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Original Article

EVALUATION OF SINIGRIN EFFECT IN NEUROPROTECTION AGAINST PARKINSON'S DISEASE AND NEUROPATHIC PAIN

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ABSTRACT

Objective: The present study aims to evaluate the neuroprotective activity of Saponin: Sinigrin against Parkinson's disease (PD) and associated neuropathic pain in rat model. A correlation between Parkinson's disease (PD) associated neuropathic pain and predicting antioxidant, neuroprotective effects of Saponin: *Sinigrin* and its interspecific relation with the underlying mechanism.

Methods: Excitotoxicity with Mono Sodium Glutamate (MSG) (2 g/kg i. p) and neurotoxicity with Acrylamide (ACR) (30 mg/kg,i. p) was induced in rats, treated with standard dextromethorphan (30 mg/kg p. o), and Pregabalin (10 mg/kg,po) and test compound *(Sinigrin* 75 mg/kg) were tested for behavioral parameters viz: muscle rigidity, locomotor activity, mechanical hyperalgesia, cold allodynia, etc. and biochemical estimation from brain and sciatic nerve homogenate by sacrificing animals was done. Estimation of brain neurotransmitters (Dopamine, Gamma-Amino Butyric Acid (GABA) antioxidants, Glutathione (GSH) and Catalase(CAT), and oxidative stress Super Oxide Dismutase (SOD), Nitric oxide (NO) concentration, Thiobarbituric Acid Reactive Substances (TBARS) and Myloperoxidase activity(MPO) was done. Statistical analysis was done using Analysis of Variance (ANOVA) followed by Tukey's multiple comparison tests.

Results: *Sinigrin* showed a significant neuroprotective activity in rats compared to monosodium glutamate (2 gm/kg i. p. It was observed from the study that test drug *Sinigrin* produced a significant ($p \leq 0.05$) reduction in muscle rigidity, increased locomotor activity, left hind paw lifting duration, improved cold allodynia, and thermal hyperalgesia. Brain neurotransmitter levels antioxidant ($p \leq 0.01$) were increased and oxidative stress ($p \leq 0.01$) was also reduced to that of the standard drug dextromethorphan.

Conclusion: The study suggests that *Sinigrin* is neuroprotective and can be used in the treatment of Parkinson's Disease (PD) and associated Neuropathic Pain (NP).

Keywords: *Sinigrin*, Neuroprotective, Excitotoxicity, Parkinson's disease (PD), Neuropathic pain (NP), N-methyl-D-aspartate receptor (NMDARs) antagonism, Dextromethorphan, Pregabalin

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INTRODUCTION

Modern life has become increasingly stressful with every passing day. Lack of physical movement and mental relaxations lead to all the stress-related disorders. It is estimated that 6.3 million people have Parkinson's worldwide, affecting all races and cultures. The three main strategic developments in drug discovery that have advanced the progress in therapeutic management of Parkinson's Disease (PD) patients have focused on the alleviation of motor symptoms by the use of dopaminergic mimetics, the development of novel non-dopaminergic drugs for symptomatic improvement, and lastly, the discovery of neuroprotective compounds that have disease-modifying effects in PD. The conventional medicines and treatment, available today have proven ineffective in curing the multifunctional pathological mechanisms of PD [1]. Clinically, is characterized by motor symptoms such as resting tremor, bradykinesia and rigidity of skeletal muscle, postural instability, stooped posture, and freezing of gait. Furthermore, patients with this disease can show non-motor symptoms, including cognitive and behavioral problems, as well as sensory impairment (pain), and they may also suffer from sleep disorders or autonomic dysfunction. One of the major problem associated is 'pain' a common symptom in PD, and is often related to the illness itself. Mechanisms implicated in the disease process include oxidative stress, mitochondrial dysfunction, protein aggregation and misfolding, inflammation, excitotoxicity, and apoptosis [2]. Glutamate and dopamine are both important central neurotransmitters in mammals. A lack of enzymatic decomposition of extracellular glutamate results in glutamate accumulating at synapses, which is mainly absorbed by Excitatory Amino Acid Transporters (EAATs), leading to nerve damage and slowly neuronal death [3]. Neuroprotection is a broad term to cover any therapeutic strategy to prevent nerve cells called neurons from dying, and it usually involves an intervention, either a drug or treatment. Glutamate-mediated excitotoxicity is involved in many types of neurodegenerative diseases. Currently, there is no safe and effective drug to prevent excitotoxicity. Since over-activation of N-methyl-D-aspartate receptor (NMDARs) is considered to be the main factor causing glutamate excitotoxicity, blocking these receptors is a potential strategy to prevent excitotoxicity [4, 5]. The goal of neuroprotection is to limit neuronal dysfunction after injury caused due to Glutamate excitotoxicity and attempt to maintain the possible integrity of cellular interactions in the brain, resulting in undisturbed neural function. These products may be of various kinds and can be classified as free radical scavengers, anti-excitotoxicity agents, apoptosis inhibitors, neurotrophic factors etc. The objective of the present study was to evaluate the neuroprotective activity of saponins glycoside (glucosinolate) *Sinigrin* against PD [6]. The mechanism(s) underlying neuropathic pain are not completely understood but are considered to be complex, multifactorial and to evolve over time [7]. Bioactivity evaluation of saponins glycoside (glucosinolates: *Sinigrin*) was done by *in vivo* models which were approved by IAEC, like neuroprotective activity was evaluated using Mono Sodium Glutamate (MSG)-induced neurotoxicity (induced excitotoxicity) in rats and neuroprotective activity against NP involved in PD was evaluated using acrylamide-induced Neuropathic Pain (NP) model in rats. Various behavioral parameter *in vivo* biochemical estimations of biochemical parameters were performed. The result of neuroprotection against PD for *in vivo* model were analyzed in Graph Pad prism software using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

