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Protective Effect of Andrographolide in 3-Nitropropionic Acid Induced Huntington Disease and Associated Neurodegenerative Changes in Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Authors NB and RKD designed the study. Author GB performed the experimental work. Author NK performed the statistical analysis. Authors AK and WUK wrote the protocol and wrote the first draft of the manuscript. Authors NB and MUK managed the analyses of the study. Author GB managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Oxidative stress and activation of pro-apoptotic mediators have been associated with the pathogenesis of Huntington's disease. Andrographolide (ANDRO) is a well-known antioxidant and inhibitor of pro-apoptotic mediator, nuclear factor kappa B (NF-kB).
Study Design: The present study was hence designed to evaluate the effect of ANDRO in Huntington's disease.
Place and Duration of Study: Department of Pharmacology, Khalsa College of Pharmacy, Amritsar, India between March 2018 and July 2018.

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Methodology: Five groups (n=6) of Sprague dawley rats were used. Normal group animals were kept untreated. The control group was administered 3-Nitro propionic acid (3-NP) for seven days. The three treament groups received 3-NP followed by ANDRO intraperitoneally for seven days. On 8th day, behavioral and coordination parameters were evaluated using multiple tests. Oxidative stress and anti-oxidant enzyme levels in brain tissue were also evaluated. Brain tissues were also evaluated using Haematoxylin-eosin staining.
Results: Administration of 3-NP resulted in motor incoordination and muscle weakness as indicated by behavioral tests. Biochemically, there was an increase in the oxidative stress and depletion of free radical scavenging. Histopathologically, there was severe neuronal degeneration and neural tissue apoptotic changes in the rat striatum. ANDRO administration resulted in significant decrease in the muscle incoordination in the behavioral tests and also decreased prooxidative biochemical changes. Brain tissues of the ANDRO treated animals showed protection against neuronal damage and neurodegeneration.
Conclusion: The results indicate that the use of ANDRO may afford protection against Huntington's disease associated muscle incoordination and subsequent neurodegeneration. Present study provides a lead for further investigation of role of NF-kB inhibitors in Huntington's disease and possible development of low-cost natural medication.

Keywords: ANDRO; Huntington disease; oxidative stress; nuclear factor kappa B; antioxidants.

1. INTRODUCTION

Huntington's disease (HD) is a chronic progressive autosomal dominant neurodegenerative disorder that is characterized by a progressively worsening chorea, cognitive and psychiatric disturbances. This involves the basal ganglia, cerebral cortex [1] and striatal-specific degeneration. The pathological changes manifest clinically in midlife as a triple combination of cognitive decline, psychiatric disturbance and impairment of motor activities. Symptomatically, HD is characterized by ataxia, choreiform movements, and dementia [2,3].

Pathologically, Huntington's disease (HD) is caused by expansion of a CAG trinucleotide repeat in the huntingtin (HTT) gene, which translates into an abnormally long polyglutamine (polyQ) tract in the Huntingtin (Htt) protein [4]. It has been proposed that mutation in the Huntingtin gene and consequent transcription product attribute to defective activation of NF-k) in the synapses, thus, altering synaptic transmission [5,6]. In the neuronal tissues, NF-kB regulates a wide range of genes that are involved in the neuro-inflammation [7,8] and pathophysiological events associated with neurodegeneration.

Moreover, reports also suggest that excitotoxic events, oxidative stress, alterations in energy metabolism and mitochondrial dysfunction could be involved in the pathophysiology of HD. The generation of reactive oxygen species (ROS) and the oxidative damage play a central role in the process of neurodegeneration. Overproduction of ROS in neurons is physiologically blocked by free radical scavengers, such as glutathione and superoxide dismutase, in the body. ANDRO, a diterpene of the labdane family, is responsible for most of the biological effects of *Andrographis paniculata* [9]. This molecule has been reported to exert neuroprotective effects against inflammation-mediated neurodegeneration [10], oxidative stress in the brain and cerebral ischemia [11]. It is interesting to note that ANDRO has also been documented to inhibit the activation of NF-kB at the intracellular level [12].

Symptoms of HD and associated pathophysiological changes can be produced in rodents by systemic injections of 3-NP, a mitochondrial toxin that causes striatal neuropathy similar to that seen in clinical HD [13]. A major advantage of 3-NP model over other models of HD is that the lesions produced are bilateral, striatal specific and develop spontaneously after systemic administration of 3-NPA.

