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Original Research Article

PHARMACOLOGICAL SCREENING OF POLYHERBAL PREPARATION ON ROTENONE INDUCED PARKINSON'S DISEASE IN RAT

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Abstract: Parkinson's disease (PD) is a slowly progressive multisystem disorder affecting dopaminergic neurons of the substantia nigra pars compacta (SNpc) and is characterized by a decrease in dopamine (DA) in their striatal terminals. This study aimed to pharmacological effect of polyherbal preparation on rotenone induced Parkinson's disease in rats. Thirty rats of both sex were assigned to five groups (n=6). Group 1 served as control group which received sunflower oil (1 ml/kg s.c) along with normal saline (5 ml/kg p.o) for 12 days. Group 2 served as rotenone treated disease control which received rotenone (2.5 mg/ml/kg emulsified in sunflower oil with DMSO by subcutaneous route) on alternate days for 12 days. Group 3 served as standard drug which received [levodopa + Carbidopa (100 mg+ 25 mg/kg)] for 12 days along with rotenone (2.5 mg/ml/kg) on alternate days for 12 days. Group 4 served as test drug 1 which received methanolic extract of Momordica Dioica fruit pulp + methanolic extract of Salvia Rosmarinus leaves (75 mg+25mg/kg p.o) for 12 days along with rotenone (2.5 mg/ml/kg) on alternate days for 12 days . Group 5 served as test drug 2 which received methanolic extract of Momordica Dioica fruit pulp +methanolic extract of Salvia Rosmarinus leaves (100 mg+25 mg/kg p.o) for 12 days along with rotenone (2.5 mg/ml/kg) on alternate days for 12 days. Rats treated with polyherbal extract showed significant effect on behavioural activity, biochemical parameters and body weight. Histopathological examination of substantia nigra region of rat brain showed decrease in formation of lewy bodies in rats treated with polyherbal extract. Conclusions: Polyherbal preparation possess antiparkinson's activity. However, its underlying mechanisms of action need further investigation.

Keywords: Parkinson's disease, Rotenone, Levodopa, Carbidopa, Polyherbal preparation, Momordica dioica, Salvia rosmarinus, Dopamine, Substantia nigra, Lewy bodies, Neuroprotection

INTRODUCTION

Alzheimer's, Parkinson's, Huntington's, and multiple sclerosis are neurodegenerative illnesses that cause memory loss and cognitive impairment due to selective neuronal cell death and protein aggregation (1). Parkinson's disease (PD) is the second most common neurodegenerative disease after Alzheimer's disease (AD), which was first described by an English physician and surgeon, James Parkinson, who wrote his essay on shaking palsy in 1817, and later referred to as Parkinson's disease (2). PD is a slowly progressive multisystem disorder, rather than just a disease, leading to massive neuropathological degeneration of dopaminergic neurons of the snpc and their terminals in the striatum. Parkinson's disease (PD) is primarily a motion disease, but non Motor symptoms are also present. It affects dopamine producing neurons in the brain and their Loss causes symptoms of Parkinson's disease. Loss of dopaminergic neurons can lead to muscle damage Stiffness, tremor and bradykinesia as well as cognitive, mental and behavioral problems

such as Sadness and fear. Parkinson's disease is an age-related condition, with a significant increase in prevalence among those over 60 (3,4). The exact cause of Parkinson's disease is currently unknown. Alpha synuclein becomes the predominant protein in Lewy bodies, playing a significant role in the pathogenesis of both familial and sporadic Parkinson's disease (5). There is currently no cure for Parkinson's disease, and treatment options include dopamine agonists and MAO-B inhibitors, which only provide symptomatic relief. Considering the promise that some natural products have yielded in treating various maladies, such products as herbs, medicinal plant extracts, and other similar botanicals are being investigated for their therapeutic potentials in neurological disorders. Several plants have been discovered to be good neuroprotectants. Momordica dioica, also known as "Kakrol," is traditionally used in Ayurvedic medicine for its neuroprotective properties, while Salvia Rosmarinus is commonly used Called rosemary, it contains compounds that have been shown to have the potential to improve cognitive abilities Function and reduction of oxidative stress in Parkinson's disease (6). In addition, current research results have shown that the combination of Momordica dioica and Salvia Rosmarinus extracts may contribute have a synergistic effect in reducing symptoms associated with Parkinson's disease (7). This synergistic impact is thought to be owing to the complimentary methods of action of the bioactive chemicals found in Momordica dioica and Salvia Rosmarinus, which target distinct pathways implicated in Parkinson's disease progression(8). Furthermore, continuing research suggests that Momordica Dioica antioxidant qualities and Salvia Rosmarinus' anti inflammatory activities may provide a novel therapeutic approach for controlling neurodegenerative illnesses other than Parkinson's disease (9).