MATERIALS AND METHODS

Drugs and chemicals

Sinigrin was procured from Sigma Aldrich, MSG was purchased from Himedia Lab., Dextromethorphan hydrochloride chewable tablets (Lastuss CT, FDC), Dopamine,(Sigma Aldrich), 5,5-dithiobis[2-nitrobenzoic acid] (Sigma aldrich). Dextromethorphan (Lastussct FDC mfg.), Seligiline, Pregabalin (gift sample obtained from Ranbaxy Research Laboratories, Gurgaon, India), Acrylamide was purchased from Sigma-Aldrich. The wistar rats of either sex, weighing 200-250g of 2-3 mo old were procured from Bharat serums and vaccines. The rats were brought to animal house of Dr. L. H. Hiranandani College of pharmacy, Ulhasnagar-03. In an animal house, these rats and mice were acclimatized under standard conditions of husbandry, i. e., 24±10 °C room temperature, 45-55 % relative humidity and 12:12 h light/dark cycle. In strict hygienic condition, the animals had free access to food and water supplied libitum. Before behavioral experiments, animals were acclimatized for at least two weeks. The study protocol was approved by the college Institutional Animal Ethics Committee (IAEC) and the experiments were conducted as per the guidelines of CPCSEA.

Experimental design

In vivo studies

In vivo models like neuroprotective activity was evaluated using MSG-induced neurotoxicity (induced excitotoxicity) in rats, and neuroprotective activity against neuropathic pain involved in PD was evaluated using acrylamide-induced NP model in rats was evaluated using acrylamide-induced NP model in rats.

Monosodium glutamate (MSG)-induced excitotoxicity in rats

Excitotoxicity was induced in rats with MSG (2 gm/kg i. p.); treated with standard dextromethorphan (30 mg/kg p. o.), test compound (Sinigrin 75 mg/kg) for 7 d and tested for behavioural parameters on 8th day of treatment. Following study protocol was used [8]:

Group I (Vehicle): 1% CMC

Group II. (Toxic group): MSG 2 gm/kg i.p.

Group III (Standard group): 2 gm/kg i.p. MSG+30 mg/kg (p.o.) Dextromethorphan

Group IV (Test Group): 2 gm/kg i.p. MSG+75 mg/kg (p.o.) Sinigrin

Behavioral assessment and biochemical estimation

Behavioral parameters observed were muscle grip strength by Rota rod, locomotor activity by actophotometer, mechanical hyperalgesia using Pin Prick Test, cold allodynia using acetone solution, hot plate (Thermal hyperalgesia). *In vivo* estimation of biochemical parameters: On 9th d of *in vivo* study, animals were sacrificed, brains and sciatic nerve were removed and homogenate was prepared. Following estimations were done viz: estimation of dopamine [9], Catalase (CAT), Glutathione (GSH), Super Oxide Dismutase (SOD), Nitric oxide (NO) concentration, Gamma-Amino Butyric Acid (GABA) levels, Thiobarbituric Acid Reactive Substances (TBARs) and Myloperoxidase (MPO) [10].

Statistical analysis

Value were expressed as Mean±SEM for 6 rats in each group. Significance was determined by one-way Analysis of Variance ANOVA followed by Tukey's multiple comparison test's *p≤0.05, **p≤0.05, ***p≤0.05, when compared with vehicle as well as disease group.

Acrylamide (ACR)-induced neuropathic pain in rat model

The NP was induced by intraperitoneal (i. p.) injection of ACR for 24 consecutive days (Gold *et al.*, 2004; Ling *et al.*, 2005). The slight modification was carried out in the present study; briefly, the ACR (30 mg/kg; i. p.) was administered once a day, for 24 consecutive days for the induction of painful neuropathy. All the groups of animals were subjected to assess the degree of nociceptive threshold at different time intervals, that is, days 0, 6, 12, 18, and 24. Following study protocol was used [11].

