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ISOLATION OF PHYTOCONSTITUENTS AND EVALUATION OF *JUSTICIA GENDARUSSA* LEAF EXTRACTS FOR ANTI PARKINSONIAN ACTIVITY

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ABSTRACT: The present study investigates the anti-parkinsonian activity of various extracts of *Justicia gendarussa* (JG) leaves in MPTP-induced neurotoxicity mice. Parkinson's disease (PD) is a neurodegenerative syndrome belongs to a group of motor symptoms disorders. In PD, brain cells degenerated in some parts of the brain which produce dopamine. JG herb is widely distributed in tropical regions of the world. The various parts of the plant are employed traditionally in the treating of various disorders. The present study was designed with 11 group's mice with 6 each. Group 1 (control), Group 2 (MPTP 20 mg/kg IP four injections at 2 h intervals), Group 3 (L-dopa), and Group 4 to Group11 were administered with Ethanol, Ethyl acetate, Aqueous and n-hexane extracts for 7 days with dose 200 and 400 mg/kg orally along with MPTP 4 injections on day 1. After 7 days, Neurotransmitters, in vivo antioxidant levels, histopathology observation, and isolation of Phytoconstituents by column and characterization studies were performed. The results of the study showed that significant alterations in neurotransmitter levels increased Malondialdehyde (MDA) and decreased reduced glutathione levels and degeneration of neurons. All the neurotransmitters and reduced glutathione levels were remarkably ($P<0.001$) and dose-dependently restored, and MDA levels were decreased with different extracts of JG at selected doses. The effective neuroprotective activity was observed with ethanol extract at 400 mg/kg, isolation of ethanol extract showed the presence of naringenin, Isoflavone. Neuroprotective activity of JG leaves could be due to the presence of isolated compounds and other L-dopa-like constituents; hence JG extracts has a prominent neuroprotective effect in PD.

INTRODUCTION: Motor disorders like Parkin son's disease is the second most prevalent neuro-degenerative disorder, character rized by depletion of dopaminergic inputs in the substantia nigra pars compacta and basal ganglia, which causes subsequent degeneration of catech olaminergic neurotransmitter called Dopamine (DA) from the striatum [1].

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DA has clinical importance in motor functioning, resulting in the clinical manifestations of PD, which include slowness of movement (bradykinesia), postural instability, resting tremor, and muscular rigidity [2]. Further depletion of DA causes pathogenesis of PD such as oxidative stress, neuroinflammation, abnormal protein aggregation, apoptosis, Iron deposition, environmental toxins, and mitochondrial respiration defect [3].

Among all, oxidative stress is predominant in the pathological mechanism of PD. This oxidative damage is a causative agent for the formation of reactive oxygen species [4], which metabolizes DA, nitric oxide, and lipid peroxidation, and reduced level of endogenous antioxidant enzymes such as

superoxide dismutase and glutathione (GSH) in the brain might be responsible for neuronal death [5] oxidative stress also has been linked with aging, ischemic injury cancer at herosclerosis inflammation and other neurodegenerative diseases [6]. Based on these findings, the usage of antioxidants as remedial interventions in PD is in practice. As natural plants produce a wide variety of natural antioxidants with the least side effects among the natural antioxidants flavonoids, polyphenolics, anthocyanins and flavones are potent antioxidants which scavenge free radical hence it could be a beneficial agent in the treatment of PD [7].

The conventional therapy of PD is Levodopa; however, prolong usage of them results in causes high incidence of pharmaco resistance, accumulation of intracellular oxidative stress, depression, sleep disturbances, and dyskinesia in the majority of PD patients [8]. To overcome this drawback, researchers are interested in screening bioactive molecules isolated from therapeutically important medicinal plants used to treat neurodegenerative disorders, including Parkinson and schizophrenia.

The main focus of the present findings is to investigate the neuroprotective effect of JG, which is a shade quick-growing, evergreen shrub found throughout Indian and other places and found to possess a broad spectrum of activities due to presence of steroids, carbohydrates, carotenoids, alkaloids, terpenoids, flavonoids, phenolic compounds, and aromatic amines, Apigenin and Vitexin 9 are specific to JG. Leaves of JG contain lignans, triterpenoid, stigmasterol, lupeol, and 16-hydroxy lupeol, and with high naringenin and kaempferol contents were successfully screened [10].