1) Materials And Methods:

1.1) Animals:

Wistar Albino Rat of weight (180-200 g) of both sexes were used in the study. Animals were obtained from the Crystal biological solution, Pune. The animals were housed in polypropylene cages, approximately six per cage, under conditions of controlled temperature (22- 26˚C) and humidity (50-60%), with a 12-h light/dark cycle, and free access to water and the specified diet ad libitum of water. All the experimental procedure were approved by Institutional Animal Ethics Committee (IAEC) with proposal no: CPCSEA/CBPL/AH/82. All the animals were allowed to acclimatize to the housing facility for 15 days before initiation of the protocol. The experiment protocol lasted for 12 days. Thirty rats of both sex were assigned to five groups (n=6). Group 1 served as control group which received sunflower oil (1 ml/kg s.c) along with normal saline (5 ml/kg p.o) for 12 days. Group 2 served as rotenone treated disease control which received rotenone (2.5 mg/ml/kg emulsified in sunflower oil with DMSO by s.c)) on alternate days for 12 days. group 3 served as standard drug which received [levodopa+ carbidopa (100 mg+ 25 mg/kg)] for 12 days along with rotenone on alternate days for 12 days. group 4 served as test drug 1 which receive methanolic extract of Momordica Dioica fruit pulp + methanolic extract of Salvia Rosmarinus leaves (75 mg+25mg/kg p.o) for 12 days along with rotenone (2.5 mg/ml/kg) for on alternate days for 12 days . Group 5 served as test drug 2 which received methanolic extract of Momordica Dioica fruit pulp +methanolic extract of Salvia Rosmarinus leaves (100 mg+25 mg/kg p.o) for 12 days along with rotenone (2.5 mg/ml/kg) on alternate days for 12 days.

1.2) Neuro-Behavioral Parameters:

1.2.1) Rotarod activity: The grip strength of all animals was tested using a rotarod. The rotarod test is commonly used in mice to assess "minimal neurological deficit" such as motor function and coordination. Prior to beginning therapy, each rat received a training session to acclimate them to a rotarod apparatus (EIE instrument, India). Animal was placed on the rotating rod with a diameter of 7cm (speed 25rpm). Each rat was subjected to three separate trials at 5min interval on days 6 and 12 and cut-off time (180s) was maintained throughout the experiment. The average results were recorded as fall of time (10).

1.2.2) Catalepsy: Catalepsy was assessed using the bar test, in which the rats were placed in half- rearing position with both front paws on a horizontal bar 9 cm above and parallel to the ground. On days 6 and 12, rats were monitored using a stopwatch to record the time they removed one paw off the bar. The maximum cut off period for observation was set at 180 seconds (11).

1.2.3) Locomotor Activity: The spontaneous locomotor activity was measured using a digital actophotometer (Hicon Instrument, India) with infrared sensitive photo cells. The gear was installed in a darkened, light- and sound-proofed, and ventilated testing room. Each interruption of a beam on the x or y axis generated an electric impulse that was shown on a digital counter. Each animal was monitored over a period of 5 minutes on days 6 and 12 (12).

1.3) Biochemical Estimation:

1.3.1) Lipid Peroxidation: Lipid peroxidation was estimated by measuring TBARS and was expressed in terms of malondialdehyde (MDA) content. Thiobarbituric acid-reactive substance content was measured in a medium containing To100 µL of tissue homogenate (10%), 15 µL of 8.1% SDS, 60 µL of acetic acid buffer (2.5 M, pH 3.4), and 115 µL of 0.81% thiobarbituric acid. The mixture was heated at 95 °C for 120min in a water bath. After cooling to room temperature, absorbance was measured in the supernatant at 532 nm. The content of malonaldehyde (MDA), expressed as n moles formed per milligram of protein in the tissue, was calculated using the formula:

$$\text{Concentration} = A \times (V/E) \times P$$

Where, A is the volume of solution, E is extinction coefficient (1.56 x 105m-1 cm-1) and P is the protein content of tissue calculated as milligram of protein per gram of tissue(13)(14).

1.3.2) GSH Level (Reduced Glutathione): The GSH content was estimated using method of Beutler et al. (1963), the tissue homogenate was mixed with TCA (10%w/v) in ratio 1:1 ratio. The tubes were centrifuged at 1000g for 10min at 40C . 0.5ml of the supernatant obtained was mixed with 2 ml of 0.3 M disodium hydrogen phosphate. Then 0.25ml of 0.01 M freshly prepared DTNB dissolved in 1% w/v citric acid was added and absorbance was measured spectrophotometrically at 412nm. A standard curve was plotted using 5-50µM of reduced form of glutathione and results were expressed as µmol of reduced glutathione per mg of protein(15).

