesthesia at day 22 by cervical dislocation. Brain was removed, cleaned with phosphate buffer and kept for biochemical and histological examination at -20°C in freezer. Serum samples were collected through heart puncture. The collected serum was processed for various biochemical tests.

Evaluation of behavioral effects

During the experiment various behavioral tests were performed to investigate the protective effects of plant extract against rotenone induced Parkinson disease in rats. These tests include effects on rats body weight, and catalepsy score using standard procedures.

Effects of plant extract on oxidative parameters of tissue homogenate

To investigate the protective effects of methanolic plant extract on various parameters of tissue homogenate was investigated using standard protocols. The effects of plant extract on the reduction of lipid peroxidation, standard method of Jollow *et al.* (1973) while glutathione content was determined using procedure of Iqbal *et al.* (1996). Protocols of Chance and Maehly (1955) was used to determine Catalase activity (CAT), method of Kakkar *et al.* (1984) was followed for Superoxide Dismutase (SOD) and procedures of Joseph *et al.* (2007) was used to determined Glutathione Peroxidase (GPx).

Statistical analysis

Data were expressed as mean and Standard Error (SE). Statistical analysis of parametric data for IC_{50} was carried out using graph prism pad software. Experimental results were further analyzed for Pearson correlation coefficient between TPCs, flavonoids and different anti-oxidant assays and tested for significance by Student's t-test ($p<0.05$). SPSS ver. 14.0 (Chicago, IL, USA), Microsoft Excel 2007 (Roselle, IL, USA) and origin were used for the statistical and graphical evaluations.

RESULTS

Qualitative phytoconstituent analysis

Various bioactive groups of phytoconstituents were determined using different fractions of plant extracts. The findings of the present study revealed that methanol, butanol, ethyl acetate and chloroform contain flavonoids, alkaloids, tannins, terpenoids and saponins however N-hexane fraction was deficient in terpenoids, and tannins while terpenoids and saponins were absent in aqueous fraction.

Determination of phenolic compounds and total flavonoids constituents

Phenolic and flavonoids compounds play a crucial role in the activity of various plant extracts. The findings of the present study showed that comparatively higher amount of total phenolic compounds in methanolic plant extract (215.12±1.92 mg GAE/g) followed by butanolic (123.67±2.07 mg GAE/g), chloroform (88.80±1.05 mg GAE/g), ethyl acetate (56.78±0.98 mg GAE/g), n-Hexane (37.14±0.92 mg GAE/g) and aqueous (19.73±0.75 mg GAE/g) fractions respectively. Similarly quantitative phytoconstituent analysis revealed that maximum concentrations of flavonoids were present in methanol fraction of plant extract as compare to other fractions (Table 1).

Effect of various fraction on scavenging of various free radicals

Various free radicals were used to investigate the efficacy of various plant fractions.

DPPH free radical scavenging efficacy

The effect of various fractions of plant extracts and ascorbic acid used as standards was checked on scavenging of DPPH free radical capability. The results revealed that methanolic fraction (85.24±3.21) show maximum efficacy as compare to other fractions like butanol (73.89±2.54), chloroform (65.43±2.78),

Table 1: Total phenolic and flavonoid components various fractions.

Sample	Total phenolic components as mg gallic acid equivalent (GAE mg/g extract)	Total flavonoids as mg rutin equivalent (mg/g extract)
Methanol	215.12±1.92^{e}	47.89±1.78^{f}
Butanol	123.67±2.07^{d}	32.02±2.01^{d}
Chloroform	88.80±1.05^{c}	19.12±1.38^{c}
Ethyl acetate	56.78±0.98^{b}	10.13±0.98^{b}
n-Hexane	27.14±0.92^{a}	7.53±0.45^{b}
Aqueous	19.73±0.75^{a}	3.07±0.12^{a}

Each value in the Table is represented as Mean±SD ($n=3$) Means not sharing the same letter are significantly different (LSD) at $p<0.05$ probability level in each column.

ethyl acetate (55.13±1.90), N-hexane (45.21±1.42) and aqueous fraction (36.11±1.76) as presented in Figure 1A. Ascorbic acid (94.53±2.53) used as standard positive control higher efficacy in scavenging of DPPH free radicals comparatively of all plant fractions.