The leaves of this plant have been reported for various biological activities such as antioxidant, anti-angiogenic, antifungal, anti-inflammatory antinociceptive, antisickling, anti bactericidal, anthelmintic, cytotoxicity, anti-arthritic, larvicidal, and adulticidal activities. Also, its aerial parts showed *in-vitro* HIV type 1 reverse transcriptase inhibitor, analgesic, and anti-inflammatory activities [11].MPTP is a frequently used neurotoxin to induce PD like Neurodegeneration in experimental animal models [12]. As JG has been traditionally used in the treatment of neurological disorders and also reported for Anti-anxiety [13], Anti Depressant [14] activities, and for Antioxidant and Free radical scavenging potential [15] hence this plant proved to be an effective remedy in Parkinson's disease. Based on these considerations, the present study is to evaluate its Anti parkinsonian effect of various extracts of JG against neurotoxin-induced Parkinsonism. Present literature reveals that there is no significant work has been carried out on JG leaves for Antiparkinsonian activity.

MATERIALS AND METHODS:

Animals: Male Swiss albino mice weighing between 25-30 gm were obtained from Sri Venkateswara enterprises, Bangalore, and used for the experiments. Animal House was maintained under standard laboratory conditions and was provided with a standard pellet diet and water *ad libitum*. Animal studies were conducted as per IAEC (1677/PO/RE/S/2012/CPCSEA/IAEC/05) dated 23-02-2019. MPTP Hydrochloride, other chemicals (Sigma Aldrich and Merck grade), and solvents used in this project are analytical grade. Plant material and Preparation of extract: *Justicia gendarussa* leaves were collected in bulk from Tirumala hills, Andhra Pradesh in India, in August month and authenticated by famous Botanist at Dept. of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh in India and voucher number 1610 was preserved in the herbarium.

The leaves were dried, coarsely powdered, and extracted with solvents like n-hexane, ethyl acetate, aqueous, and ethanol by successive extraction in a Soxhlet for 6 h. After each extraction, the solvent was evaporated, and the remaining extracts were concentrated to dryness at a normal temperature from 45-50 °C to obtain dried extracts; percentage yield was calculated for all four extracts and stored for further studies. Thin Layer chromatography: TLC was performed for all four crude extracts of JG and extracts were applied 1-10 µl on silica gel G coated TLC plate. Plates were placed in the TLC glass chamber; then the mobile phase was allowed to move through the adsorbent phase up to 3/4th of the plate, spots were developed in each solvent system. Ninhydrin reagent was sprayed to visualizing the spots, and the R_f values were calculated [16].

Acute Toxicity and Gross Behavior Studies: Mice were grouped into four, each 3 animals. Group-I control and received 2% v/v Tween 80.

Groups 2-4 animals received orally by gavages 500, 1000, 2000 mg/kg of all extracts suspended in Tween 80. The animals were observed continuously for 2 h and then occasionally at a gap of 1 h till 6, 12, 24, and 48 h for any neurological effects. The mice were observed for mortality at the end of 48 h to calculate LD_{50} values (OECD guidelines 420) [17].

Experimental Design: Animals were divided into eleven groups, each containing six mice. Group I- control (2% Tween 80 p.o), Group II- 20 mg/kg i.p MPTP four injections at 2 h intervals. Group III- L-dopa, Standard (100 mg/kg i.p) Group IV- XI received ethanol, ethyl acetate, aqueous, and n-hexane extracts orally for seven days along with 20 mg/kg MPTP on day 1.

At the end of 7 days experimental treatment, the mice are kept overnight fasting and sacrificed by cervical decapitation, brains were removed immediately and brain tissues were homogenized in cold acid butanol and used for the estimation of Dopamine (DA), epinephrine (EP), norepinephrine (NEP), serotonin (5-HT), protein levels, *in-vivo* lipid peroxidation, reduced glutathione levels and histopathological analysis of diseased and extract-treated groups of mice whole-brain followed by isolation and characterization studies by various analytical techniques. Methods for Extraction of DA, EP, and NEP: Tissue samples were homogenized in acid cool butanol to a concentration of 50 mg/ml. The homogenates were centrifuged at 800 rpm in a cooling centrifuge at 4 °C for 15 m.

The residue was discarded, each 2.5 ml of distilled water and n-heptane was added to the supernatants, contents were mixed thoroughly and centrifuged to 1000 rpm for 05 m. The aqueous phase was collected, 200 mg of alumina, 1.5 ml of sodium acetate was added, and PH was adjusted to 8.0 with 1N NaOH.

