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Antiparkinsonian Activity Of Vitamin D3 And Pregabalin Against Rotenone Induced Parkinsonism In Rat Model

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Abstract: -

Rotenone is a potent specific inhibitor of mitochondrial complex-1 that appears to reproduce the behavioural features of Parkinson's disease in rats. It selectively destroys dopaminergic neurons, causing a deficiency of dopamine in the striatum which leads to impaired motor functions include tremor and muscle rigidity. The purpose of this study was to assess if pregabalin affects Parkinson's disease. Long-term use of neuroleptics in psychotic disorders such as schizophrenia results in extrapyramidal symptoms such as Parkinson's disease and neuropathic pain. The present study deals with the antiparkinson effect of Vitamin D3, Pregabalin on rotenone induced Parkinson disease in wister rats. Neural degeneration was induced in a group (II, III, IV) by Rotenone (2.5mg/kg daily i.p.) for 28 days. Group II: Vitamin D3 (30mg/kg); Group III Pregabalin (30mg/kg); Vitamin D3 (30 mg/kg p.o.) and pregabalin (30 mg/kg p.o.) drug treatment significantly improved these behavioural and biochemical alterations restored mitochondrial enzyme complex activities and attenuated neuroinflammatory markers in rotenone (2.5mg/kg) treated animals as compared to control group. It can be concluded that the Vitamin D3 (30mg/kg) showed highly significant decrease oxidative stress (MDA level)) and significantly increase dopamine level of post-treatment. The pregabalin (30mg/kg) showed significant increase locomotor activity and Rota rod test.

Keywards: - Parkinson disease, Pregabalin, Rotenone, Vitamin D3

Introduction: -

In 1817, James Parkinson described Parkinson's disease (PD) for the first time as a chronic progressive neurological condition.[1] Parkinson's disease (PD), the second most prevalent neurodegenerative disorder, is distinguished by decreased motor coordination (muscle rigidity, tremor, postural imbalance, and movement slowness) and non-motor symptoms (mood disorders, sleep disturbances, and cognitive loss). [2] Parkinson's disease affects 10 million individuals globally. It affects approximately 2-3% of persons over the age of 65 [3]. Parkinson's disease (PD) is distinguished primarily by dopamine-carrying neuron loss in the substantia nigra, as well as extrapyramidal symptoms such as tremors, bradykinesia, rigidity, and inability to maintain normal posture [4]. Free radical damage causes neuronal death in Parkinson's disease, resulting in the formation of Lewy bodies [5].
In general, a combination of synthetic drugs is more effective in treating Parkinson's disease. Although levodopa is the first-line treatment for Parkinson's disease, long-term use has a number of undesirable side effects [6]. Parkinson's disease increases neurodegeneration due to dopaminergic loss, oxidative stress induced by -synuclein aggregation (Lewy body), and mitochondrial dysfunction. The Parkinson's disease (PD) demonstrates that inflammatory mediator production, elevated reactive oxygen species (ROS), and mitochondrial abnormalities are all crucial factors in the development of PD.[7,8] Consequently, the primary therapeutic targets for delaying the development and progression of Parkinson's disease are oxidative stress and inflammatory processes.[9].
As a result, numerous pharmacological strategies have been investigated to address inflammation and oxidative stress, two closely related processes. The current medications only help with the symptoms of Parkinson's disease. However, the actual cause of this chronic complex neurological disorder is uncertain; genetic mutation, environmental factors, and age are the key causes of PD[7]
The rotenone model of Parkinson's disease (PD), which established the role of systemic complex I breakdown and pesticide exposure in the the cause of the disease. Rotenone is a popular pesticide and a high-affinity inhibitor of complex I of the mitochondrial electron transport chain. Despite causing uniform complex I inhibition throughout the brain, rats treated with rotenone demonstrated many signs and symptoms of Parkinson's disease (PD), such as motor deficits, the