ABTS free radical scavenging assay

The ABTS free radical scavenging efficacy of various fractions of plant extracts and ascorbic acid was carried out. The findings shows that ascorbic acid (92.4±4.29) has maximum inhibition of ABTS free radicals scavenging as compare to various plant fraction viz; methanol (79.21±3.21), butanol (68.43±2.78), chloroform (59.45±2.76), ethyl acetate (53.54±1.89), N-hexane (32.76±1.66) and aqueous fraction (22.14±0.92) (Figure 1B).

Hydrogen peroxide (H_2O_2) scavenging ability

Various fractions of plant extracts were characterized for the assessment of free radical efficacy of H_2O_2. The data revealed that H_2O_2 free radicals were significantly inhibited by methanol fraction as compare to other fraction however, showed less inhibition as compare to ascorbic acid used as standard antioxidant positive control as shown in Figure 2A.

Superoxide radical scavenging activity

Superoxide free radical scavenging activity of various fraction of the plant and ascorbic acid was determined in the current study. Reports obtained presented that ascorbic used as standard antioxidant show higher percent inhibition of superoxide radical followed by various plant fraction in order of ascorbic acid <Methanol fraction <butanol fraction <chloroform fraction

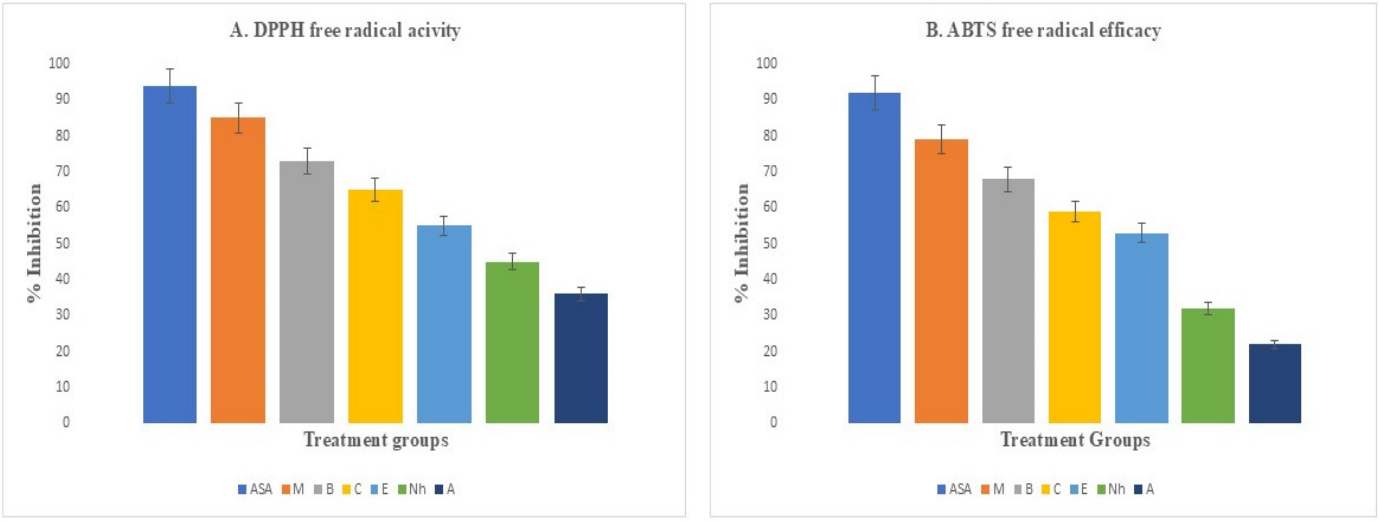

Figure 1: Antioxidant activity of various fractions of *Torilis nodosa*. (A) DPPH radical scavenging activity of different fractions compared with ascorbic acid. (B) ABTS radical scavenging activity of different fractions compared with ascorbic acid. Values are expressed as mean±SD (*n*=3).