Group I (Vehicle): 1% CMC

Group II. (Disease group): ACR 30 mg/kg, i. p.

Group III (Standard group): Pregabalin 10 mg/kg, p. o.

Group IV (Test Group): *Sinigrin* 75 mg/kg,p. o.

Behavioral and biochemical assessment

The animals were subjected to the assessment of behavioral tests such as locomotor activity by actophotometer, Motor rigidity by horizontal bartest and muscle grip strength by hang test, analgesic potential by tail immersion test, Vonfrey test and hot plate test, Skeletal muscle weakness (decreased fore and hind limb grip strength, impair locomotor activity), degree of the nociceptive pain threshold, heat thermal sensitivity of the hind paw, Mechanical sensitivity of the hind Paw, test Spinal thermal hyperalgesia was assessed by tail withdrawal reflex. *In vivo* estimation of biochemical parameters was done on 24th day of *in vivo* study; animals were sacrificed, their brains were isolated and estimations done were: estimation of dopamine [12], CAT, GSH, SOD activity based on the reduction of Nitro Blue Tetrazolium (NBT) by oxygen (O_2), GABA levels, Malondialdehyde (MDA), SOD [13, 14].

Statistical analysis

The results of anti-Parkinson's activity were expressed as mean±SEM of 6 animals in every group. Results were analyzed statistically using one–way ANOVA followed by Tukey's multiple comparison tests; all groups were compared with disease control group and $P<0.05$, $P<0.01$ was considered significant. GraphPad Prism was the software used for statistical analysis.

RESULTS

MSG-induced excitotoxicity in rats

Behavioral parameters

Results of rotarod test and actophotometer were mentioned in table 1 and fig. 1 (1.1 and 1.2). For mechanical Allodynia (Von Frey Hair Test) and cold allodynia (latency for tail flicking) in table 2 and fig. 2 and 2 (2.1 and 2.2).

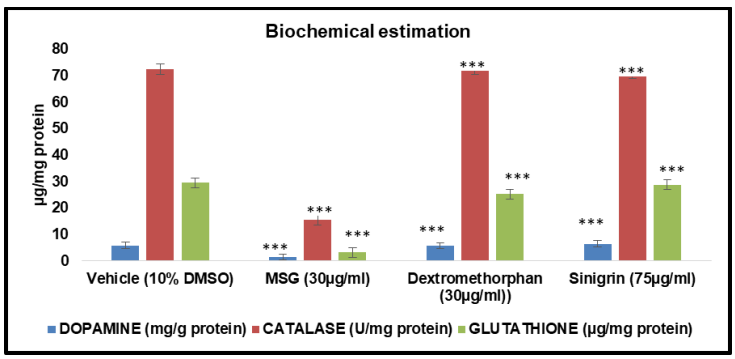

Fig. 3: Biochemical estimations of dopamine, CAT and GSH (The results of activity are expressed as mean±sem of 6 animals in every group. Significance was determined by one-way ANOVA followed by Tukey's multiple comparison test's ** p≤0.05, *** p≤0.05, when compared with vehicle as well as disease group)

Table 1: Muscle grip strength by rotarod and locomotor activity by actophotometer

Groups	Time in sec. (Fall off time)	Score of locomotor activity
Vehicle	51.66±2.87	205.5±2.00
Toxic (MSG-2g/kg i. p)	8.16±1.70***	87.33±1.93***
Standard (Dextromethorphan-30 mg/kg p. o)	49.33±0.71**	198.66±1.41***
Sinigrin (75 mg/kg p. o)	50.63±0.61***	152.26±1.65***

The results of activity are expressed as mean±SEM of 6 animals in every group. Significance was determined by one-way ANOVA followed by Tukey's multiple comparison test's **p≤0.05, ***p≤0.05, when compared with vehicle as well as disease group.

Table 2: Effect of test compound on paw withdrawal threshold and latency of tail flicking

Groups	Mechanical allodynia): (Von frey hair test)	Cold allodynia (Acetone evaporation test method)
Vehicle	50.92±2.07	20.667±0.429
Toxic (MSG-2g/kg i. p)	64.532±2.038**	10.667±1.313**
Standard (Dextromethorphan-30 mg/kg p. o)	39.50±3.05***	5.66±0.668***
Sinigrin (75 mg/kg p. o)	23.72±1.11***	4.33±0.612***

The results of activity are expressed as mean±SEM of 6 animals in every group. Significance was determined by one-way ANOVA followed by Tukey's multiple comparison test's **p≤0.05, ***p≤0.05, when compared with vehicle as well as disease group.