1.3.3) Catalase Activity: Pipet 25 µL of samples or appropriate standards into duplicate wells in the plate. Pipet 25 µL of Assay Buffer into duplicate wells as the Zero standard. Add 25 µL of the supplied Hydrogen Peroxide Reagent to each well using a repeater pipet. Incubate at room temperature for 30 minutes. Add 25 µL of the supplied Substrate to each well using a repeater pipet. Initiate the reaction by adding 25 µL of the prepared HRP Reagent to each well using a repeater pipet. Incubate at room temperature for 15 minutes. Read the optical density at 560 nm
+/- 20 nm (acceptable Range 540-580 nm) (16)(17).

1.4) Histopathological Studies

The brains from control and experimental groups were fixed with 10% formalin and embedded in paraffin wax and cut into longitudinal section of 5 µm thickness. The sections were stained with hemotoxylin and eosin dye for histopathological observation (18).

1.5) Statistical analysis

The results were expressed as the mean ± standard error mean (SEM). Statistical analyses were performed using one way analysis of variance (ANOVA)between groups, and unpaired comparisons were analyzed using the least significant difference method t-test. A P-value of 0.0001 or less was considered statistically significant. All analyses were conducted using Tukey's test Prism GraphPad software Version 9.2.0.

RESULT

2.1Effects of herbal drug on neurobehavioural parameter and Weight

Neurobehavioral analysis include assessing motor and non-motor symptoms to better understand the disease's course and underlying causes. These analyses aid in discovering prospective treatment targets and determining the efficacy of drugs. The muscle coordination strengths were assessed using a rotarod test. On

12th day Group 5 had higher muscle strength (128±0.9) compared to Group 4 (108.22±1.4) ($p<0.01$) and Group 2 (35.88±1.47). These data reveal that the grip strength of the Group 5 rat is more than the Group 4 and Group 2. Group 5 required shorter time to withdraw the front paw (10.05±0.31) compared to Group 4(13.77±0.44) and Group

2(25.61±0.59). Motor impairment and rigidity was assessed using catalepsy test. On 12th day Group 5 animals removed front in less time (10.05±0.31) as compared to Group 4 (13.77±0.44) ($p<0.01$) and Group 2 (25.61±0.59). These data reveal that the time required to remove front paw to Group 5 rats is less than the Group 4 and Group 2. Group 5 requires shorter time to withdraw the front paw (10.05±0.31) compared to Group 4(13.77±0.44) and Group 2(25.61±0.59). Actophotometer were used to detect locomotor activity. On 12th day Group 5 (366.6±1) animal showed more number of movements as compare to group 4 (308.8±3)and group 2 (124.88±97) rats. Group 5 animals had more locomotor activity in rats as compared to group 4 and group 2. Body weight of rat were weighed using digital weighing balance on 12thday of the study. Animals in Group 5 (229.33±9.43) gained considerably more weight than Groups 4 (229.33±9.43) and Group 2 (146.66±34.51).

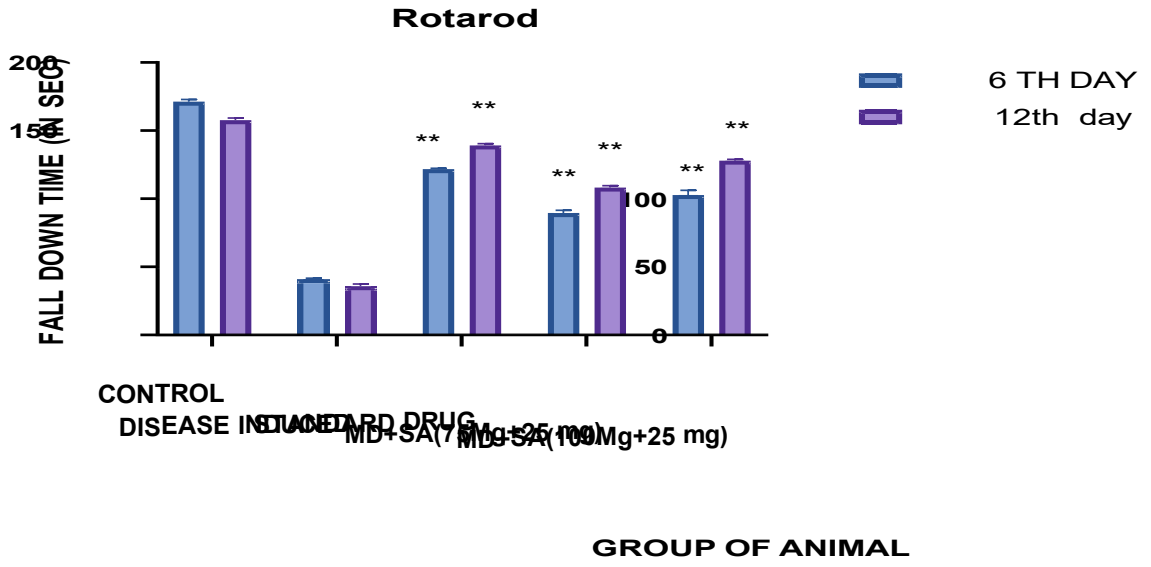
2.2Effects of herbal drug on Biochemical Estimation