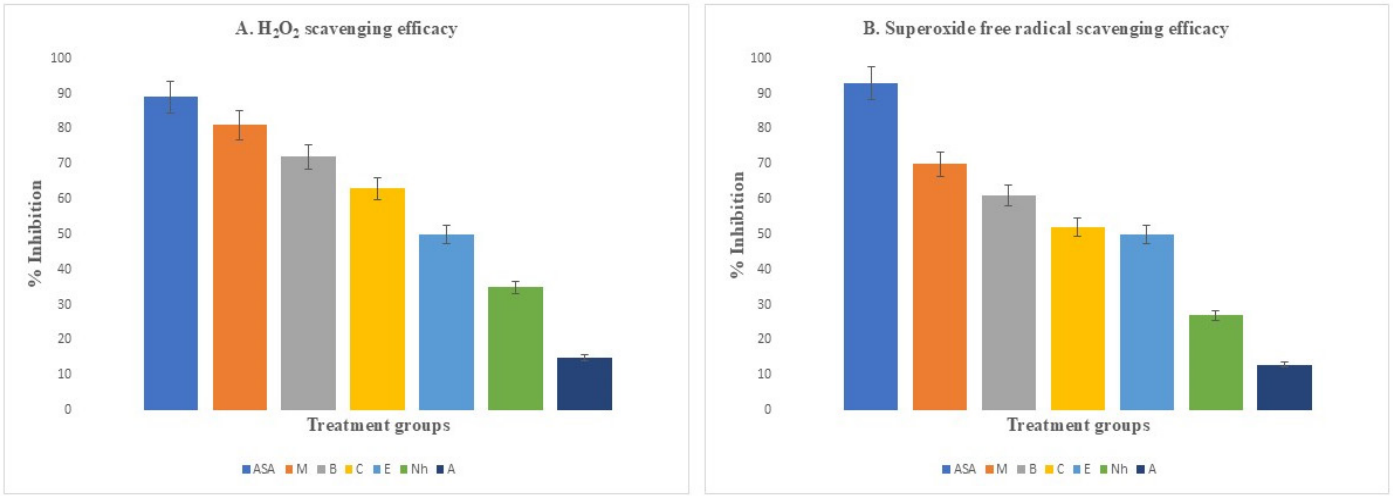

Figure 2: Antioxidant activity of various fractions of *Torilis nodosa*. (A) Effects of various fractions and ascorbic acid on scavenging of H_2O_2, (B) Superoxide radical scavenging ability of ASA and various fractions; Values are expressed as mean±SD (*n*=3).

<ethyl acetate fraction <N-hexane fraction <aqueous fraction respectively (Figure 2B).

Total Antioxidant Activity Assessment of Various fractions

Total antioxidant activity of various fractions and ascorbic used a standard was calculated as presented in Figure 3. The data showed that various fraction have the potency to remove free radical however revealed less efficacy as compare to standard antioxidant ascorbic acid. The comparative analysis among the various fractions of plant showed that methanol extract has higher potency as compare to other fractions.

Determination of hydroxyl radical inhibition of various fractions

Various fractions of plant extracts and ascorbic acid revealed were characterized using hydroxyl radicals. The findings revealed that methanol fraction (72.51±3.21) among the various fractions of plant extract showed higher hydroxyl free radical scavenging potential as compare to other fractions viz; followed by butanol (61.67±2.68), chloroform (50.31±2.11), ethyl acetate (39.21±1.89), N-hexane (23.98±0.89) and aqueous fraction (14.32±0.23) respectively as shown in Figure 4.