Table 3: Biochemical estimations of dopamine, CAT and GSH

Groups	Dopamine (mg/g protein)	Catalase (U/mg protein)	Glutathione (µg/mg protein)
Vehicle (10% DMSO)	5.82±0.045	72.3±1.89	29.44±1.889
MSG (30µg/ml)	1.37±1.057***	15.54±1.76***	3.17±0.583***
Dextromethorphan (30µg/ml))	5.68±0.037***	72.27±1.416***	25.22±3.104***
Sinigrin (75µg/ml)	6.37±0.026***	70.90±1.989***	28.78±1.788***

The results of activity are expressed as mean±SEM of 6 animals in every group. Significance was determined by one-way ANOVA followed by Tukey's multiple comparison test's *p≤0.05, **p≤0.05, ***p≤0.05, when compared with vehicle as well as disease group.

Table 4: Locomotor activity using actophotometer

Day / Groups	0	7	15	21
	Score of locomotor activity (Counts/5 min)			
Vehicle (1%CMC)	426.25±8.40	424.27±11.20	425.45±8.576	453.84±14.6
Disease control (ACR 30 mg/kg)	75.15±2.00*	78.27±6.63*	83.7±4.24*	89.95±4.50*
Pregabalin (10 mg/kg p. o)	400.99±14.11**	412.25±11.69**	415.47±4.12**	421.24±3.24**
Sinigrin (75 mg/kg p. o)	398.19±14.67**	409.24±19.37**	415.38±13.61**	420.55±11.62**

The results of activity were expressed as MEAN±SEM of 6 animals in every group. Results were analyzed statistically using one–way ANOVA followed by Tukey's multiple comparison tests; all groups were compared with disease control group and *P<0.05, **P<0.01 was considered significant. GraphPad Prism was the software used for statistical analysis.

Table 5: Muscle grip strength by horizontal bar test

Day / Groups	0	7	15	21
	Fall off time (Sec)			
Vehicle(1%CMC)	200.25±0.57	208.32±1.48	2128.67±0.95	209.15±0.95
Disease control (ACR 30 mg/kg)	64.67±1.33***	53.25±0.26***	48.33±0.18***	42.53±0.42 ***
Pregabalin (10 mg/kg p. o)	132.25±2.02***	144.67±1.76***	152.48±0.59***	180.25±0.29 ***
Sinigrin (75 mg/kg p. o)	124.25±1.52***	145.45±0.64***	194.67±0.72***	200.85±0.34 ***

The results of activity are expressed as MEAN±SEM of 6 animals in every group. Significance was determined by one-way ANOVA followed by Tukey's multiple comparison test's ***p≤0.05, when compared with vehicle as well as disease group.

Table 6: Hang test

Day / Groups	0	7	15	24
	Falloftime (Sec)			
Vehicle(1%CMC)	20.83±3.55	21.24±2.33	22.64±2.9	20.33±3.14
Disease control (ACR 30 mg/kg)	7.27±2.67*	6.27±1.18*	5.78±1.07*	4.87±0.93*
Pregabalin (10 mg/kg p. o)	19.97±0.99**	18.67±1.76**	17.83±2.01**	18.47±2.83**
Sinigrin (75 mg/kg p. o)	18.16±2.77**	17.66±1.60**	18.33±3.26**	18.67±2.90**

The results of activity were expressed as MEAN±SEM of 6 animals in every group. Results were analyzed statistically using one–way ANOVA followed by Tukey's multiple comparison tests; all groups were compared with disease control group and *P<0.05, **P<0.01 was considered significant. GraphPad Prism was the software used for statistical analysis.

Table 7: Von frey hair test (Mechanical allodynia)

Treatment days	Day 0	Day 6	Day 12	Day 24
Vehicle (1%CMC)	52.54±3.19	52.03±7.15	52.29±3.29	52.92±2.07
Disease control (ACR 30 mg/kg)	62.34±3.00*	63.71±4.11*	63.09±2.828*	65.532±2.038*
Pregabalin (10 mg/kg p. o)	49.52±3.00**	48.16±3.59**	47.26±2.046**	46.50±3.05**
Sinigrin (75 mg/kg p. o)	50.45±3.76**	50.53±8.07**	50.44±2.37**	50.14±1.32**

The results of activity were expressed as mean±SEM of 6 animals in every group. Results were analyzed statistically using one–way ANOVA followed by Tukey's multiple comparison tests; all groups were compared with disease control group and *P<0.05, **P<0.01 was considered significant. GraphPad Prism was the software used for statistical analysis.