Effect of lipid peroxidation on rat brain tissue showed the decrease in the level of MDA of group 5(0.647 ±0.012) as compare to group 4(0.710 ±0.003) and group 2(0.845 ±0.010). Effect of Reduced Glutathione on rat brain tissue showed the increase in the level of glutathione in the group 5(47.81 ±1.72) rats as compare to group 4(43.61 ±1.63) and group 2(20.66 ±1.21) rats. Effect of catalase on rat brain tissue showed the increase in level of catalase in group 5(0.380

±0.04) rats as compare to group 4(0.364 ±0.06) and group 2(0.156 ±0.04) rats.

(A)

(A)



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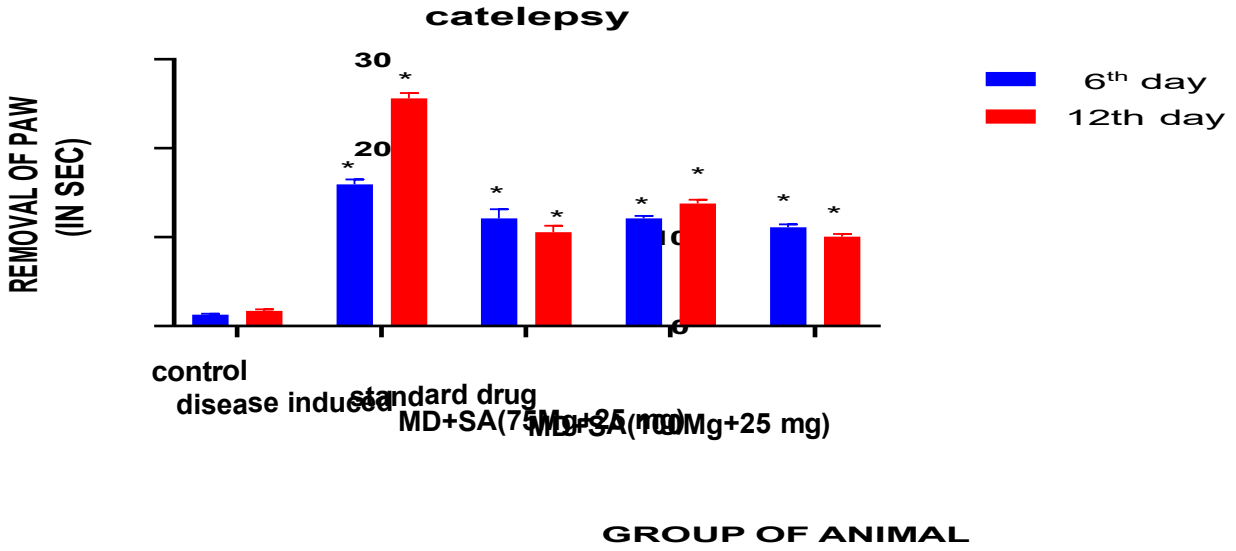