Despite extensive research, HD a devastating hereditary disease remains incurable, warranting further studies to determine the causes and cure of HD. As it was observed from the documented literature that pathogenesis of HD involves both the ROS and pathological activation of NF-kB in neural tissue and ANDRO possesses both anti-oxidant as well as NF-kB inhibitory action. Therefore, the present study was designed to evaluate the possible protective effect of ANDRO in 3-NP induced HD and associated neurodegeneration in rats.

2. MATERIALS AND METHODS

2.1 Animals

Sprague Dawley Rats (either sex), weighing between 300-350 g, were procured from the Institute of Himalayan Bioresource Technology (IHBT), Palampur, Himachal Pradesh, India. The animals were quarantined and housed in Central Animal House Facility (CPCSEA Registration no. 1753/PO/E/S/14/CPCSEA) for acclimatization for seven (07) days prior to experimentation. Animals were housed in polypropylene cages with dust free rice husk as a bedding material and maintained under standard laboratory conditions with controlled temperature (23 ± 2°C), humidity (40 ± 10%) and natural (12 h each) light-dark cycle. The animals were fed with standard rodent pellet diet and water *ad libitum*. The experiments were carried out between 09:00 and 18:00 h.

2.2 Drugs and Chemicals

The drug, ANDRO, was purchased from Tokyo Chemical Industry Co. Ltd, Chennai, India and 3-NP was purchased from Sigma-Aldrich Inc, Missouri, and USA. For the biochemical estimation's chemicals like ferric chloride, iodine, sodium hydroxide, hydrochloric acid, sodium carbonate, haematoxylin, potassium hydroxide was purchased from Qualikems Fine Chemicals, Pvt. Ltd. Nandesari, Gujrat. Potassium dihydrogen phosphate, formaldehyde, potassium chloride and di-potassium hydrogen phosphate were purchased from Thermo Fischer Scientific India Pvt. Ltd., Mumbai. Reduced glutathione (GSH), N-1-napthylethylene diamine dihydrochloride and NADPH were obtained from Central Drug House Pvt. Ltd. New Delhi. Di-sodium hydrogen phosphate and picric acid were purchased from Merck Specialties Pvt. Ltd. Mumbai. Sodium chloride was purchased from RFCL Ltd. Gujrat. 5.5'Dithiobios (2-nitrobenzoic acid) DTNB was purchased from Alfa Aesar, England. Dopamine hydrochloride was procured from Hi Media Laboratories, Mumbai. All the chemicals and reagents employed in the study were of analytical grade.

2.3 Experimental Protocol

The animals were divided into 5 different groups (n=6). 3-NP (25 mg/kg) was administered through Intra-peritonial route daily to separate groups of rats for seven successive days. The dose of 3-NP was selected on the basis of previous literature reports [14,15]. Group 1 and 2 were naïve animals and 3-NP treated animals respectively. The animals in group 3, 4 and 5 were treated with 3-NP in the same manner as in group 2. In addition, they also received ANDRO in the dose of 2 mg/kg, 3 mg/kg and 4 mg/kg respectively, daily 30 min before the administration of 3-NP for seven days.

After twenty-four hours of the last dose the animals were put on to the battery of behavioral parameters, followed by euthanasia and dissection of the brain tissue for the biochemical estimations like thiobarbituric acid reactive substances (TBARS), GSH & Histopathology.

2.4 Behavioral Models for Evaluation of HD

2.4.1 Balance beam test

The balance beam test was used to measure the mental alertness and muscle coordination of the animal to cross a narrow beam (3 cm × 100 cm) horizontally suspended 1m above the ground. In this test the travel of animal from one end to the other without falling was recorded [16,17]. During testing the rats were 2 min to traverse the beam. If the rat did not complete the task in 2 min or it fell off the beam, the trial was ended and the rat was placed back in the cage. For the successful performers the time was recorded.