Table 8: Estimation of brain neurotransmitters (ng/g of tissue)

Groups	Dopamine (ng/g tissue)	Serotonine (ng/gtissue)	Gaba (ng/g tissue)
Vehicle control	345.23±1.06	369.13±1.88	398.67±1.31
(Acrylamide 30 mg/kg)	125.33±1.30***	159.47±1.16***	137.53±1.11***
Pregabalin (10 mg/kg)	289.67±1.66***	296.33±1.10***	268.33±1.22***
Sinigrin (75 mg/kg)	279.67±2.70***	285.67±0.42***	154.33±0.51***

The results of activity were expressed as mean±SEM of 6 animals in every group value are expressed as mean±SEM for 6 rats in each group. Significance was determined by one-way ANOVA followed by Tukey's multiple comparison test's *p≤0.05, **p≤0.05, ***p≤0.05, when compared with vehicle as well as disease group.

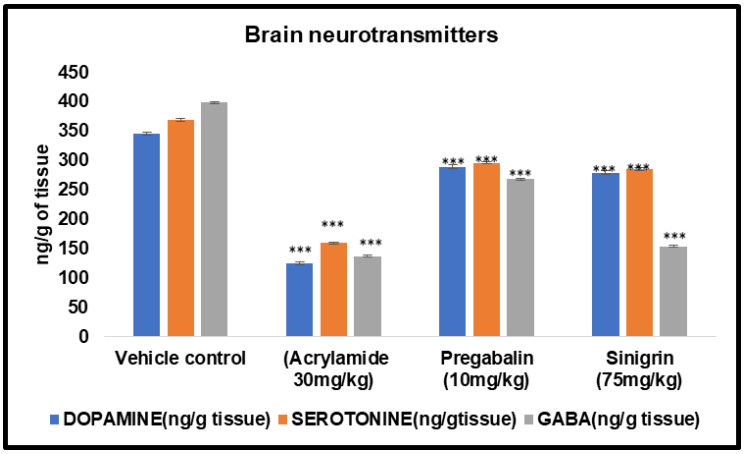

Fig. 8: Estimation of brain neurotransmitters (ng/g of tissue), the results of activity were expressed as mean±SEM of 6 animals in every group value are expressed as mean±SEM for 6 rats in each group. Significance was determined by one-way ANOVA followed by Tukey's multiple comparison test's ***p≤0.05, when compared with vehicle as well as disease group

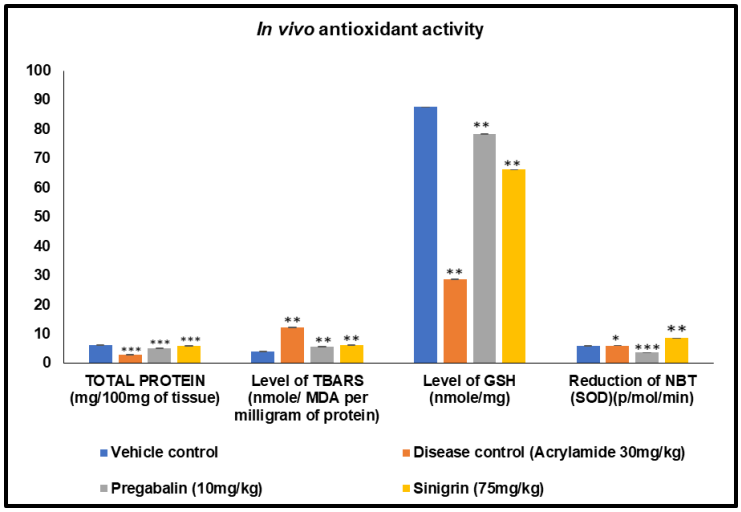

Fig. 9: Estimation of *in vivo* antioxidant activity (The results of activity were expressed as mean±SEM of 6 animals in every group. The results of activity were expressed as mean±SEM of 6 animals in every group. Results were analyzed statistically using one–way ANOVA followed by Tukey's multiple comparison tests; all groups were compared with disease control group and *P<0.05, **P<0.01 was considered significant. GraphPad Prism was the software used for statistical analysis

Table 9: Estimation of *in vivo* antioxidant activity

Groups	Total protein (mg/100 mg of tissue)	Level of TBARS (nmole/MDA per milligram of protein)	Level of GSH (nmole/mg)	Reduction of NBT (SOD)(p/mol/min)
Vehicle control	6.23±0.26	4.00±0.1167	87.55±0.29	5.796±1.46
Disease control (Acrylamide 30 mg/kg)	2.85±0.11***	12.12±0.3602**	28.70± 0.23**	5.997±1.41*
Pregabalin (10 mg/kg)	5.09±0.08***	5.69±0.19**	78.37±0.25**	3.56±1.34***
Sinigrin (75 mg/kg)	5.84±0.11***	6.1817±0.28**	66.15±0.44**	8.51±2.28**

The results of activity were expressed as mean±SEM of 6 animals in every group. The results of activity were expressed as mean±SEM of 6 animals in every group. Results were analyzed statistically using one–way ANOVA followed by Tukey's multiple comparison tests; all groups were compared with disease control group and **P*<0.05, ***P*<0.01 was considered significant. GraphPad Prism was the software used for statistical analysis.