formation of ubiquitin and synuclein-positive nigral inclusions, and selective nigrostriatal dopaminergic degeneration [10] Alternatively, oxidative stress may be the cause of rotenone poisoning.
The brains of individuals with Parkinson's disease (PD) exhibit indications of oxidative stress, such as reduced glutathione levels and oxidative changes to proteins, lipids, and DNA.[11] Reactive oxygen species (ROS) are produced by mitochondrial respiration and dopamine metabolism. Impaired complex I activity increases the generation of ROS[12].Within complex I, upstream of the rotenone-binding site, is a region of electron leakage that produces ROS[13]. The pregabalin is antiseizure and antinociceptive drug. Pregabalin is a voltage-gated Ca2+ channel antagonist that exclusively binds to the alpha-2-delta subunit to provide antiepileptic and analgesic effects. Pregabalin's mode of action for pain relief: Pregabalin inhibits the VGCC, reducing glutamate and sensory neuropeptide release at the synapse via decreasing Ca2+.[14] The genesis and progression of several movement disorders, including Parkinson's disease (PD), have been connected to VD3 deficiency [15]. In a mouse model of PD, VD3 insufficiency has been shown to favourably modify and enhance neurotransmission as well as behavioural impairments [16]. A steroid called cholecalciferol (VD3) has been shown to enhance cell viability through encouraging repair and upregulating genes related to protein synthesis. We proposed that because of its functions in Ca2+-related indicating, radical detoxification, and overall brain health VD3 may help with dyskinesia. The effects of VD3 on enzymes related to oxidative stress, behavioural changes, and dopamine metabolism.[17]

Materials and Methods: -

Wistar rats(200-250g) were obtained from (YSPM'S YTC, Faculty of pharmacy, Satara). These rats were procured from registered breeder and were acquainted in the quarantine area for one week. After acquaintation, animals were transferred to the standard laboratory conditions of 22□2□C temperature, 50□15% of relative humidity,12 hr dark/12hr light cycle and the animals had free access for Purpose Control and Supervision of Experiments on Animals Guidelines. The experiment was performed as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Experimental design: -
Rotenone was first dissolved in dimethyl sulfoxide at 50× stock solution and diluted in sunflower oil to obtain a final concentration of 2.5 mg/mL. For the induction of PD in rats, ROT (2.5 mg/kg body weight) was administered intraperitoneally (i.p) once daily for 4 weeks. All animals were in 5 Groups (n=6). Group-I (Normal Control)- was administered Vehicle/Saline once a day for two weeks.

Group II Toxicant control rotenone (2.5mg/kg i.p.) once a day for two weeks. Standard control (Group-III) administered with Levodopa/carbidopa (125mg/kg once a day for two weak 30 min before rotenone). Test 1 (Group-IV) oral administration of Vitamin D3 (30mg/kg) once a day for two weak 30 min before rotenone). Test 2 (Group-V) oral administration of Pregabalin (30mg/kg) once a day for two weak 30 min before rotenone). During a 28-day course of treatment, all 5 animal groups participated in behavioural evaluation tests, which included the High Bar Test (Catalepsy test), locomotor activity using actophotometer test and Rotarod tests (fall of time) were used. Oxidative stress (MDA level) and neurochemical test (dopamine level) evaluations were also carried out. [18]-[19]-[20]-[21]

Cataleptic Activity: - The catalepsy was measured using the bar test. To cause catalepsy, rotenone was employed. The rats were positioned in the bar test with both front paws on a horizontal bar that was parallel to and 9 cm above the base. One-centimetre-wide wooden bar. When the animal moves its head or removes both front paws from the bar, the activity comes to a stop. There was a 300-second time limit. In the period between the two assessments, the animals were placed back in their own cages. Every measurement was made between 23 and 25 °C in a quiet setting. When it came to scoring, the animal was classified as cataleptic and the duration was measured in seconds if it kept the forced position for at least 20 seconds. [22]-[23]

Actophotometer test: - A locomotor ability is any physical action that necessitates the animal
to move from one place to another. With an actophotometer (activity cage), it is easy to measure. Actophotometer can identify akinesia or hypokinesia. Six light beam transmitters make up the device's cage, and the receiver is positioned so that when an animal cross one of the beams, only a single beam is interrupted at a time. One cell completes when the photo beam reaches the acceptor photocell. The difference was noted when the animal cut off the beam of light. Each animal's total photo beam interference during a 10-minute period was recorded as activity.[24]