2.4.2 Limb withdrawal test

This test is considered to be an important parameter to measure functional abnormalities of the hind limbs, which are indicative for the extent of striatal degeneration [18]. In this behavioural test, the animal was placed on a 20 cm high, 30 cm× 30 cm Perspex platform containing four holes, two holes of 5 cm diameter for the hind limbs and two holes with a diameter of 4 cm for the forelimbs. The rat was placed on the platform by positioning first the hind limbs and then the forelimbs into the holes. The time taken by the animal to retract its first hind limb and the contra lateral hind limb were recorded. The difference between the retraction times of both hind limbs was determined. The test was performed three times with a 45 min interval and the average values were reported.

2.4.3 Inclined plane test

Inclined plane test, as described [19], was used to assess motor function, motor coordination and muscle strength in rats. The apparatus consists of two rectangular boards connected to each other by a hinge. A mat with ridges 0.6 cm in

height was fixed to the movable panel and two protractor-like plywood side panels with angles 0 to 90° marked on its face were fixed on the base. The maximum inclination at which a rat could maintain itself for 5 s with the body axis perpendicular to the axis of the plane was considered as the 'capacity angle' for the animals. The angle was increased or decreased by a margin of 0.5° gradually until the rat could maintain its position on the inclined plane for 5 s without sliding downwards.

2.4.4 String test for grip strength

The latency to hold the grip on a horizontal wire is considered an indirect measure of grip strength [20]. The rat was allowed to hold (with the forepaws) a steel wire (2 mm in diameter and 35 cm in length), stretched horizontally at a height of 50 cm over a cushion support. The length of time for which the rat was able to hold the wire was recorded.

2.4.5 Motor activity

The motor activity of the animal was assessed by putting them into the digital actophotometer. The number of crossings of the infrared beam were calculated for 2 min. After assessing each animal, the actophotometer was cleaned with ethanol.

2.5 Biochemical Estimations

2.5.1 Brain thiobarbituric acid reactive substances

The animals were euthanized. Whole brain tissue was dissected out after removal of the skin from the skull followed by midline incision in the skull using scalper and bone cutting scissors. Whole brain samples were dissected by cutting the spinal cord at the base of the brain adjacent to the atlas vertebrate. The sample collected was washed twice with Phosphate buffer saline blotted and used for biological and histological evaluation. Samples were homogenized thoroughly. Later it was centrifuged at 10, 000 × g for 30 min and the supernatant was utilized for biochemical estimation. Briefly, 1 ml of supernatant and 1 ml of Tris HCL was incubated at 37ºC for 2 h. After incubation, 1 ml of 10% Trichloroacetic acid (TCA) was added and centrifuged at 10, 000× g for 10 min. To 2 ml of supernatant 2 ml of 0.375%w/v thiobarbituric acid was added and the tubes was kept in boiling water for 10 min. After cooling 1 ml of distilled water were added and absorbance was measured at 532 nm using a spectrophotometer (Shimadzu, Japan). Extent of lipid peroxidation was expressed as nanomoles of MDA (malondialdehyde level) consumed per minute at 25ºC.

$$nmoles\ MDA/ml = \frac{6.41 \times \text{Absorbance sample} \times \text{Total volume}}{\text{volume of sample}}$$

2.5.2 Estimation of GSH [21]

Briefly, 3 ml of potassium phosphate (0.2 M, pH 7.6) buffer was taken in the test tube, followed by the addition of 1 ml supernatant and 0.5 ml Ellman's reagent (19.8 mg of DTNB (0.001 M) in 100 ml of 0.1% sodium citrate) was added. An absorbance of reaction product in the cuvette was read after 5 min at 412 nm using Shimadzu 1601 UV/Visible double beam spectro-photometer.

2.6 Haematoxylin and Eosin Staining

The Brain tissues preserved in 10% formalin were dehydrated in graded concentrations of ethanol, immersed in xylene and then embedded in paraffin. The sections of 4 µm thickness were cut and placed on slide using commercial Baker's mounting fluid. Paraffin wax was removed by warming the slide gently, until the wax melted and then was washed with xylene. This was followed by washings with absolute alcohol and water to hydrate the sections and stained with haematoxylin and eosin [22]. The hydrated sections were stained with haematoxylin for 15 min. The stained sections were washed with water and treated with 1% acid alcohol mixture for 20 s. The acid alcohol mixture was washed off with water and sections were counterstained with 1% aqueous solution of eosin for 2 min. After washing with water to remove excess of eosin, the sections were dehydrated using absolute alcohol and then mounted using Canada balsam as mounting agent. The slides were observed for gross histopathological changes and neutrophil accumulation.