The samples were centrifuged again at 1000 rpm for 5 min (1.5 ml supernatant was collected and used for the estimation of serotonin (5-HT). The alumina was washed twice with 2 ml of distilled water by overtaxing the tube and centrifuged to 1000 g for 5 m. The supernatant was discarded, and the walls of the tube were blotted with strips of filter paper. The monoamines were eluted by shaking the alumina with 2 ml of 2 N acetic acid.

The supernatant was collected in a tube, 100 µl of EDTA was added, and pH was adjusted to 6.3, 100 µl of iodine solution was added to the above content, and content was mixed thoroughly. The samples were to stand at ambient temperature to 02 m, finally the required quantity of alkaline sulphite solution was added. The contents were shaken well and allowed to stand at normal temperature for 02 m, and pH was adjusted to 5.4 with 5 N acetic acid. The fluorescence of EP (410, 500 nm) and NEP (385 and 485 nm) was read in Spectroflourimeter (ELICO SL-174) with excitation and emission wavelength. The same samples were again heated for 05 m in a boiling water bath and cooled; the fluorescence of DA was read at excitation and emission wavelengths of 320 and 370 nm18.

Estimation of 5-HT: To 1.5 ml of above collected supernatant, 100 µl of Cysteine, 1.5 ml of HCL and 100 µl of O-Phthalaldehyde (OPA) solution was added. The tubes were kept at ambient temperature for 20 m. The above tube 100 µl of 1M sodium Metaperiodate was added and heated to 80° in a water bath for 20 m. The samples were cooled, and the fluorescence of serotonin was read at 360, and 470 nm, and the amount of serotonin was calculated and expressed in µg/g wet weight of tissue [18].

Estimation of Proteins: Brain homogenates 5% w/v were prepared in trichloroacetic acid (TCA). 0.2 ml supernatant was collected, and 04 ml of alkaline copper sulfate was added, kept at RT for 20 m. Then 0.4 ml of folin phenol reagent was added and kept at RT for 20 m. The color developed was observed at 600 nm in Spectrophotometer [19].

***In-vivo* Antioxidant Studies:**

Preparation of Mice Brain Homogenate: Brain tissue was homogenized in 50 mmol phosphate buffer (pH 7.0) containing 0.1 mM EDTA to give 5% (W/V) homogenate. The homogenate was centrifuged at 10,000 rpm for 10 m at 0° in a cooling centrifuge; the obtained supernatant was utilized for further studies.

***In-vivo* Lipid Peroxidation:** Malondialdehyde level (MDA) was performed quantitatively at 532 nm as per method [20] by Spectrophotometer and expressed as n mol/mg of proteins in the tissue.

Reduced Glutathione Level: GSH levels were analyzed according to method [21] at 412 nm in UV-Visible Spectrophotometer and expressed in µ moles/mg protein.

Histopathological Analysis of Brain: Small portion of the isolated brain from normal control, diseased and experimental groups were immediately kept in 10% formalin and washed in running tap water, dehydrated with low grades of alcohol, and cleared in Xylene. The brain tissues were enclosed in liquid paraffin wax and allowed to cool and cut into a thickness of 10 µm. The section was dried and stained with Eosin dye for histopathological changes [22] by Fluorescent microscope, and photographs were taken.

Isolation of Active Constituents and Characterization: Active extract was subjected for isolation by column chromatography [23,] and characterization studies were performed by FTIR (Bruker, Perkin Elmer Spectrum Version 10.5.1, spectra within wave number range 4000 – 400 cm-1), NMR (DRX – Bruker 400 Mhz), CDCL3 and DMSO (Sigma Aldrich, Bengaluru, India), were used as solvents and TMS as internal standard and Mass spectroscopy by (AGILENT-LCMS).

Statistical Analysis: The results were analyzed using Graph pad prism version 8.1.5. All values are expresses as Mean ± Sem. The data of biochemical estimations were analyzed using one way (ANOVA) test for multiple comparisons followed by the Tukey-Krammer test. In all the tests, the criteria for statistical significance was P<0.05.

RESULTS: The percentage yield of Ethanol, ethyl acetate, Aqueous extract, and n-hexane were found to be 9.4, 7.2, 7.9, and 8.1% (w/w). Phytochemical screening of all four extracts JG leaves revealed the presence of flavonoids, phenols, amino acids, glycosides, cardiac glycosides, proteins, carbohydrates, alkaloids, steroids, terpenoids, and tannins as major important constituents. Therefore it serves for locating the pharmacologically active chemical compounds.