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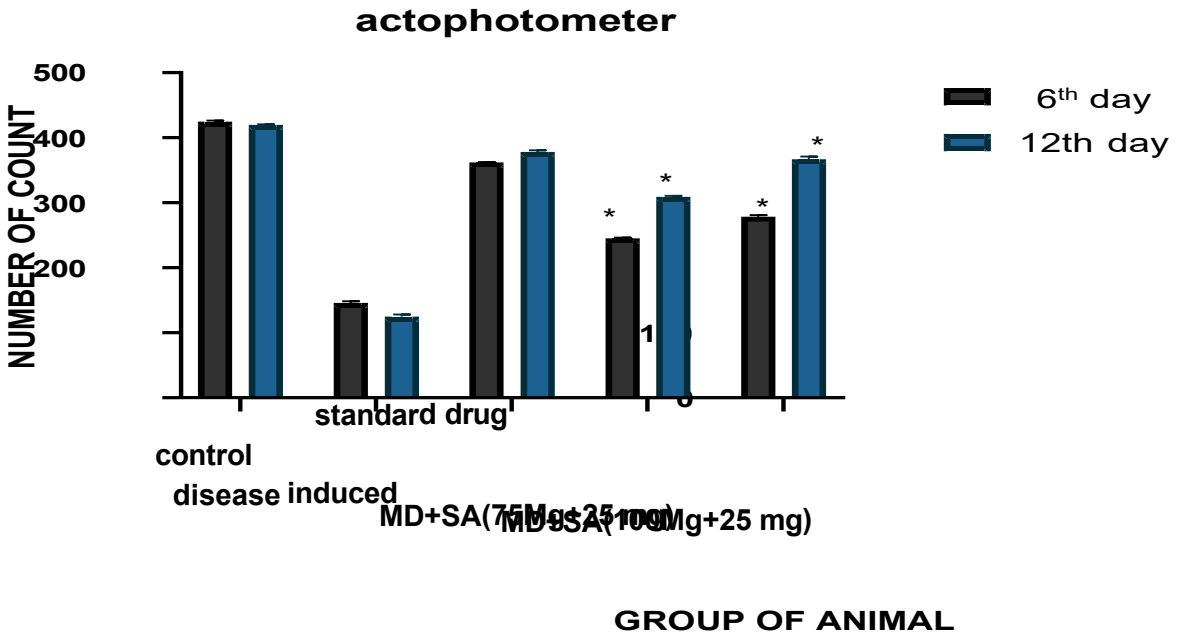

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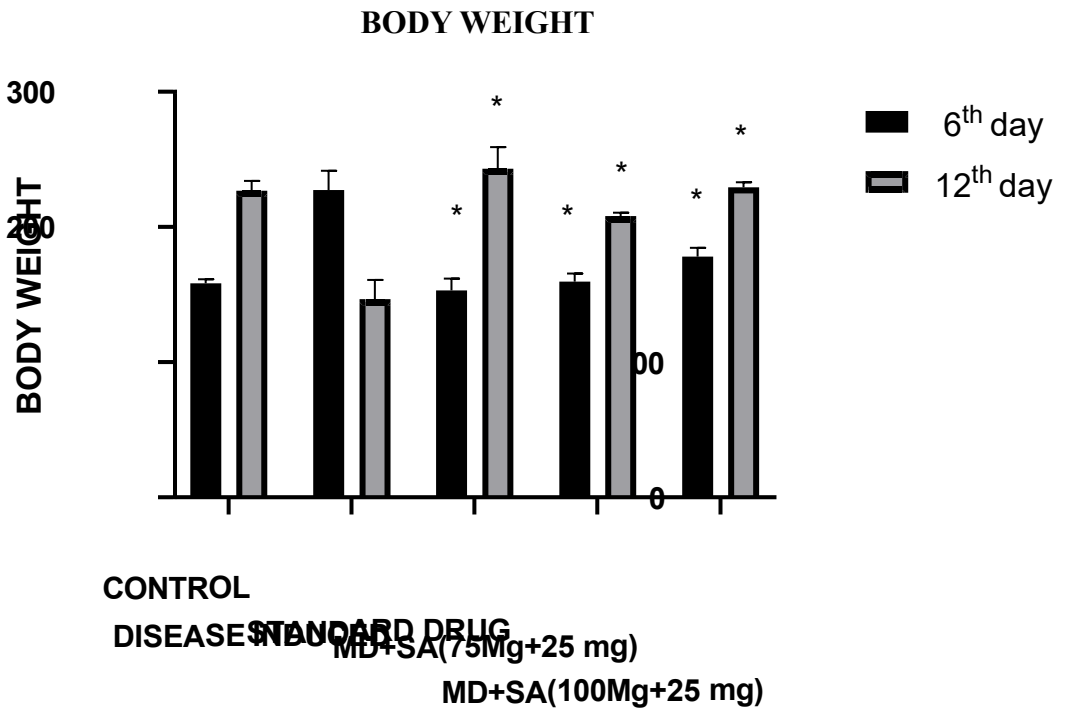