2.7 Statistical Analysis

All the result data was analyzed for statistical significance using Sigma - Stat® software employing one way ANOVA followed by *post hoc* analysis by Tukey's test for single as well as multiple comparison parameters. The p value ≥ 0.05 was considered to be statistical significant.

3. RESULTS AND DISCUSSION

3.1 Effect of ANDRO on 3-NP Induced Alterations in Motor Activity

In the present study, administration of 3-NP (25 mg/Kg *i.p*) to the control group resulted in a significant decrease in the motor activity (as evaluated using digital actophotometer) as compared to normal group indicating a significant lack in the central nervous system stimulation in the 3-NP treated group as compared to normal group. Administration of ANDRO in doses of 02 mg/Kg, 03 mg/Kg and 04 mg/Kg *i.p* in rats resulted in a significant dose dependent increase in the motor activity as compared to 3-NP treated group (Fig. 1) indicating improvement in the central nervous system stimulation under the influence of ANDRO.

3.2 Effect of ANDRO on 3-NP Induced Reduction in Mental Alertness

In the present study, administration of 3-NP (25 mg/Kg *i.p*) to the control group resulted in a significant decrease in the mental alertness and muscle coordination of animals to cross a narrow beam (as evaluated using Balance beam test) compared to normal group indicating a significant lack in the central nervous system stimulation in the 3-NP treated group as compared to normal. Administration of ANDRO in doses of 02 mg/Kg, 03 mg/Kg and 04 mg/Kg *i.p* in rats resulted in a significant dose dependent increase in the muscle coordination and mental alertness as compared to 3-NPtreated group (Fig. 2) indicating improvement in the mental alertness and muscle coordination under the influence of ANDRO.

3.3 Effect of ANDRO on 3-NP Induced Functional Abnormalities of Hind Limbs

In the present study, administration of 3-NP (25 mg/Kg *i.p*) to the control group resulted in a significant increase in the functional abnormalities of the hind limbs (as evaluated using limb withdrawal test) compared to normal group indicating a significant lack in the movement of hind limbs in the 3-NP treated group as compared to normal. Administration of ANDRO in doses of 02 mg/Kg, 03 mg/Kg and 04 mg/Kg *i.p* in rats resulted in a significant dose dependent increase in the movement of the hind limbs as compared to 3-NP treated group (Fig. 3) indicating improvement in the hind limb movement under the influence of ANDRO.

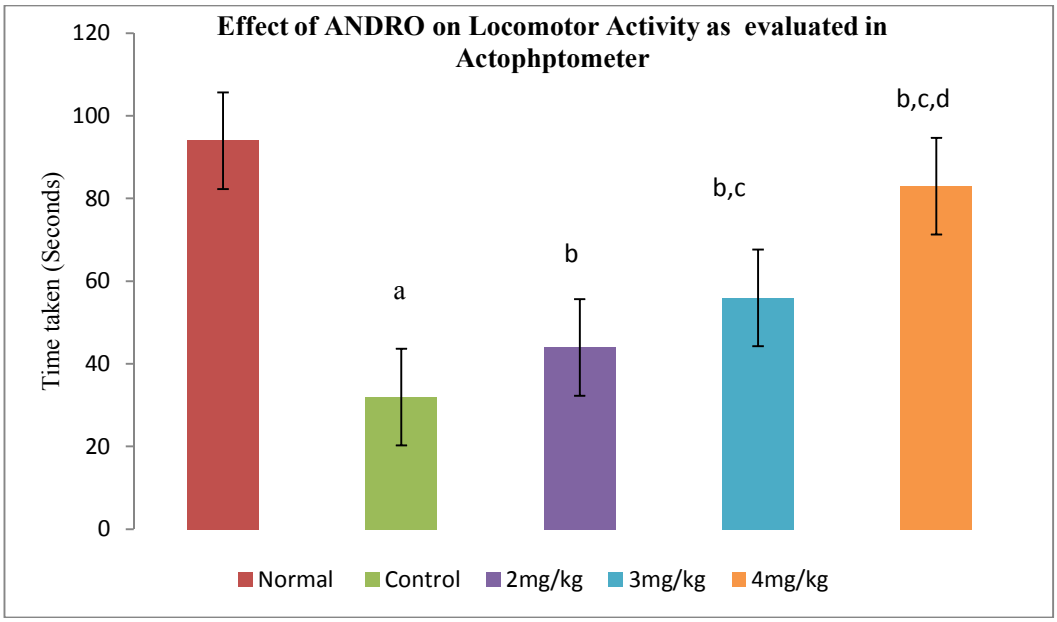


Fig. 1. Changes in motor activity score the normal, control and ANDRO treated groups