Thin Layer Chromatography: TLC analysis of JG extracts revealed the presence of various constituents were along with L-dopa and R_f values of ethanolic extract (0.86, 0.67,0.40, 0.28), ethyl acetate (0.86, 0.63,0.44), aqueous extract (0.67, 0.45) n-hexane (0.72) of JG with a solvent system n-hexane : acetone (8:2). R_f value of L-DOPA was found to be 0.62, with the solvent system of Butanol: Acetic acid: water (12:3:6).

Acute Toxicity and Gross Behavioral Changes: All the extracts of JG were found to be safe since no animal died even at the maximum single dose of 2000 mg/kg orally. The animals did not show any significant gross behavioral changes at the doses tested. Hence, doses of the various extracts selected for the present study were 200 and 400 mg/kg (OECD Guidelines, 420). The plants and plant extracts which showed CNS stimulant effect was screened for anti- Parkinsonian activity.

Results of Neurotransmitter Levels In Brain: Dopamine level was much affected, and other amine levels *viz* EP, NEP, and 5-HT were slightly altered on MPTP (P<0.001) intoxication (1.623 ± 0.040) at 20 mg/kg as compared to control (5.228 ± 0.19) and L-Dopa (5.318 ± 0.17) groups because dopamine is the main targeted neurotransmitter in the brain. While, dopamine level was tremendously increased (P<0.001) and all other amine levels (P<0.01) were improved significantly and dose-dependently on pretreatment with JG extracts compared to control, MPTP and L-dopa and here, dopamine values at highest dose for all the active extracts were presenting, ethanol extract showed 5.008 ± 0.13µg/g, ethyl acetate 4.238 ± 0.14, aqueous 4.14 ± 0.17 and n-hexane extract showed very negligible effect on all neurotransmitters 1.907±0.03, all the values are depicted in **Fig. 1**, **Table 1**.

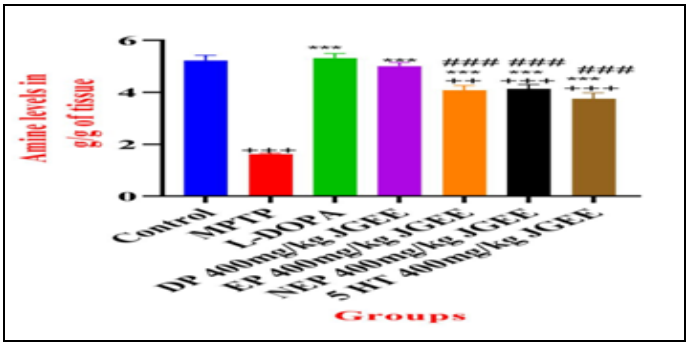

FIG. 1: THE EFFECT OF ETHANOL EXTRACT OF JG ON VARIOUS BRAIN AMINE LEVELS AT *400mg*/Kg

Each column represents as Mean ± Standard error of mean (n=6) +++, ++ (P<0.001,P<0.01) *vs.* Control; ***(P< 0.001) Vs MPTP; ###(P<0.001) *vs.* L- Dopa groups.

TABLE 1: EFFECT OF VARIOUS EXTRACTS OF *JUSTICIA GENDARUSSA* LEAVES ON BRAIN AMINE LEVELS IN MPTP INDUCED NEUROTOXICITY IN MICE

Plant Extracts	Doses mg/kg	Dopamine	Epinephrine	Nor Epinephrine	Serotonin
		µg/g Wet Weight of Brain Tissue			
	Control	5.228±0.19	5.228±0.19	5.228±0.19	5.228±0.19
	MPTP	1.623±0.04^{+++}	1.623±0.04^{+++}	1.623±0.04^{+++}	1.623±0.04^{+++}
	L-Dopa	5.318±0.17***	5.318±0.17***	5.318±0.17***	5.318±0.17***
Ethanol	MPTP+200	4.138±0.07$^{+++,***,##}$	3.367±0.27$^{+,***}$	3.110±0.14$^{+++,***,###}$	2.088±0.22$^{++,**,###}$
	MPTP+400	5.008±0.13***	4.082±0.17$^{***,#}$	4.132±0.16$^{++,***}$	3.747±0.23***
Ethyl	MPTP+200	3.69±0.14$^{+++,***}$	2.118±0.17$^{+++,***,###}$	2.183±0.11$^{+++,###}$	1.175±0.04$^{+++,###}$
	MPTP+400	4.238±0.14$^{++++,***}$	4.238±0.14$^{++++,***}$	3.207±0.22$^{+++,***,###}$	2.288±0.34$^{+++,***,###}$
acetate	MPTP+200	3.655±0.16$^{+++,***}$	2.013±0.09$^{+++,***,###}$	2.060±0.10$^{+++,###}$	1.203±0.06$^{+++,###}$
Aqueous	MPTP+400	4.142±0.17$^{+++,***}$	2.532±0.12$^{+++,***,###}$	2.580±0.11$^{+++,**,###}$	2.288±0.34$^{++,***,###}$
n-hexane	MPTP+200	1.415±0.08***	0.545±0.04$^{+++,###}$	0.565±0.04$^{+++,**,###}$	0.6392±0.14$^{+++,###}$
	MPTP+400	1.907±0.03***	1.508±0.32$^{+++,###}$	1.572±0.31$^{+++,###}$	1.023±0.07$^{+++,###}$