In vivo anti-Parkinson activity

Effects of Torilis nodosa on Body Weight

The body weight of trial rats plays a crucial part in the anti-parkinson effects of plant extracts. The rats' body weight was aimed to evaluate their overall healthiness. Induction of rats with 1.5 mg/kg body weight rotenone triggered substantial decrease in body weight of rats. Co-treatment with 150 mg/kg and 300 mg/kg body weight plant extracts and 5 mg/kg body weight stalevo significantly improved the abnormality caused by rotenone depends on the amount of dose as shown in Table 2.

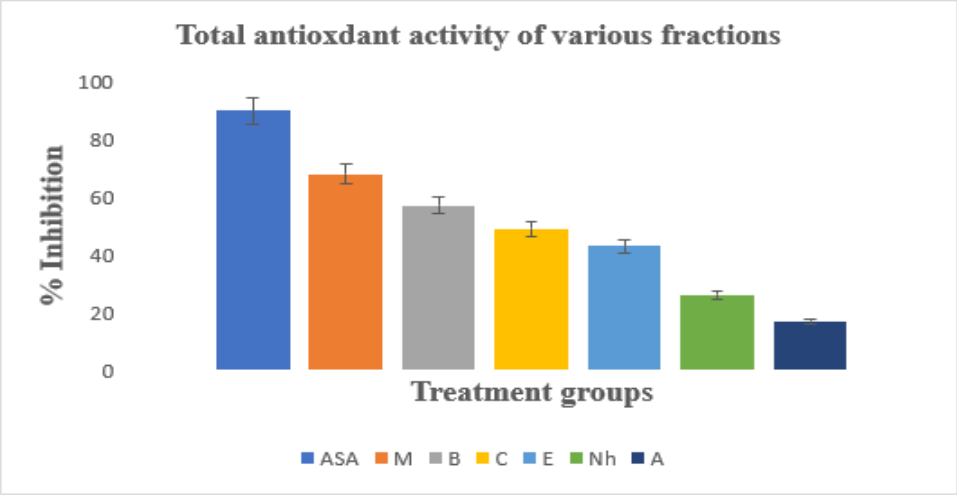

Figure 3: Total antioxidant activity of ASA and various fractions; Values are expressed as mean±SD (*n*=3).

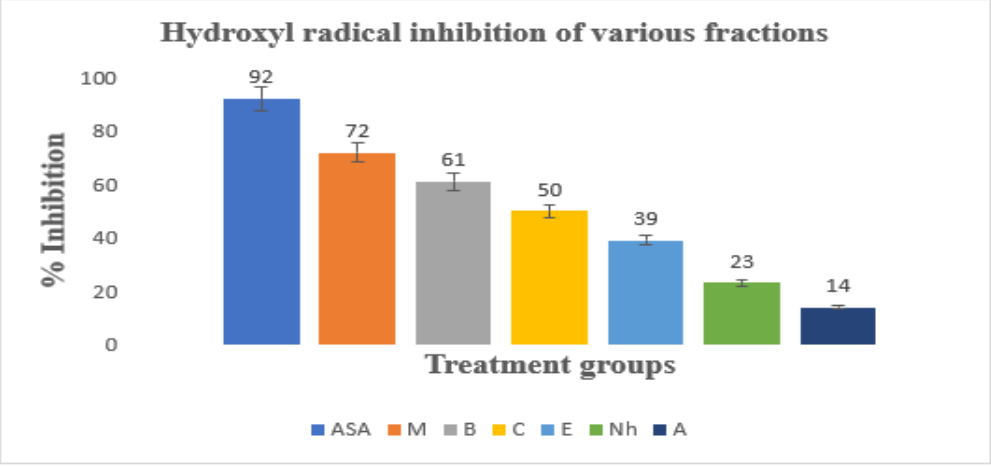

Figure 4: Hydroxyl free radical scavenging assay of ASA and various fractions; Values are expressed as mean±SD (*n*=3).