Values are represented as the mean ± S.D., n = 5 in each group, [a] p=.05 as compared to the Normal group; [b] p=.05, as compared to Control group; [c] p=.05, as compared to 3-NP + ANDRO Low dose group (2mg/Kg i.p); [d] p=.05, as compared to 3-NP + ANDRO Middle dose group (3mg/Kg i.p)

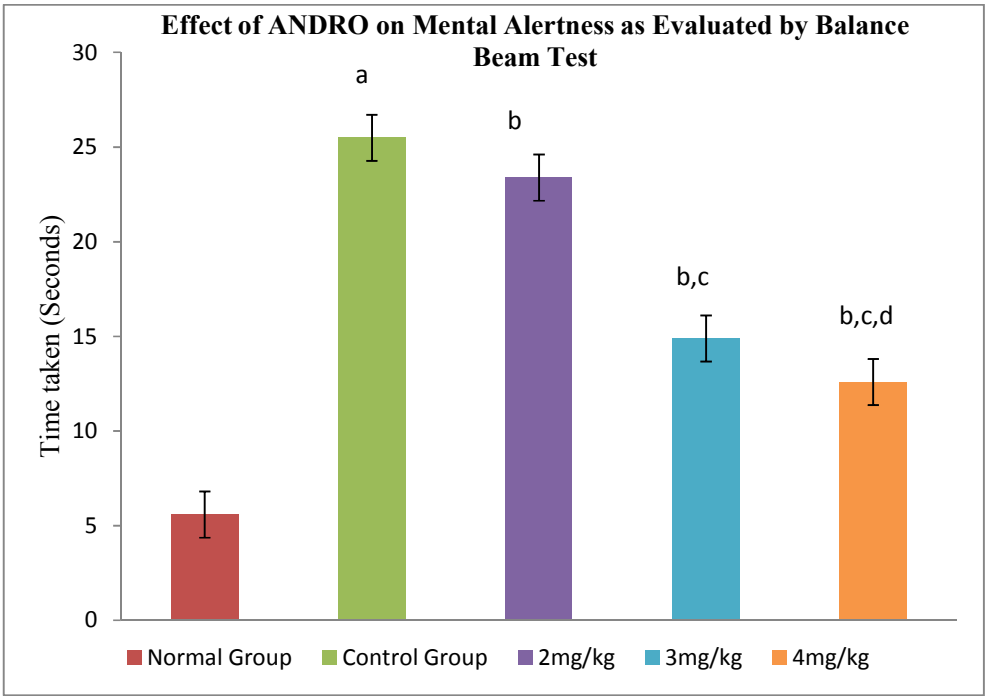


Fig. 2. Changes in mental alertness in the normal, control and ANDRO treated groups

Values are represented as the Mean ± S.D., n = 5 in each group, [a] p=.05as compared to the Normal group; [b] p=.05, as compared to Control group; [c] p=.05, as compared to 3-NP + ANDRO Low dose group (2mg/Kg i.p); [d] p=.05, as compared to 3-NP + ANDRO Middle dose group (3mg/Kg i.p)