Each value expresses the Mean ± SEM (n=6) +,++,+++(P< 0.05, P<0.01,P<0.001) Vs Control; **,***(P< 0.01, P<0.001) Vs MPTP; #,##, ###(P<0.05,P<0.01,P<0.001) Vs L- Dopa groups.

Effect on MDA and GSH levels: MDA level was significantly (P<0.001) elevated in MPTP-induced animals (1.75 ± 0.01) as compared to the control group (0.82 ± 0.08). MDA level was decreased significantly (P<0.001) and dose-dependently with all three extracts, ethanol extract of JG (0.65 ± 0.05), with ethyl acetate 1.02 ± 0.07, aqueous 1.13 ± 0.09 and n-hexane extract showed only minute effect 1.24 ± 0.13 at 400 doses. Reduced glutathione level was significantly (P<0.001) decreased in MPTP (0.51 ± 0.01) as compared to the vehicle group (4.06 ± 0.11). GSH level was restored significantly (P<0.001) and dose-dependently with all the three extracts, ethanol 3.41 ± 0.04, ethyl acetate 2.85 ± 0.02 and aqueous extract 2.15 ± 0.016 and n-hexane extract 1.84 ± 0.015 showed minute action at 400 mg/kg dose compared to other treated groups **Fig. 2**.

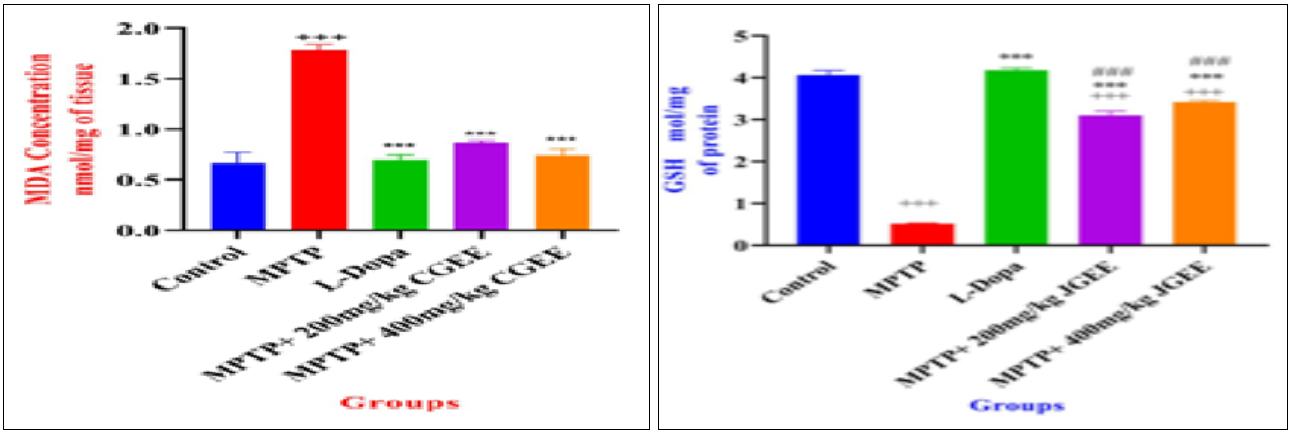

FIG. 2: THE EFFECT OF ETHANOL EXTRACT OF JG ON LIPID PEROXIDATION (MDA) AND REDUCED GLUTATHIONE LEVELS (GSH) AT 200 AND 400 mg/kg

Each column represents as Mean ± Standard Error of Mean (n=6) +++(P< 0.001) *vs* Control; ***(P< 0.001) *vs* MPTP; ###(P<0.001) *vs* L- Dopa groups.