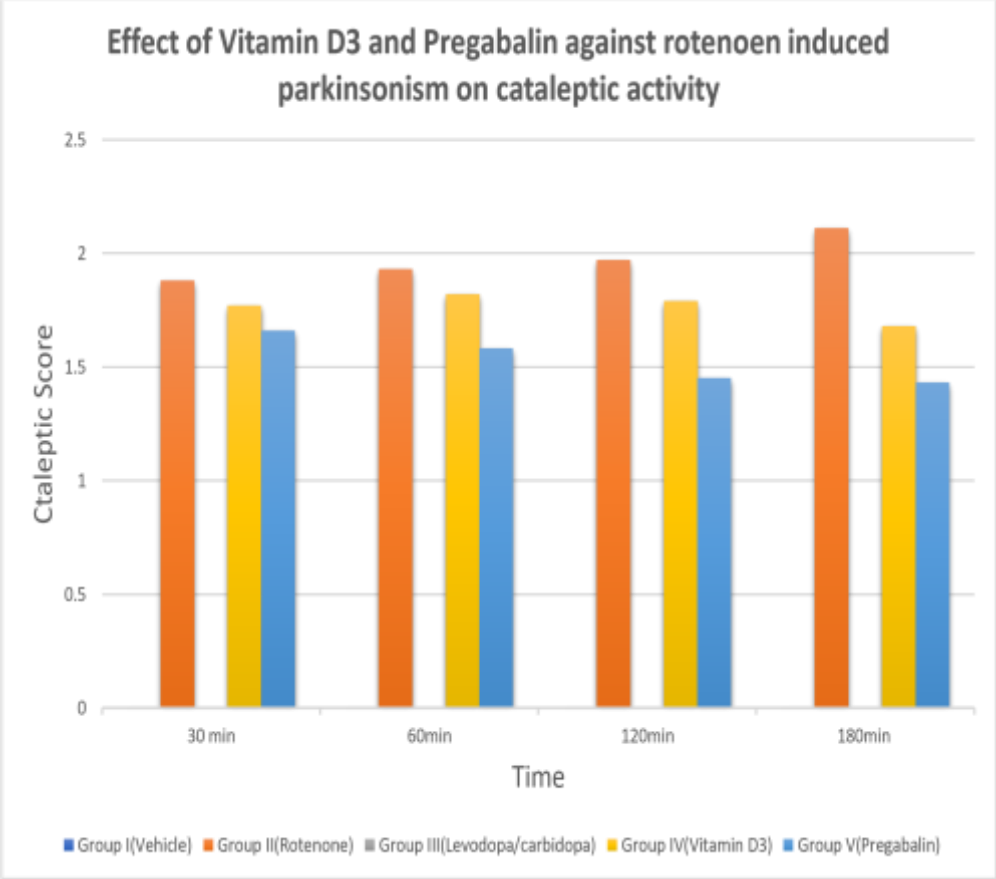
Rota-rod:-
The effect of rotenone, Vitamin D3 and Pregabalin on muscle rigidity. The Rota-rod test was used to assess the strength of muscles. The rats were trained to maintain posture on a rota-rod 6 centimetres across. The rod is divided into five parts

by a partition disc with a diameter of 10.5 cm. To stop animals from jumping from it, the rod was placed 50 cm above the ground. during two initial training sessions lasting 300 s, spaced around 10 minutes apart. After the first training trials, a 120-second baseline trial was performed. Each animal's duration on the rotarod was timed, and those that able to stay on it for the entire duration were awarded a maximum score of 120 seconds. All of the rats underwent five trials before the evaluation. For around five minutes, the control rat stays fastened to the pole. At regular intervals, the medicated rats were placed on the revolving rod, and the fall-off time was noted.[25]

Assessment of dopamine:- The dissected brain samples were weighed, homogenised in one millilitre of ice-cold water, and chilled at 80°C until analysis.0.2 µg/ml isoproterenol hydrogen and 0.1 mmol/L ethylenediaminetetraacetic acid (EDTA) in a 0.1 mmol/L perchloric acid solution. After centrifuging tissue homogenates for 30 minutes at 4°C at 15,000 g, the supernatant was filtered and stored at -80°C until the test. Using an electrochemical detector and a 25 cm × 0.5 cm I.D column, high performance liquid chromatography was used to measure the levels of dopamine and DOPAC. The sample peak obtained is expressed in micrograms per gramme of tissue weight and is compared with the standard peak.[26]