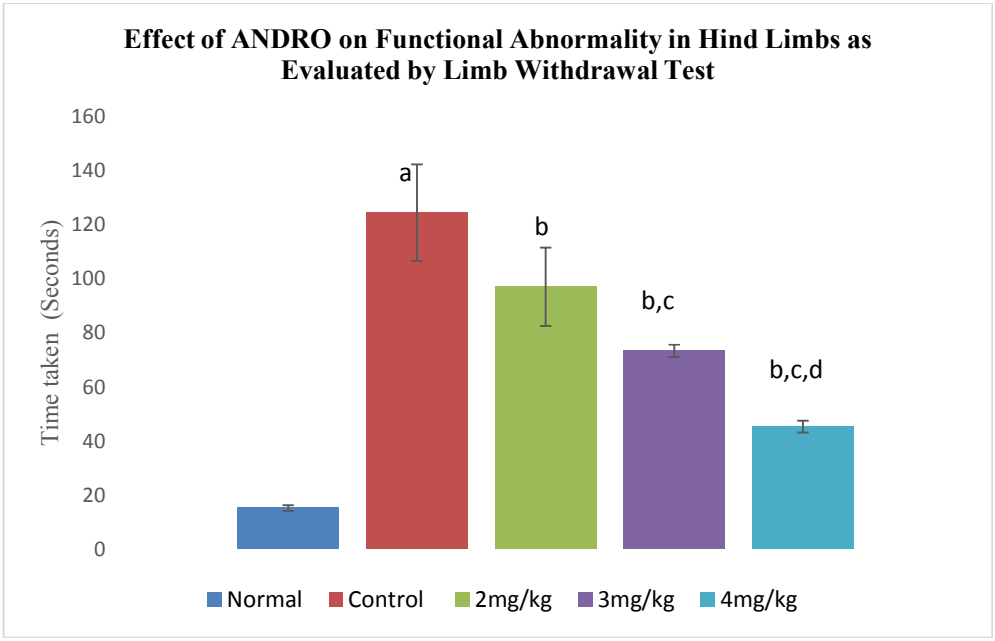


Fig. 3. Changes in functional abnormality in hind limbs in the normal, control and ANDRO treated groups

Values are represented as the Mean ± S.D., n = 5 in each group, [a] p=.05as compared to the Normal group; [b] p=.05, as compared to Control group; [c] p=.05, as compared to 3-NP + ANDRO Low dose group (2 mg/Kg i.p); [d] p=.05, as compared to 3-NP + ANDRO Middle dose group (3 mg/Kg i.p)

3.4 Effect of ANDRO on 3-NP Induced Alterations in Motor Functions

In the present study, administration of 3-NP (25 mg/Kg *i.p*) to the control group resulted in a significant decrease in the motor function, coordination and muscle strength (as evaluated using inclined plane test) compared to normal group indicating a significant lack in the motor function, coordination and muscle strength in the 3-NP treated group as compared to normal. Administration of ANDRO at doses of 02 mg/Kg, 03 mg/Kg and 04 mg/Kg *i.p* in rats resulted in a significant dose dependent increase in the motor function, coordination and muscle strength as compared to 3-NPtreated group (Fig. 4) indicating improvement in the motor function, coordination and muscle strength under the influence of ANDRO.

3.5 Effect of ANDRO on 3-NP Induced Alterations in Grip Strength

In the present study, administration of 3-NP (25 mg/Kg *i.p*) to the control group resulted in a significant decrease in the grip strength (as evaluated using string test) compared to normal group indicating a significant lack in the grip strength in the 3-NP treated group as compared to normal. Administration of ANDRO at doses of 02 mg/Kg and 04 mg/Kg *i.p* in rats resulted in a significant increase in the grip strength as compared to 3-NPtreated group (Fig. 5) indicating improvement in muscle strength under the influence of ANDRO.

3.6 Effect of 3-NP Induced Changes in Brain Tissue Biochemical Levels

In the present study, there was a significant increase in the brain tissue Thiobarbituric Acid Reactive Substances (TBARS) levels in 3-NP treated group as compared to normal group indicating a significant increase in the generation of free radicals in the brain tissue in 3-NP treated group. Administration of ANDRO 2 mg/Kg, 3 mg/Kg and 4 mg/kg *i.p*.in rats resulted in a significant dose dependent decrease in brain tissue TBARS levels as compared to 3-NP treated group (Fig. 6).