Histopathology Observations: Histopathological analysis of stained sections illustrated that normal neuronal morphology in the control group, but the animal group induced with MPTP showed marked neuronal damage and degeneration of neurons in a significant number, mice upon treatment with L-Dopa and other treated groups reversed the neuronal damage occurred by MPTP toxicity and showed good regeneration of neurons. Normal and good neuron structure was observed with ethanol extract of JG with 400 mg/kg dose and shown in **Fig. 3**.

Isolation of active constituents and their characterization: All the analyzed extracts showed significant anti-parkinsonian activity, and ethanol extract demonstrated maximum neuroprotective effect duly it was subjected to isolation and characterization studies to notice the active constituents responsible for the present activity.

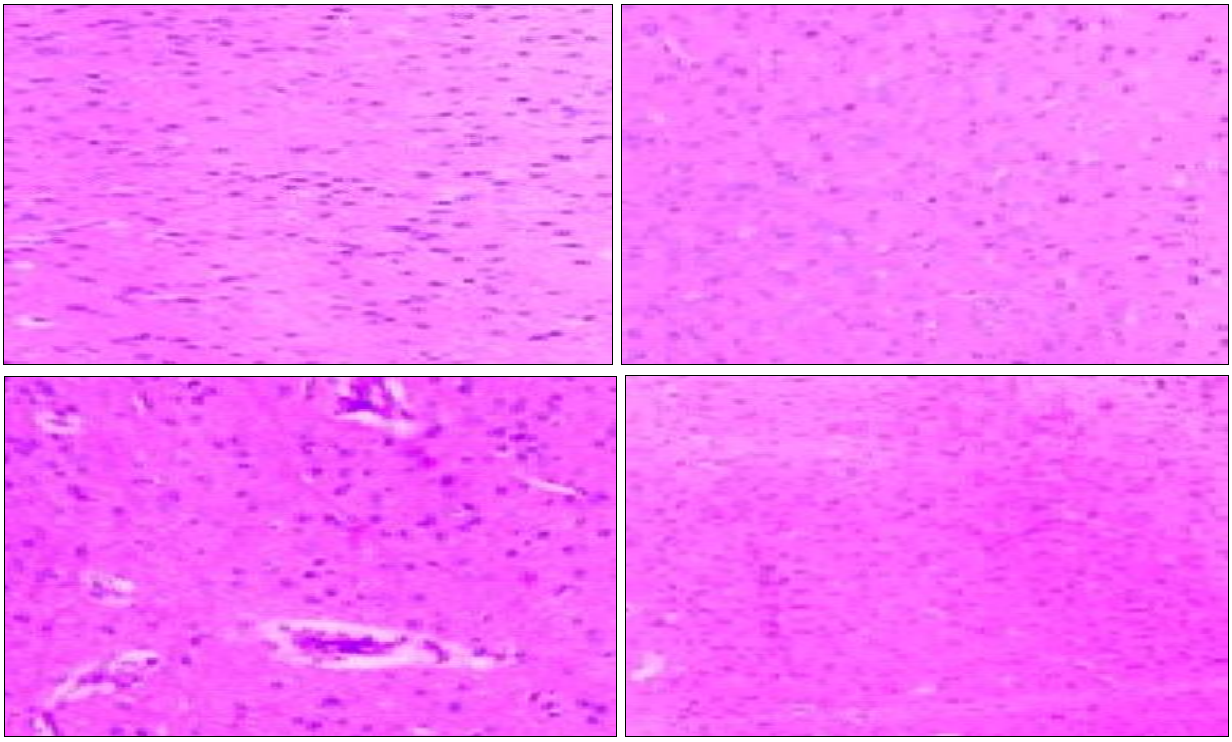

FIG. 3: PROTECTIVE EFFECT OF ETHANOL EXTRACT OF JG AT 400 mg/kg ON BRAIN NEURONS

The isolated compound of JG F8 compound 1 showed (greenish-black with a percentage yield of 0.34) with a solvent system of chloroform: acetic acid as mobile phase (9:1) R_f value 0.86, compound 2 F10 shows (a yellowish colored compound with a percentage yield of 0.22% with a solvent system of ethyl acetate: acetic acid (9:1) R_f value 0.67.