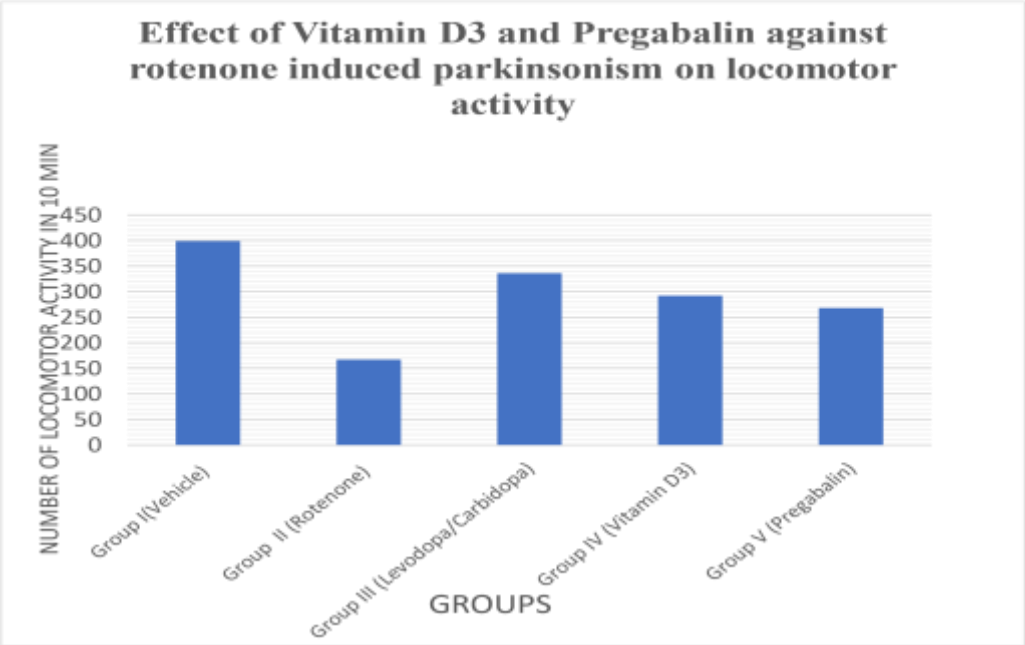
Biochemical evaluation:-
MDA Level: - MDA, a marker of lipid peroxidation, was quantified spectrophotometrically using the Colado et al. (1997) technique and 1, 1, 3, 3-tetraethoxypropane as a standard. MDA is measured in n moles per mg protein. 500 l of phosphate buffered tissue homogenate (pH 7.4), 300 l of 30% trichloroacetic acid (TCA), 150 l of 5 N HCl, and 300 l of 2% w/v 2-thiobarbituric acid (TBA) were added, followed by 15 minutes at 90 °C heating. For 10 minutes, the mixture was centrifuged at 12,000 g. A pink supernatant was produced and spectrophotometrically quantified at 532 nm.[27]

Results: -
Cataleptic activity: -



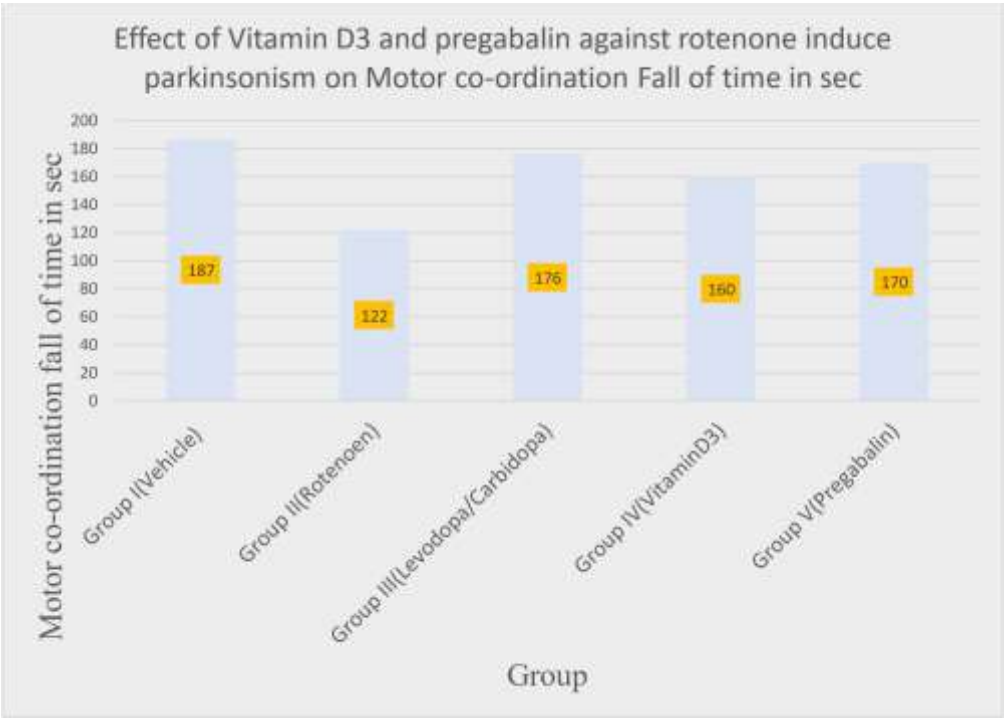
Data are expressed as Mean □SEM (n=6) NC=Negative Control, PC=Positive Control
***P<0.01 ###p Negative Control compared with Normal Control
##p <0.01 Standard control and Test Control compared with Negative control.