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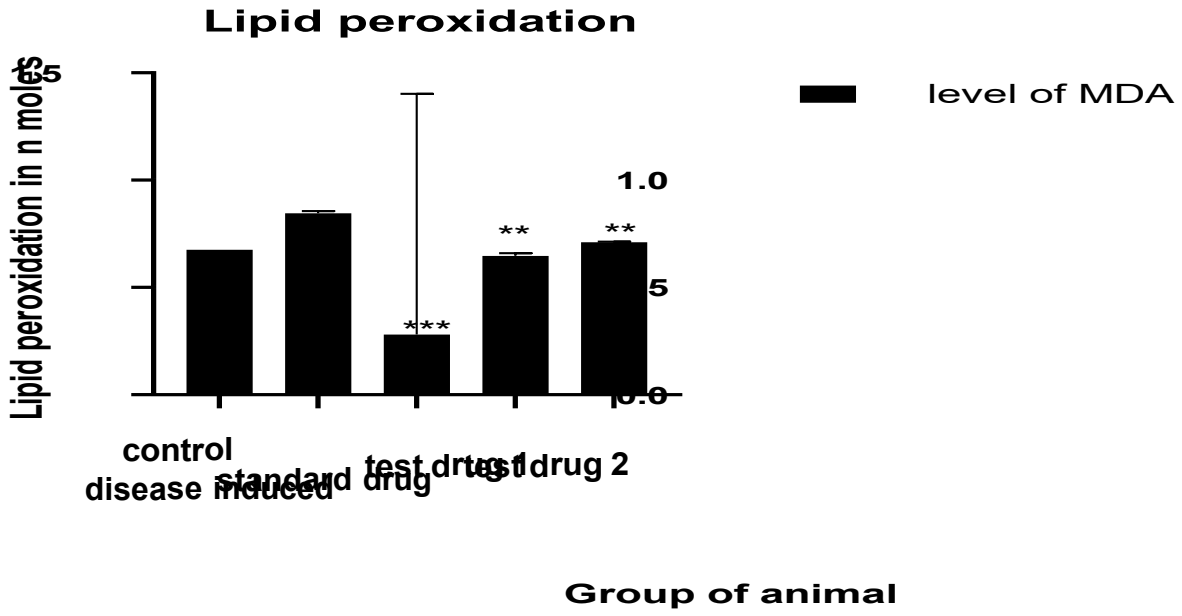

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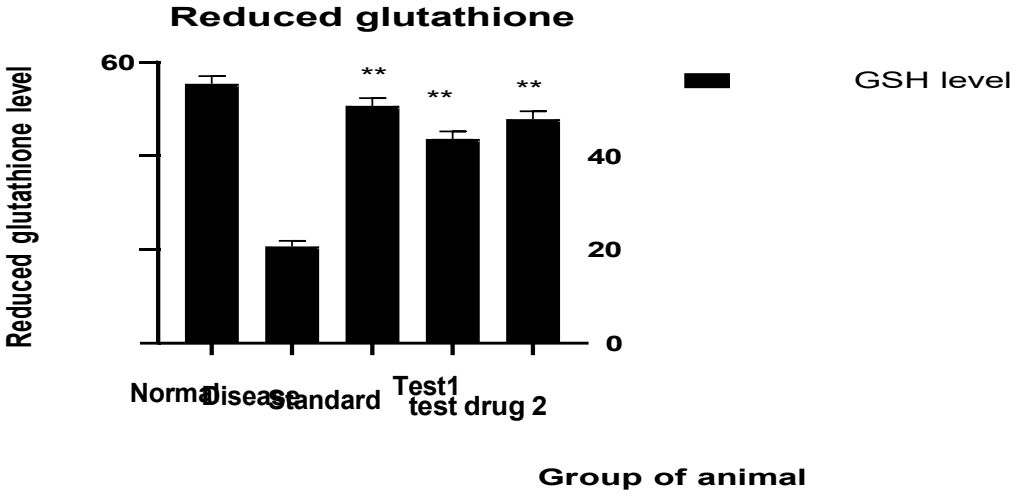

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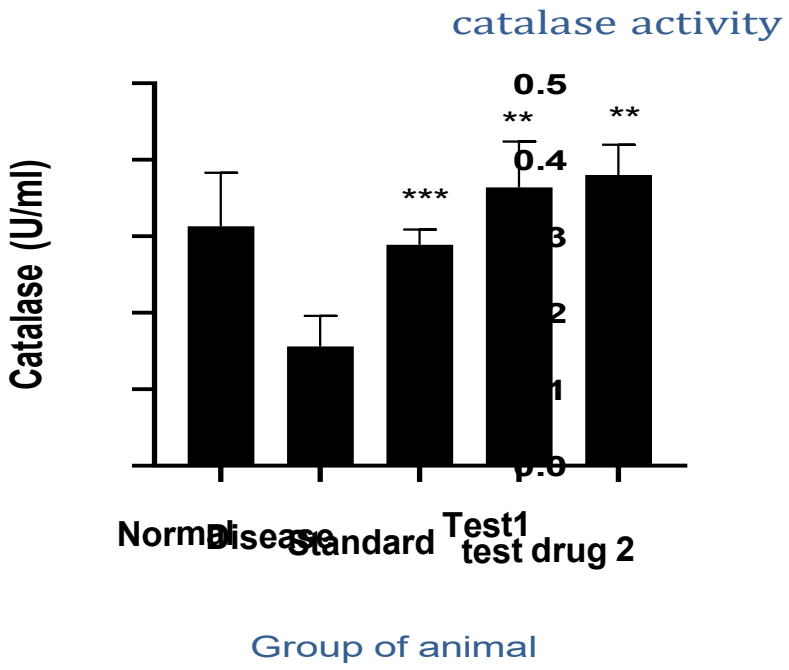