ACR-induced neuropathic pain in rat model

Results of locomotor activity test using actophotometer, motor grip strength by horizontal bar test, muscle grip strength by hang test, Von frey Hair Test (Mechanical allodynia), were mentioned in table 4,5,6,7 and fig. 4,5,6,7.

Biochemical estimation

Estimation of Serotonin, GABA and Dopamine were evaluated in preparation of tissue extracts were mentioned in table 8 and fig. 8.

***In vivo* antioxidant activity**

Estimation of total protein, TBARS, GSH, and Nitro Blue Tetrazolium (NBT) were evaluated in preparation of tissue extracts were mentioned in table 9 and fig. 9.

DISCUSSION

The burden of PD is likely to increase in the years to come as many countries, particularly those in Asia, face an ageing population. One of the major problem associated is 'Pain' a common symptom PD, and is often related to the illness itself. Although pain in PD is common and a significant source of disability, its clinical characteristics, pathophysiology, classification, and management remains to be defined. Mechanisms implicated in the disease process include oxidative stress, mitochondrial dysfunction, protein aggregation and misfolding, inflammation, excitotoxicity, and apoptosis. Saponins, an important group of bioactive plant natural products. *Sinigrin* is one of the glucosinolates of which the bioactivity was as a neuroprotective against PD and NP. The known therapeutic activities of *Sinigrin* revealed are anticancer, anti-inflammatory, antibacterial, antifungal, antioxidant, and wound healing effects activity enhanced through optimal delivery to the human body research. This study was designed to evaluate the neuroprotective activity of saponins glucosinolates: *Sinigrin* against Parkinson's disease and neuropathic pain involved in PD.

In this study, MSG was used to induce Parkinsonian activity using excitotoxicity model. Excitotoxicity results from the over-activation of glutamate receptors (such as NMDA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid AMPA and KARs) leading to increased Ca+2 and Na+levels and decreased K+level in the cells resulting to cellular swelling and neuronal death [15]. Glutamate is the main neurotransmitter responsible for excitotoxicity mechanism. Excess release of glutamate leads to overstimulation of NMDA receptors, which was created by sodium salt of MSG resulted to excitotoxicity. In the present study, we had focused upon exploring the effect of Sinigrin (75 mg/kg, p.o.) for the anti-Parkinsonian activity using excitotoxicity model. Excitotoxicity was induced in rats with the help of MSG (2 gm/kg, i.p.). Dextromethorphan an established neuroprotective agent, was used as a standard in the present research study. Dextromethorphan, a synthetic opioid agonist, functions additionally as an NMDAR antagonist was used as standard drug against MSG-induced excitotoxicity.

In rats, MSG induced behavioral and physiological changes like decreased motor activity (actophotometer), muscle strength and muscle grip strength (rotarod), it was also observed to decrease significantly the locomotor activity as compared to normal animals (table 1). Treatment with test drug *Sinigrin* (75 mg/kg, p. o.) and standard drug dextromethorphan (30 mg/kg p.o.) increased the fall-off time, improved muscle strength and muscle grip strength (table 1) when compared to disease group.

When studied the neuropathic pain parameters to understand the relation between pain and PD, relatively it was observed to be able toattenuate MSG-induced rise in nociceptive pain threshold by measuring paw withdrawal threshold using Vonfrey hair test (mechanical allodynia) and latency of tail flicking by acetone evaporation test method (cold allodynia) [16] evidently showed significant change when compared to disease group (table 2). The effect of MSG-induced excitotoxicity on neuropathic pain associated to PD was supported evidently when treated with test drug *Sinigrin* (75 mg/kg, p. o.) and standard drug dextromethorphan (30 mg/kg p.o.) showed significant effect by attenuating nociceptive pain withdrawal threshold (table 3)as compared to disease group.

The fact that the reactive oxygen species are involved in the pathogenesis of MSG-induced neurotoxicity decrease in brain tissue dopamine level along with decrease in levels of GSH, and CAT is considered as an indication of the oxidative stress and neuronal damage in PD was proven by observing the reduced the level of GSH and CAT in the present study in MSG-treated animals as compared to normal group (table 3). The GSH and CAT are major scavengers of free radicals in cytoplasm and an important inhibitor of free radical. Hence, if the activity of CAT is not adequate to degrade H2 O2, more H2O2 is converted into toxic hydroxyl radicals. GSH, and CAT levels were significantly lower in the treated group compared with normal group (table 3).