Locomotor activity: -



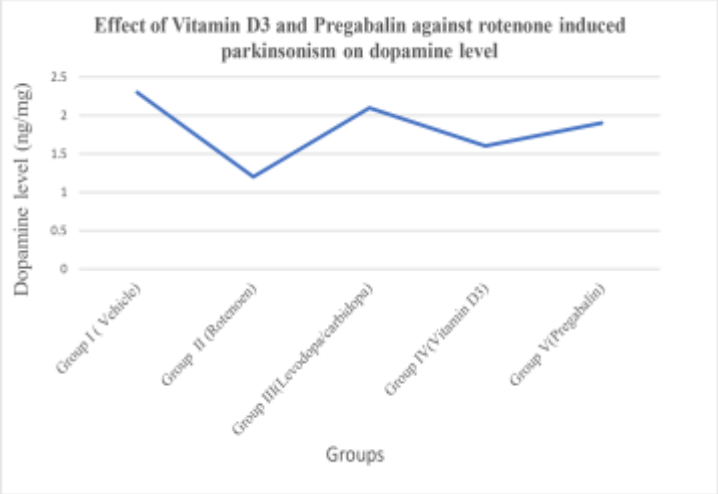
Data are expressed as Mean SEM (n=6) NC=Negative Control, PC=Positive Control
***P<0.01,**p<0.01 Negative Control compared with Normal Control
##p <0.01 Standard control and Test Control compared with Negative control.

Rota-Rod:-



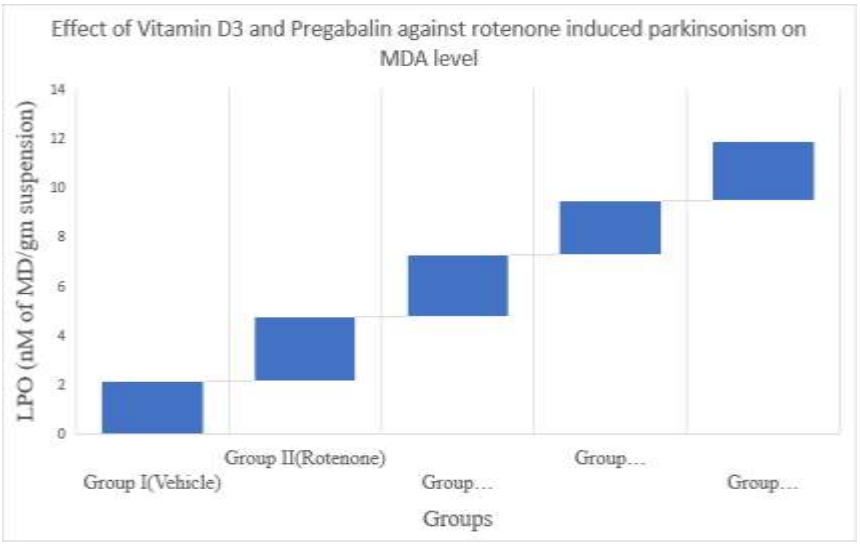
Data are expressed as Mean SEM (n=6) NC=Negative Control, PC=Positive Control
***P<0.01,**p<0.01 Negative Control compared with Normal Control
##p <0.01 Standard control and Test Control compared with Negative control.

Dopamine:-



Data are expressed as Mean SEM (n=6) NC=Negative Control, PC=Positive Control
***P<0.01 **p<0.01 Negative Control compared with Normal Control
##p <0.01 Standard control and Test Control compared with Negative control.