Catalepsy score

Catalepsy score plays a key role in behavioral study of Parkinson activity. In the study bar test was used to study the effects of various fractions of plant extract. Table 3 revealed that administration of 1.5 mg/kg b.w., rotenone group significantly increased cataleptic score as compared to the normal control group. Rats treated with 150 and 300 mg/kg body weight of plant extracts as well as 5 mg/

Table 2: Effects of on body weight (g).

Groups	After 7 days	After 14 days	After 21 days
Control	190.78±2.78	198.55±2.42	214.24±2.52[b]
1.5 mg/kg Rotenone	188.23±3.11	178.24±4.27	170.11±1.43[a]
1.5 mg/kg Rotenone +5 mg/kg stalevo	193.38±3.34	196.91±2.04	212.03±4.13[b]
1.5 mg/kg Rotenone +150 mg/kg *T. nodosa*	191.12±4.73	198.23±3.92	208.27±2.98[b]
1.5 mg/kg Rotenone +300 mg/kg *T. nodosa*	190.53±3.04	193.75±2.77	213.65±3.57[b]

Each value in the Table is represented as Mean±SD (n=3) Means not sharing the same letter are significantly different (LSD) at p<0.05 probability level in each column.

Table 3: Effects of on cataleptic score.

Groups	After 3rd day	After 6th day	After 9th day	After 12th day	After 12th day	After 12th day	After 12th day
Control	2.12±0.06	4.68±0.19	7.12±0.26	8.43±0.12	9.92±0.98	10.24±0.87	12.87±1.75
1.5 mg/kg Rotenone	5.21±0.09**	10.12±0.31**	13.33±0.33**	16.11±0.32**	18.23±1.14**	21.28±1.76**	25.21±1.98**
1.5 mg/kg Rotenone +5 mg/kg stalevo	3.11±0.21++	5.25±0.28++	7.23±0.71++	9.12±0.27++	10.67±0.58++	11.72±1.23++	13.74±0.89++
1.5 mg/kg Rotenone +150 mg/kg *T. nodosa*	4.42±0.12+	7.62±0.27+	8.56±0.58++	10.14±0.57++	12.54±1.02++	14.64±1.24++	16.26±2.01++
1.5 mg/kg Rotenone +300 mg/kg *T. nodosa*	2.67±0.09++	4.35±0.13++	7.11±0.08++	8.52±0.67++	9.24±1.21++	11.39±0.78++	13.10±1.02++

Mean±SE (n=3); ** revealed significant difference (p<0.01) as compared to rotenone group, +, ++ revealed significant difference (p<0.05) and (p<0.01) as compared to control group respectively.

Table 4: Effects of *Torilis nodosa* on Oxidative markers in PD model rats.

Groups	TBARS nM /min/mg protein)	GSH (M /g tissue)	SOD (U/mg protein)	CAT(U/min)	GPx (nM /min/mg protein)
Control	23.5±1.15++	33.5±1.3++	27.13±0.87++	26.70±0.82++	34.14±1.45++
1.5 mg/kg Rotenone	56.7±2.23**	16.5±1.1**	10.45±0.25**	12.01±0.91**	14.76±0.94**
1.5 mg/kg Rotenone +5 mg/kg stalevo	22.8±1.45++	32.5±1.7++	26.78±1.17++	24.97±0.78++	33.57±1.17++
1.5 mg/kg Rotenone +150 mg/kg *T. nodosa*	32.6±1.51++	23.6±2.1+	19.58±0.91+	22.23±0.88++	26.65±1.22++
1.5 mg/kg Rotenone +300 mg/kg *T. nodosa*	26.5±1.56++	31.2±1.6++	24.93±1.02++	25.29±0.95++	30.71±1.16++

Mean±SE (n=3); ** revealed significant difference (p<0.01) as compared to rotenone group, +, ++ revealed significant difference (p<0.05) and (p<0.01) as compared to control group respectively.