The known effects of MSG causing neurotoxicity and damaging dopaminergic neurons by generating free radicals produced due to over-activation of glutamate pathways was proven when significantly increase in dopamine levels along with GSH, and CAT in *Sinigrin* and standard dextromethorphan treated groups as compared with disease group was observed (table 3). The increased levels of GSH and CAT are free radical scavengers and important brain antioxidants were proved to be increased by *Sinigrin* when compared to standard drug Dextromethorphan and MSG-induced excitotoxicity disease group (table 3).

Dextromethorphan was used as a standard drug in the MSG-induced neurotoxicity model. The dextromethorphan is an NMDARs antagonist and has been reported to possess neuroprotective property [17]. Over activation of NMDARs is responsible for the alteration of these electrolyte levels in the neurons. Hence, NMDARs antagonists may be beneficial in protecting neurons from damage by inhibiting glutamate pathway-induced neurotoxicity and free radical generation. *Sinigrin* and dextromethorphan are found to be protective against nerve damage, indicating that *Sinigrin* and dextromethorphan act similarly, mediating there effect through NMDARs antagonism, thus inhibiting glutamate pathways. Thus, it can be said *Sinigrin* significantly improved symptoms of Parkinson's disease and attenuated neuropathic pain by inhibiting glutamate pathway mediated by inhibiting NMDARs and its significant antioxidant activity against generated free radicals due to excitotoxicty related to PD.

Further study involved the assessment of effect of *Sinigrin* on Acrylamide (30 mg/kg) induced neuropathic pain model in wistar rats to evaluate reduction in pain involved in Parkinson's disease

and to prove the association of neuropathic pain with PD. Acrylamide is one of the neurotoxic agents known to cause the potential distal multifocal axonal degeneration that leads to ''dying back'' (i. e. progressive axonal loss) neuropathy along with changes in neuronal cellular environment by altering the biochemical and neuronal cytoskeleton process in rats. ACR induces the oxidative stress associated with elevation of neuronal intracellular calcium ion concentration along with ''dying back'' neurodegeneration [18].

ACR neurotoxicity is associated with the enhancement of lipid peroxidation and the reduction of the anti-oxidative capacity distal axon and nerve terminal regions. The neurotoxic effects of acrylamide involving both the peripheral and central nervous system have been documented in both humans and animals. regabalin an anticonvulsant, widely used for the management of NP [19]. ACR can cause Parkinsonian-like movement impairments, such as bradykinesia and hypokinesia. In one study, chronic exposure to ACR caused severe un-coordination of muscle movement [20, 21]. Conventional therapeutic agents have ameliorated the neuropathic pain symptom such as anticonvulsant (i. e. pregabalin, gabapentin, and carbamazepine), anti-depressants (i.e. amitriptyline and duloxetine), topical medicines (i. e. lidocaine and capsaicin patch), and opioids [22]. However, all these medications have been shown to possess wide spectrum of adverse effects and limit their full clinical exploitation in the management of painful neuropathy. Therefore need for newer drugs in the treatment of neuropathic pain, which would target the exact pathogenesis of PD for pain associated with the disease rather only combat symptomatic pain, was required.

In the present study, Pregabalin a ligand to the a2δ subunit of the voltage-gated calcium channel inhibiting excitatory neurotransmitters [23] was used as a standard drug. *Sinigrin* at a doses of 75 mg/kg and standard drug Pregabalin (10 mg/kg, p. o.) was used in the present research study. Acrylamide (30 mg/kg; i. p.) induced nociceptive pain-sensitive changes and resulted reduction in locomotor activity. Disruption to the dopamine nerve cells by producing free radicals alters locomotor activity; when compared to normal group, a significant decrease was noted after the last injection for locomotor activity by actophotometer ($P<0.05$), Von Frey Hair (Mechanical allodynia), motor rigidity by horizontal bar test and muscle grip strength by hang test, analgesic potential by tail immersion test at different time intervals, viz;, 0, 6, 12, 18, and 24th day (table 4-7). ACR produces skeletal muscle weakness and ataxia that is related to selective damage to distal axon and nerve terminal regions.

Sinigrin and Pregabalin pretreatment attenuated ACR-induced rise in the nociceptive pain threshold in paw by tail immersion test and mechanical allodynia test as compared to ACR disease group (table 4-7). Treatment with *Sinigrin* and Pregabalin elevated the period of falling from the rotating rod apparatus when compared with the treated induction group, respectively. The rota-rod paradigm a reliable test to study motor function and balance an unaltered central function, motor coordination and skeletal muscle weakness (decreased fore and hind limb grip strength, impair locomotor activity) due to ACR model, was found to be elevated by *Sinigrin* and Pregabalin. Thus, the alteration of Acrylamide-induction of neuropathic pain and neurotoxicity and leading to low muscle grip activity and, therefore, can conclude *Sinigrin* can act as NMDARs antagonist and also shows protection against PD excitotoxicity.