MDA:-



Data are expressed as Mean SEM (n=6) NC=Negative Control, PC=Positive Control
***P<0.01 ,**p<0.01 Negative Control compared with Normal Control
##p <0.01 Standard control and Test Control compared with Negative control.

Discussion:-

The goal of current investigation was to assess VitaminD3 and Pregabalin influence on rotenoneinduced behavioural changes. For the purpose of establish a PD model in rats, rotenone, a pesticide and selective inhibitor of mitochondrial complex-1, has been employed. It specifically kills DA-ergic neurons[28] (Yong et al., 2005) and results in the motor function impairment that is symptomatic of Parkinson's disease (PD)[29]. When compared to control group of rats in the current investigation, DA content was shown to decrease significantly in the animals treated with rotenone, revealing impairment to the DA-ergic system. .. During rotenone given to rats, there was a significant increase in catalepsy (bar test), decrease in locomotor activity (using
Actophotometer), and decrease in muscle activity (rota-rod test). According to the available data, rats given rotenone appeared to have impairment to their motor control system (DA-ergic neurons) and the onset of behavioural signs like those of Parkinson's disease. The pathophysiology of Parkinson's disease is significantly influenced by oxidative stress that is produced as a result of mitochondrial failure, namely mitochondrial complex-1 impairment[30]. In the present study, oxidative stress was assessed using the MDA assay. It was found that, in comparison to control animals, the MDA level significantly increased in rotenone-treated animals, indicating enhanced oxidative stress in those animals. Lipids,

particularly polyunsaturated fatty acids, are very vulnerable to ROS formation via a variety of enzymatic and non-enzymatic processes, which can lead to peroxidation.
By scavenging reactive oxygen species, vitamin D3 might provide protection against oxidative stress-induced cellular damage.[31]
Pregabalin is a voltage-gated antagonist of the calcium channel that only binds to the alpha-2delta subunit, which has analgesic and antiepileptic properties. The way that pregabalin relieves pain [32].
Rats given Rotenone (2.5 mg/kg i.p.) had a higher cataleptic score in the high bar test than the normal control group received. Comparing Group II to the other groups, there is an apparent rise in paw retention time on bar and a markedly higher catalepsy in the disease control group.Test 1(Group-IV) treated by Vitamin D3 (30 mg/kg p.o.) did not show significant reduction in cataleptic activity at 14th day. Test 2(Group-V) treated by Pregabalin (30mg/kg p.o.) showed significant reduction in cataleptic activity at 28th day.
In locomotor activity by actophotometer was decreased in rotenone (2.5mg/kg i.p.) administered rats as compared to normal control (Group-I). Group-III treated by Levodopa/Carbidopa (120mg/kg p.o.) showed significant increase in locomotor more significant increase in locomotor activity at 28th day. Test 1(Group-IV) treated by vitaminD3 (30 mg/kg p.o.) did not show significant increase in locomotor activity at 28th day. Test 2 (Group-V) treated by Pregabalin (30mg/kg p.o.) can show significant increase in locomotor activity at 28th day.
Rotenone(2.5 mg/kg i.p.) showed a decrease in retention time on the Rota rod apparatus, which clearly shows the muscle co-ordination property dysfunction due to dopaminergic depletion. Group-III treated by Levodopa/Carbidopa (120mg/kg p.o.) can increase motor coordination activity (fall-off time) at 28th day. Test 1 (Group-IV) treated by Vitamin C (30mg/kg p.o.) did not showed significant increase motor coordination activity (fall-off time) at 28th day. Test 2 (Group-V) treated by (30 mg/kg p.o) showed more significant increase motor coordination activity (fall-off time) at 28th day.
Rotenone (2.5mg/kg i.p.) treated animals shows decrease the dopamine. Group-III treated by Levodopa/Carbidopa (120mg/kg p.o.) showed more significant increase in dopamine level.
Group IV-V showed slightly significant increase dopamine level. Administration with rotenone (2.5mg/kg) show increase the MDA level. Test 1 (Group-IV) treated by Vitamin D3 (30 mg/kg
p.o.) Showed more significant decrease in MDA level. Test 2 (Group V) treated by pregabalin did not show significantly decrease MDA level.

Conclusion:-
The present study can be concluded that pregabalin may posses symptomatic antiparkinson activity, Vitamin D3 can be shows antioxidant activity in Parkinson disease against rotenone induce parkinsonism in rats. However further investigation is required to establish this neuroprotective response in another experimental animal model.

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