Table 5: Effects of *Torilis nodosa* on blood profile test.

Groups	SGPT(ALT) (U/L)	Serum Albumin (µmol/L)	Total Proteins (g/dL)	T. Bilirubin (mg/dL)	Serum Creatinine (mg/dL)	Blood Urea (mg/dL)
Control	25.45±1.87++	7.32±0.76++	9.56±0.56++	2.33±0.45++	0.89±0.45++	23.9±0.93++
1.5 mg/kg Rotenone	55.35±2.05**	2.95±0.34**	4.17±0.71**	8.21±0.57**	1.72±0.16**	43.5±0.57**
1.5 mg/kg Rotenone +5 mg/kg stalevo	28.23±0.98++	7.78±0.19++	9.98±0.99++	2.73±0.67++	0.90±0.34++	26.4±0.55++
1.5 mg/kg Rotenone +150 mg/kg *T. nodosa*	38.01±1.98+	4.98±0.28+	6.12±0.77+	6.12±0.78+	1.21±0.25+	37.7±0.71+
1.5 mg/kg Rotenone +300 mg/kg *T. nodosa*	29.67±0.95++	7.12±0.17++	9.43±0.58++	3.81±0.56++	0.94±0.38++	27.9±1.52++

Mean±SE (n=3); ** revealed significant difference (p<0.01) as compared to rotenone group, +, ++ revealed significant difference (p<0.05) and (p<0.01) as compared to control group respectively.

kg body weight stalevo, showed significantly (p<0.01) decrease against catalepsy by decreasing cataleptic score (Table 3).

Oxidative Biomarkers Test

Rat brain samples were examined for oxidative stress indicators such as antioxidant enzyme activities, TBARS and GSH contents. Rats treated with 5 mg/kg body weight rotenone caused significant increase (p<0.01) in TBARs and reduced the level of GSH contents. Co-treatment of rats treated with 150 mg/kg and 300 mg/kg body weight *Torilis nodosa* showed significant improvement (p<0.01) by reversing the abnormal change caused by rotenone in the activities of antioxidant enzymes, level of TBARs and GSH as compared to the rotenone-treated group rats. Similarly, markedly decreased (p<0.01) TBARs levels and increased GSH levels were reported in rats treated with rotenone as compared to normal non treated control rats (Table 4). Activities of antioxidant enzymes revealed the efficiency of body defense system. Administration of 1.5 mg/kg body weight rotenone caused significant reduction (p<0.01) in the activities of antioxidant enzyme such as Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione Peroxidase (GPx). The administration of *Torilis nodosa* (150 mg/kg and 300 mg/kg) body weight significantly increased (p<0.01) the activities of SOD, CAT and GPx. Similar observations were recorded by the treatment of 5 mg/kg body weight stalevo (Table 4).

Liver Profile Test

In our study, the liver profile test was also taken, but the liver profile test was not directly related to Parkinson's disease. Administration of 1.5 mg/kg body weight of rotenone caused significant changes (p<0.01) in the liver profile of rats. Treatment of 150 mg/kg and 300 mg/kg body weight *Torilis nodosa* improved the abnormalities in SGPT, serum albumin, total protein, and total bilirubin as compared to rotenone treated rats. Similar effects were reported in rats treated with 5 mg/kg body weight stalevo.

Kidney function and profile play a key role in the health of any individual. Administration of 1.5 mg/kg body weight rotenone caused significant elevation (p<0.01) in serum creatinine and blood urea level. Co-treatment of 150 mg/kg and 300 mg/kg body weight *Torilis nodosa* significantly reversed (p<0.01) the serum creatinine and blood urea dose dependently. Similar reports were obtained from the rats treated with 5 mg/kg body weight stalevo (Table 5).