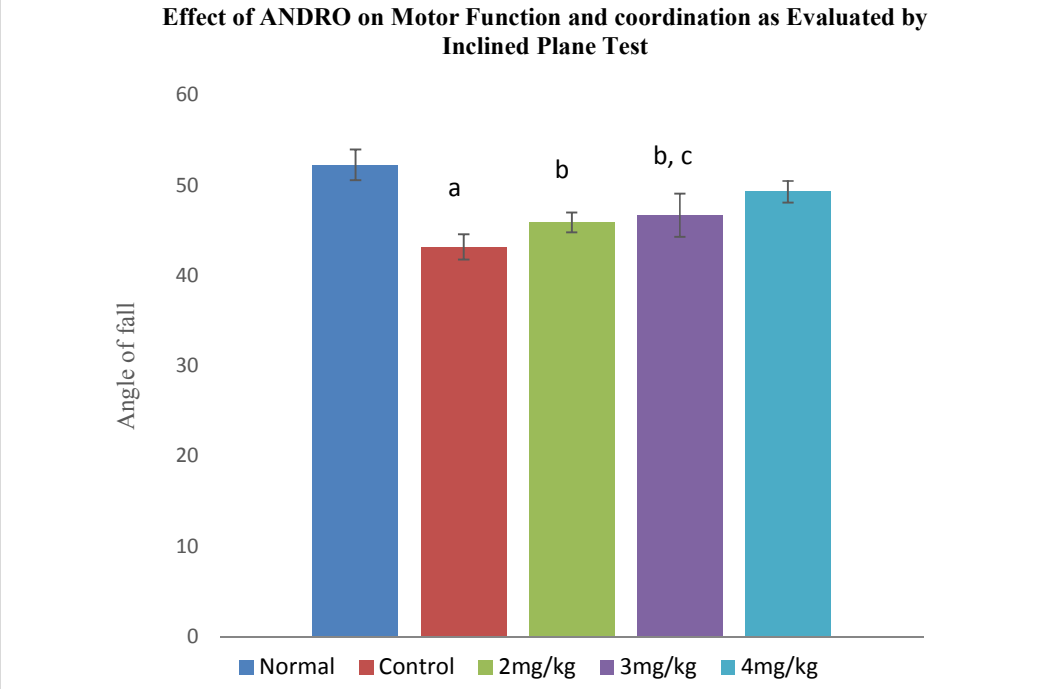


Fig. 4. Changes in motor function and coordination in the normal, control and ANDRO treated groups

Values are represented as the Mean ± S.D., n = 5 in each group, [a] p=.05as compared to the Normal group; [b] p=.05, as compared to Control group; [c] p=.05, as compared to 3-NP + ANDRO Low dose group (2 mg/Kg i.p); [d] p=.05, as compared to 3-NP + ANDRO Middle dose group (3 mg/Kg i.p)