On 24th d of *in vivo* study, animals were sacrificed, their brains were isolated and furthermore, the biochemical estimation of brain neurotransmitters viz; Dopamine, serotonin and GABA (table 8). *In vivo* antioxidant activity was done were parameters viz; TBARS, GSH, total calcium, NO activity and total protein levels were estimated from the sciatic nerve tissue sample on 24th day showed significant change in the levels. ACR induction of NP resulted in TBARS levels rise (an index of lipid peroxidation) and total calcium free radicals have been reported to increase intracellular Ca2+concentration along with activation of NMDARs, which has been considered having consanguineous relation with pain modulation [24] and fall in the GSH, an endogenous anti-oxidant level) was observed when compared to normal group. Treatment with *Sinigrin* and standard drug Pregabalin raised brain neurotransmitter dopamine significantlyas compared to disease group; thus can be said can play important role in improving PD-like symptoms. Several studies have indicated that serotonergic dysfunction in PD is associated with the development of motor and non-motor symptoms and complications [25]. Serotonin have shown to play in modulating other neurotransmitter systems, including dopamine, GABA, and glutamate, have meant an expansion in efforts to develop potential of drugs acting on serotonergic system for both motor and non-motor symptoms in PD [26]. *Sinigrin* and Pregabalin raised serotonin and GABA levels significantly ($P<0.01$) as compared to Acrylamide induced disease group. Sinigrin and Pregabalin attenuated ACR-induced rise in sciatic nerve tissue TBARS, total calcium and decrease in GSH levels. In the present study resulted rise in TBARS levels (an index of lipid peroxidation) and total calcium and fall in the reduced glutathione (GSH, an endogenous anti-oxidant level) when treated with Sinigrin and Pregabalin in ACR induced NP, thus supports the contention that free radicals contribute in pathogenesis of NP involved in PD.

This harmful effect of MSG and ACR are related to the over stimulation of glutamate pathway and oxidative stress, respectively, resulting to excitotoxicity neuronal death. *Sinigrin*, standard dextromethorphan and Pregabalin significantly reduced the levels of dopamine (neurotransmitter), CAT and GSH (antioxidant enzymes) in rats in both MSG and ACR models. Therefore, it concludes that Sinigrin and dextromethorphan prevents MSG-induced excitotoxicity. Also *Sinigrin* and Pregabalin prevent ACR-induced oxidative stress and neurotoxicity.

CONCLUSION

From this study, we conclude that *Sinigrin* was found to be neuroprotective as it reduces excitotoxicity score and NP, thus prevents PD. Treatment with *Sinigrin* also elevates glutamate level as a resulted from MSG-induced excitotoxicity and ACR neurotoxicity. The ameliorative effect of Sinigrin may be due to its potential antioxidative, inhibition of lipid peroxidation, calcium channel blocking, and neuroprotective action. Nevertheless, more extensive studies are needed to explore full therapeutic potential molecule and exact molecular mechanism in the management of NP. These findings provide a preliminary evidence for its potential as neuroprotective anti-parkinsonian medication and also has potential to decrease neuropathic pain associated in Parkinson's disease and can be a major strategy in the prevention and improvement of symptoms and disease.

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ABBREVIATIONS

MSG-Monosodium Glutamate, ACR-Acrylamide NMDARs-N-Methyl-D-Aspartate Receptors, PD-Parkinson; s Disease, NP-Neuropathic Pain, AMPA-Alpha-Amino-3-Hydroxy-5-Methyl-4-Isoxazolepropionic Acid, EAATs-Excitatory Amino Acid Transporters, GSH-Glutathione, CAT-Catalase, TBARS-Thio Barbituric Acid Reactive Substances, SOD-Superoxide Dismutase KARs-Kainate Receptors, NBT-Nitro Blue Tetrazolium, O_2-Oxygen.

AUTHORS CONTRIBUTIONS

The author Dr. C. D. Upasani provided author Mrs. Rachana Deepak Sarawade guidance about the contents of the research article. The author Mrs. Rachana D Sarawade obtained the data, analyzed the data and compiled the data in proper manner and computed the data as per journals Instructions. The author Dr. C. D. Upasani supervised the author Mrs. Rachana D. Sarawade computed data with Plagiarism and Technical check. Dr. Paraag Gide, (Principal, DLHHCOP) for providing the animal and laboratory facility required to complete the work.

CONFLICTS OF INTERESTS

Declared none

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