DISCUSSION

Parkinson disease is a neurodegenerative dysfunction that effected huge population worldwide. In market several therapeutic allopathic drugs are available but have side effects. Therefore, scientists are busy in the development of new beneficial drugs from medicinal plants which has less or no side effects and have easy access (Ferreira and Massano, 2017; Garabadu and Agrawal, 2020). In the present study *Torilis nodosa* plant was analyzed for its phytochemical composition, antioxidant capacity and antiparkinsonian activity. Phytochemical analysis of different fractions showed that alkaloids, tannins, glycosides, saponins and flavonoids are present in the crude methanol extract of the plant. Other phytochemicals, including steroids, terpenoids, and saponins are also found in other fractions of plant extract. Previous studies have shown that plant extracts contain many phytochemicals, including steroids, terpenoids, flavonoids, saponins and alkaloids. These phytochemicals are important in the prevention and treatment of infectious diseases and act as free radical scavengers (Farombi, 2003).

Quantitative evaluation showed that the methanol fraction of *Torilis nodosa* plant extract composed of maximum quantity of

phenol and flavonoids contents as compare to other fractions. Similar data were reported in other studies (Khan *et al.*, 2012).

In this study, various fractions of *Torilis nodosa* exhibited antioxidant activity measured by DPPH, ABTS, H_2O_2 and other scavenging methods. Methanol has the highest value in DPPH; in ABTS, butanol showed the highest percentage of inhibitory activity, but in H_2O_2, ethyl acetate showed the highest percentage of activity. Previous studies have shown that antioxidant behavior, one of the most frequently observed functions of bioactive substances which reduces the effects of oxidative stress (Madihaand Haider, 2019).

In the present study due to higher activity of methanol fraction, it was preceded for *in vivo* anti-Parkinson activities. In the Parkinson activity the methanol fraction of *Torilis nodosa* markedly improved the abnormalities in the body weight of rats treated with rotenone. Similarly, it was reported that rats administered with both safflower flavonoid extract and madopar showed significant improvement as compared to rats in the rotenone group which are in consistent with our findings (Rodriguez, 2008; Nour *et al.*, 2013).

Behavioral marker plays a crucial role in the assessment of Parkinson activities. In the recent study the rotenone-treated group rats showed lowest time on rotarod as compared with the normal control group rats. *Torilis nodosa* plants showed marked improvement in rod spinning and sucrose feeding tests. Similar findings were obtained in the study conducted before and after the addition of quercetin in rotenone-induced Parkinson's disease (Procaccio *et al.*, 2024).

The impact of the therapy on memory performance was investigated using the Morris water maze and footprint test. Data of the current study revealed that rotenone injection considerably increased ($p<0.01$) escape latency and dramatically decreased ($p<0.01$) time spent in and crossings over the target quadrant in comparison to the control rats. *Torilis nodosa* (150 g/kg and 300 mg/kg body weight) has been shown to have a significant effect in the improvement of this alteration caused in rats. Our results are consistent with previous studies showing that rotenonen treated rats exhibited higher tonicity scores and beam walking latencies compared to control rats which were restored (Seppi *et al.*, 2019).

Injection of 1.5 mg/kg body weight caused significant differences in front and hind limb step length and front limb base width compared to normal controls. *Torilis nodosa* treatment had a positive effect on the rotenone-induced footprint test in mice. The results of this study are consistent with those of Madiha *et al.* (2019) showed decreased walking and reduced forward movement in rotenone-treated rats.

The antioxidant defense system plays an important role in eliminating harmful chemicals entering the brain. In this study, rotenone-induced parkinsonian effects also caused oxidation, ultimately reducing the activity of antioxidant enzymes such as SOD and CAT. Various treatments of *Torilis nodosa* and stalevo showed significant improvement in antioxidant enzyme activity. Similar findings are found in previous reports (Shen *et al.*, 2016; Liu *et al.*,2020).