Spectral Analysis Data: The IR spectrum showed the presence of bandsat (2922.60 cm^{-1}) aromatic CH stretch, OH Stretching (3413.57cm^{-1}), ketone (1243.70 cm^{-1}) carbonyl stretching (1733. 78 cm^{-1}) Aromatic C=CH (1651.27 cm^{-1}).The NMR spectra exhibited 1H NMR (400 MHz, CDCl3) δ 8.12 (s, 1H), 7.40 (d, J = 6.9 Hz, 1H), 7.38 (d, J = 8.2 Hz, 2H), 7.24 (s, 1H), 7.15 (s, 1H), 7.10 (t, J = 7.5 Hz, 1H), 7.00 (d, J = 7.8 Hz, 2H), 4.53 (s, 1H), 2.28 (s, 2H).

Based on mass spectra, the molecular ion peak was determined at m/z 272 consistent with the molecular weight formula of 272.25 g/mol and designated compound one as Naringenin, and spectral data was given in **Fig. 4**.

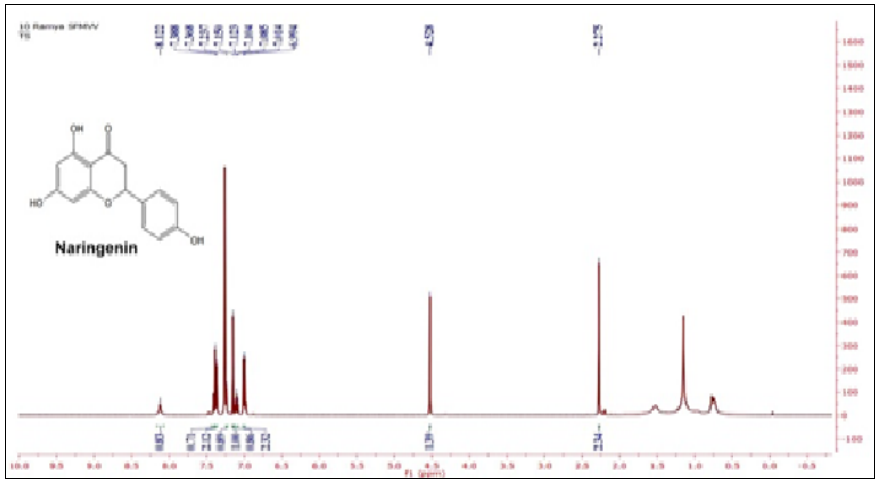

FIG. 4: NMR SPECTRA OF NARINGENIN

FTIR spectra of JG showed bands at aromatic CH bends (1408.01, 1385.24 cm^{-1}), C-O-Stretch (1077.84 cm^{-1}), Aromatic=CH (1622.89 cm^{-1}). 1H NMR (400 MHz, CDCl3) δ 8.37 (s, 1H), 7.69 (d, J

= 7.3 Hz, 2H), 7.58 – 7.51 (m, 1H), 7.38 (t, J = 7.1 Hz, 2H), 6.95 (d, J = 10.8 Hz, 3H), 4.51 (s, 2H), 2.32 (s, 2H), 1.27 (s, 1H). On the basis of mass spectra the molecular ion peak was determined at m/z 270 consistent to molecular weight 270.20 g/mol and identified compound Two as Isoflavone **Fig. 5.**

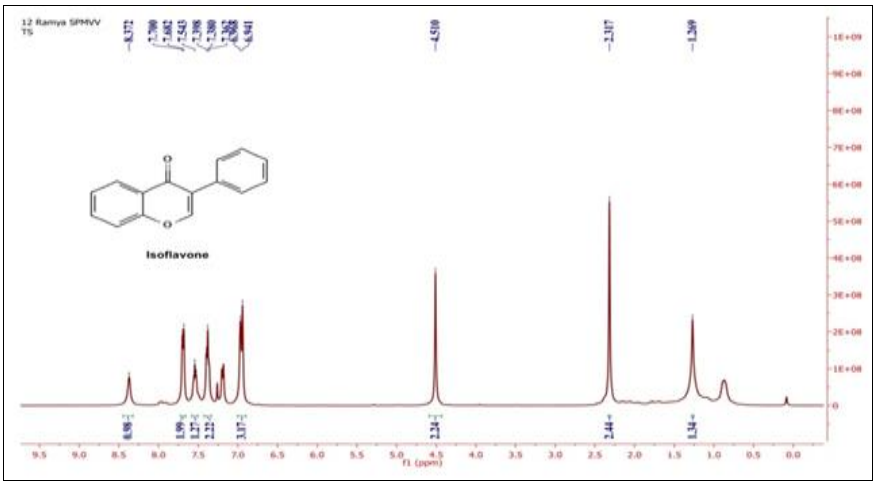

FIG. 5: NMR SPECTRA OF ISOFLAVONE

DISCUSSION: DA itself is a chief catecholamine neurotransmitter that is responsible for control body movements, death or degeneration of dopamine-producing nerve cells of substantia nigra leads to irregular body movements [24]. Excessive degeneration of dopaminergic neurons is correlated with increased reactive oxygen species (ROS) [25] which leads to oxidative damage, abnormal accumulation of cellular proteins such as α-synuclein and mitochondrial complex 1 inhibition triggers the progressive neuronal death [26]. Since medicinal plants generate a great deal of phytoconstituents such as flavonoids, phenolics, sterols, alkaloids, anthocyanins, carotenoids and glucosinolates with promising antioxidant [27].