Fig. 1. A) The muscle coordination strengths were assessed using a rotarod test. B) Motor impairment and rigidity was assessed using catalepsy test. C) Actophotometer were used to detect locomotor activity. D) Body weight of rat were weighed using digital weighing balance. E) Effect of lipid peroxidation on rat brain tissue. F) Effect of Reduced Glutathione on rat brain tissue. G) Effect of catalase on rat brain tissue All values expressed as mean±SEM; n=6 rat in each group, by one-way ANOVA followed by Dunneet's Multiple Comparison Test(compared with control group) *p<0.05, **p<0.01, and ***p<0.00.

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2) Histopathological study

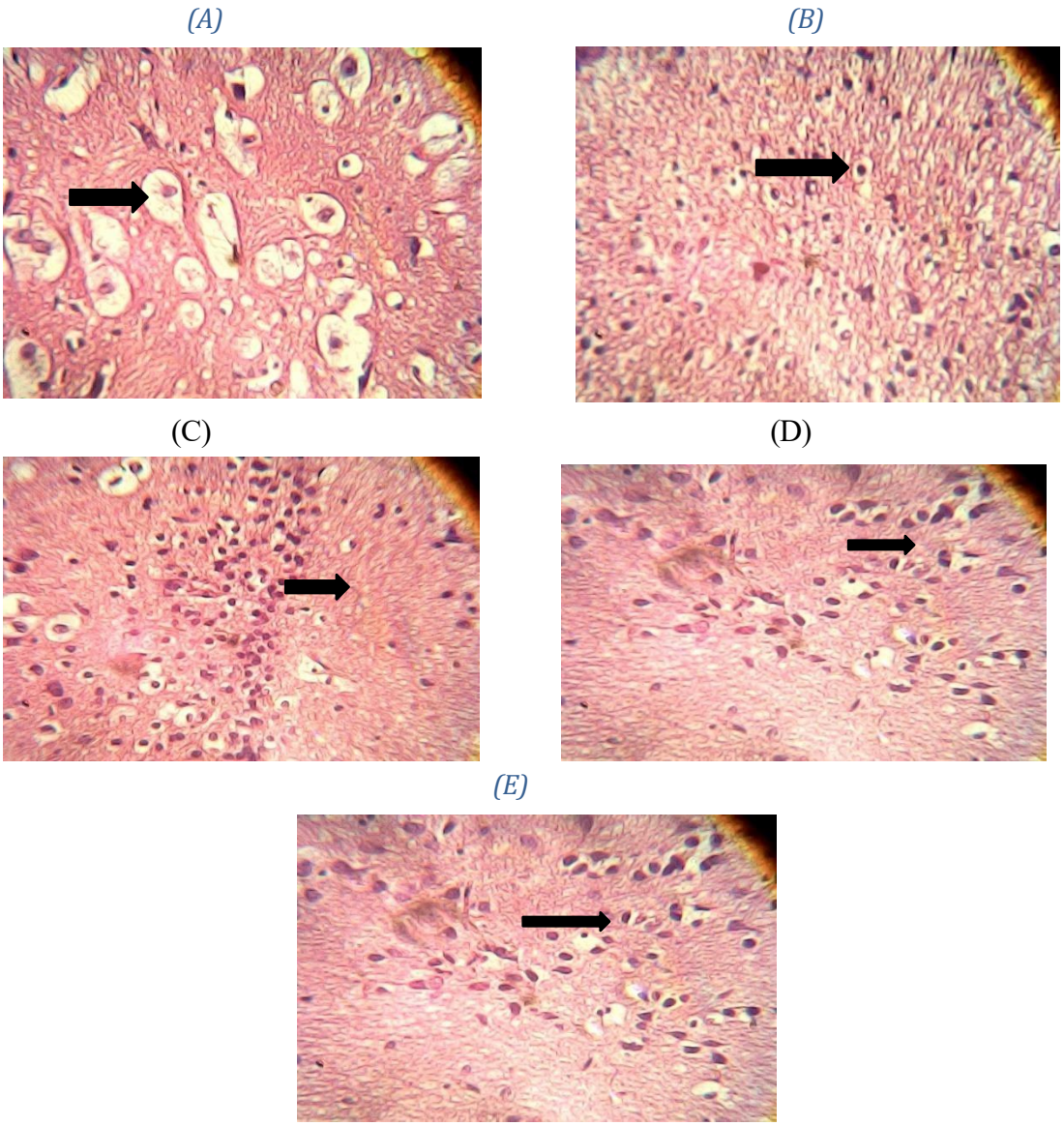

Fig. 2. A)control group showing normal brain architecture. B) Rat treated with rotenone showing degeneration of neuron increase in the number of formation of lewy bodies . C) Rats treated with rotenone and Levodopa+carbidopa (100mg+25 mg/kg) showing minimal changes in neuronal cell integrity and architecture decrease in number of lewy bodies. D) Rats treated with rotenone and momordica dioica+ salvia rosmarinus (75mg+25 mg/kg) showing mild formation of lewy bodies E) Momordica dioica + salvia Rosmarinus 100+25 mg/kg) treated rats showing minimal changes in neuronal cell populations and decrease in number of lewy bodies.

DISCUSSION

Parkinson's disease (PD) is highly prevalent neurodegenerative disorder worldwide next to Alzheimer disease (AD). To date, no treatment available for this advanced and chronic disorder that can inhibit or halt the degeneration of neurons in the Substantia nigra(19).

The purpose of present study assess the effects of methanolic extract of Momordica Dioica And Salvia Rosmarinus Linn on oxidative stress (OD) induced damage in animal model of PD. Our results exposed

that might be Momordica Dioica And Salvia Rosmarinus protective against OD induced PD in wistar rats. Momordica Dioica And Salvia Rosmarinus is a natural bioactive and most prominent compound, which has showed admirable therapeutic benefits in several diseases. Due to their active biological properties like ant-inflammatory and anti-oxidative Momordica Dioica And Salvia Rosmarinus has a significant valuable role in many pathological ailment as well as inflammatory and neurological disorders, cancer, antibacterial, antidiabetic, cardiovascular and AD(20). The potential protective effect of natural bioactive compound against rotenone induced toxicity of the nigrostriatal dopaminergic neurons. Momordica Dioica And Salvia Rosmarinus shown to be effective in the therapy for motor symptoms, along with oxidative damage and inflammation by reduction of Dopamine(21). Oxidative stress is due to formation of excessive superoxide which is mainly responsible for neurodegenerative disease and inflammatory processes are intimately linked with death of dopamine producing neurons in the brain (22). In PD pathogenesis neuroinflammation is also considered as a main component because the accumulation of cytokine and activation of microglia are found in most experimental model of PD (23). Animal models of PD centered on precise pathogenic mechanisms can successively lead to the development of neuroprotective drug for PD that delay the progression of disease (24).