Lipid peroxidation and glutathione content are very important at the cellular level. In this study, rotenone treatment caused an increase in lipid peroxidation TBARs contents and a decrease in GSH levels. Obeso *et al.* (2008) obtained similar observations when evaluating the effect of Tymol on brain homogenates from patients with Parkinson's disease.

The liver is an important organ, and its interior is very sensitive to all kinds of toxins. Rotenone-induced liver injury in mice eventually increased serum ALT, total proteins and total bilirubin levels. These findings suggests that rotenone causes damage to hepatocytes. These damages can be repaired by treatment with multiple doses of *Torilis nodosa*. Our results are consistent with those of Liu *et al.* (2020) reported that quercetin protects the liver from damage by increasing liver function biomarkers. The kidney is the powerhouse of the body and plays an important role in maintaining homeostasis and detoxification and elimination of toxic metabolites from cells. Toxic metabolites of rotenone cause significant changes in renal serum creatinine and blood urea, indicating that rotenone causes nephrotoxicity. *Torilis nodosa* and stalevo treatment reversed the anemia. Ramos (2007) and Talarowska *et al.* (2016) reported same findings during their study in rats. These findings revealed that *Torilis nodosa* has the potency to be used for the treatment of Parkison disease. Further research on the exact mechanism of action of *Torilis nodosa* and isolation of its bioactive component is recommended.

CONCLUSION

From the current data it was revealed that the methanol extract of *Torilis nodosa* significantly improved antioxidant enzymes, behavioral, learning and memory parameters due to the presence of bioactive metabolites. Further research work is needed to investigate the mechanism of action of these bioactive constituents present in the plant extract.

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ABBREVIATIONS

CNS: Central nervous system; **ND:** Neurodegenerative diseases; **PD:** Parkinson's disease; **AD:** Alzheimer's; **MS:** Multiple sclerosis; **SNpc:** Substantia nigra pars compacta; **SCNA:** α-synuclein; **LRRK2:** Leucine rich repeat kinase 2; **PINK-1:** PTEN induced

kinase 1; **NaOH:** Sodium hydroxide; **NADH:** Nicotinamide adenine dinucleotide reduced; **CAT:** Catalase; **SOD:** Superoxide dismutase; **GPx:** Glutathione peroxidase; **SE:** Standard error.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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THE AUTHOR CONTRIBUTION DECLARATION

Muhammad Nadeem Khan performed the experiments; Rahmat Ali Khan designed the study plan, Mushtaq Ahmed while Humera Nazir, and Muhammad Waseem Khan revised the manuscript, made corrections and nessecry software analysis of the manuscript.

SUMMARY

Torilis nodosa (L.) Gaertn., a medicinal plant from the Apiaceae family, has traditionally been used to treat various human diseases. This study aimed to evaluate its phytochemical composition, antioxidant potential, and neuroprotective effects against Parkinson's disease induced by Rotenone in rats.

The plant material was shade-dried, extracted with methanol, and further fractionated using solvents of increasing polarity (n-hexane, chloroform, ethyl acetate, and butanol). Phytochemical screening revealed the presence of bioactive compounds such as flavonoids, alkaloids, terpenoids, saponins, tannins, and anthraquinones. *In vitro* antioxidant assays demonstrated strong free radical scavenging activity, with the methanolic fraction showing the highest activity due to its high phenolic and flavonoid content.

For *in vivo* analysis, rats were treated with methanolic extract doses of 150 mg/kg and 300 mg/kg in a rotenone-induced Parkinsonism model. The results showed significant improvements in behavioral, physiological, and biochemical parameters. Brain tissue analysis indicated that the extract enhanced antioxidant enzyme activity and reduced lipid peroxidation caused by rotenone toxicity.

Overall, the findings suggest that *Torilis nodosa*, particularly at a dose of 300 mg/kg, exhibits strong neuroprotective effects and may serve as a promising natural source for the development of therapeutic agents to manage Parkinson's disease.

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