Chronic use of L-dopa leads to motor complications [28]. Hence there is a growing interest to screen and isolate bioactive lead molecule from the medicinally active herbs to fight against. Justicia gendarussa is a highly reputed plant used traditionally for treating of various biological diseases. Our previous study on these plant extracts showed a marked effect on Neurobehavior screening as improved motor coordination, loco-=motor activation leads to brain activation, increased alertness, and exhibited CNS stimulant activity; by all this evidence, the present study is focused on the neurobiological mechanism, and possible interaction of various extracts of JG leaves on brain neurotransmitters and antioxidant enzyme levels [29]. Dopamine was highly affected in Parkinson's disease because the Dopamine plays an important role among all the neurochemicals in PD30, and it is the targeted neurochemical in the brain; drastic reduction of brain DA and slightly alteration of other monoamines like NEP, EP and 5-HT were noticed in MPTP induced group. Reduced brain dopamine and other amine levels were recover significantly and dose-dependently by three extracts of JG, but maximum antiparkinsonian effect was perceived with ethanolic extract of JG with higher; these findings are similar to previously reported studies [31, 32].

It could be due to presences of chemical compounds isolated from ethanol extract of JG such as Naringenin (Flavonoid), and isoflavone are potent antioxidant which protects the tyrosine hydroxylase (TH)-positive cells from damage, reduce the apoptosis, maintain the mitochondrial membrane potential and improve the cell viability and increased the level of dopamine and its metabolites [33]. Further neuroprotective effect of naringenin has also been reported previously in neurotoxicity induced Parkinson's mice model, which was isolated from other plants [34]. The formation of cytotoxic free radicals is observed in substantia nigra with PD patients; these free radicals bring on lipid peroxidation and cell death, which leads to severe oxidative stress in substantia nigra. In the present study, the intoxication of

MPTP resulted in significant elevation of malondialdehyde (MDA), and depletion of reduced glutathione levels (GSH) indicates an increased level of free radicals during the process of oxidative stress. Pretreatment with all three extracts of JG, their levels were remarkably reversed, but a strong antioxidant defense mechanism was seen with ethanol extract of JG at 400 mg. A previous study supports that, Flavonoids are a powerful antioxidant that inhibits lipid Peroxidation and elevates the reduced glutathione levels at early stages by scavenging of superoxide anion and hydroxyl radicles [35].

The negligible effect was noticed with n-hexane extract of JG on brain amine and antioxidant levels, it could be due to the absence of antioxidant principles in the extract. Histopathological reports of MPTP induced groups, partial necrosis neuron cells was occurred due to MPTP induced toxicity on brain neurons.

Damaged neuronal cells were recovered and achieved to the original state with a higher dose of EEJG, as this extract contains more ethanol soluble constituents like polyphenols, flavonoids, amino acids, proteins and isolated compounds Naringenin and isoflavone, it might cross the Blood-Brain Barrier, inhibit the mitochondrial dysfunction and apoptosis of neurons and fight MPTP induced neurotoxicity and our results are coupled with previous findings reported by other scientist [36].

All the extracts of JG employed in the study showed a good anti-parkinsonian effect at both the selected doses (200,400 mg/kg). Moreover, ethanol was subjected for isolation and characterization due to its beneficial maximum neuroprotective effect. Naraginin and Isoflavone (new compound from JG) were isolated and characterized by FTIR, NMR Mass spectroscopic techniques and correlated with the present activity.

CONCLUSION: *Justicia gendarussa* is the most promising plant due to its significant anti-parkinsonian activity against MPTP-induced parkinsonism. It can be proved that ethanolic extract of JG can be employed in the treatment of parkinson's disease due to its antioxidant and neuroprotective properties; further studies are required to establish the exact mode of action and more clinical trials.

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CONFLICTS OF INTEREST: The author declares that there are no conflicts of interest regarding the publication of this paper.

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