The rotarod test is a widely used as behavioral assessment tool for evaluating motor coordination and balance in rodent models of Parkinson's disease. The test is sensitive to the effects of dopamine neurotransmission and can be modified to focus on specific aspects of motor learning, such as the acquisition of motor skills (25). The parameter of rotarod such as fall down time is used to evaluate grip strength in muscle. As the fall down time of disease induced group is less than the treatment group on the day 6 and 12 is recorded. In the rotarod test combination of Momordica dioica and salvia Rosmarinus showed significant effect on the treatment group as compared to disease control animal comparing the day 6 and 12. Catalepsy is a significant behavioral marker used in the assessment of Parkinson's disease (PD) in rat models. As we evaluated the time required to remove the front paw from the bar on the day 6

and 12, we observed the effect of combination of Momordica dioica and salvia Rosmarinus dose given group showed the significance increase in time to remove the front paw from the bar as compare to control group animal. Actophotometer is a device used to measure locomotoractivity in experimental rats. In this we observed locomotor movement of test drug group animal increased as compared to disease induced. The histopathological studies showed the presence of lewy bodies more in disease control group than the treatment group.

Lipid peroxidation occurs due to attack by radicals on double bond of unsaturated fatty acid and arachidonic acid which generate lipid peroxyl radicals and that initiate a chain reaction offurther attacks on other unsaturated fatty acid. As the biochemical estimation of Lipid peroxidation, catalase, reduced glutathione level were observed and the levels of of lipid peroxidation is increased in disease control group than the treatment group. GSH and catalaselevels were decreased in disease control group as compared with treatment group As we know,lipid peroxidation is the process of oxidative degradation of polyunsaturated fatty acids and its occurrence in biological membranes causes impaired membrane function, impaired structural integrity, decreased fluidity, and inactivation of number of membrane bound enzymes (26).

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Ethics approval:

All the experimental procedure were approved by Institutional Animal Ethics Committee (IAEC) with proposal no: CPCSEA/CBPL/AH/82.

Declaration of Competing Interest:

None.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.