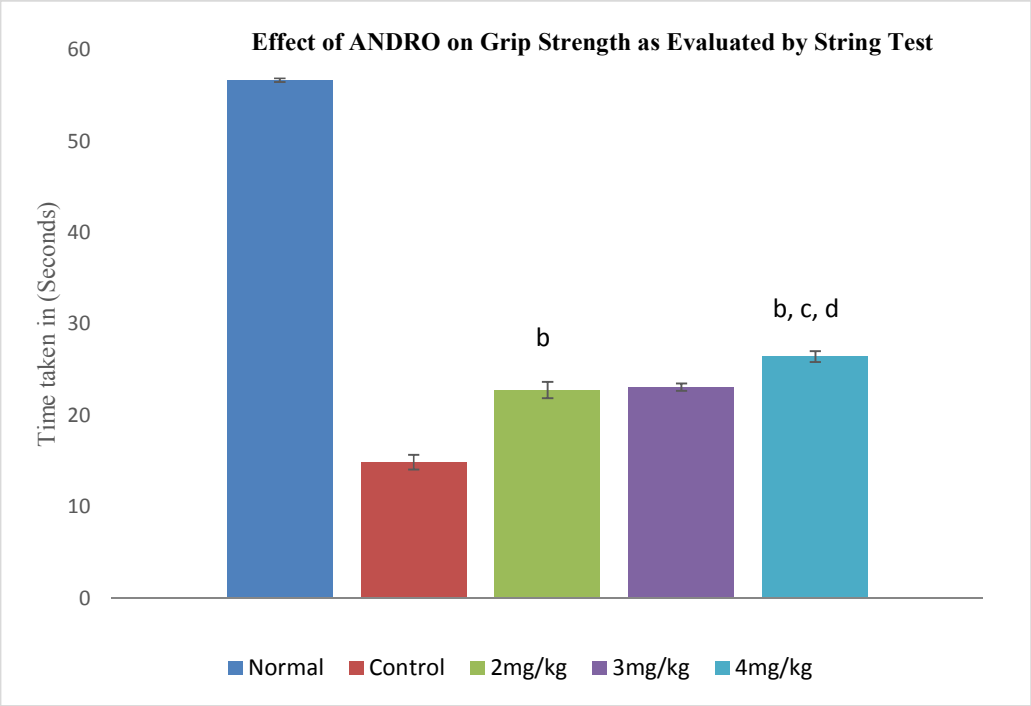


Fig. 5. Changes in grip strength in the normal, control and ANDRO treated groups

Values are represented as the Mean ± S.D., n = 5 in each group, [a] p=.05as compared to the Normal group; [b] p=.05, as compared to Control group; [c] p=.05, as compared to ANDRO + Low dose group (2mg/Kg i.p); [d] p=.05, as compared to ANDRO + Middle dose group (3mg/Kg i.p)

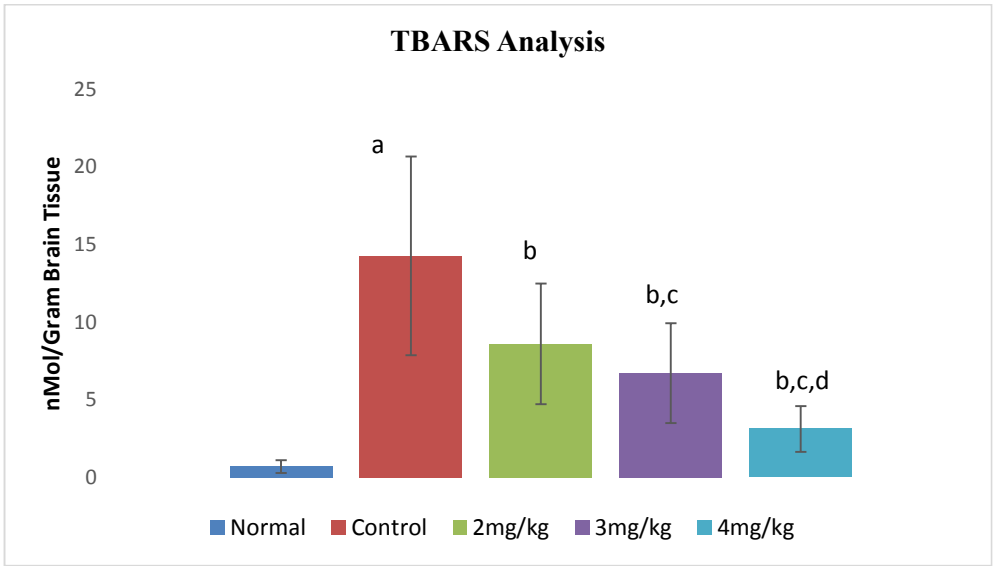


Fig. 6. Changes in brain tissue TBARS levels of normal, control and ANDRO treated groups

Values are represented as the Mean ± S.D., n = 5 in each group, [a] p=.05as compared to the Normal group; [b] p=.05, as compared to Control group; [c] p=.05, as compared to 3-NP + ANDRO Middle dose group (3mg/Kg i.p)

Furthermore, there was a significant decrease in the brain tissue reduces glutathione (GSH) levels in 3-NP treated group as compared to normal group indicating an overload of the free radical scavenging mechanisms of the brain as compared to normal group. Administration of

ANDRO at doses of 2 mg/Kg, 3 mg/Kg and 4 mg//kg *i.p.* in rats resulted in a significant dose dependent increase in brain tissue GSH levels as compared to 3-NP treated group (Fig. 7).

3.7 Effect of ANDRO on 3-NP Induced Changes in Histopathology of the Brain Tissue

Histological evaluation was performed on brain samples on the last day of the experimental protocol. Hematoxylin and eosin stained sections of tissues were evaluated for signs of neurodegenration. Comparison of brain tissue section from the ANDRO treated rats with control group showed significant improvement in the neurodegenration. The microscopic photographs are shown in (Fig. 8). Slides of Normal group showed well defined striatal cell morphology with no signs of degeneration or striatal cell margination (Fig. 8a). 3-NP treated group showed significant degenerative changes (Fig. 8b) with widespread pyknosis (arrows) of the striatal tissue multifocal vacuolization (a hallmark of neuronal apoptosis), indicating severe neuronal apoptosis (Fig. 8b). In ANDRO (2 mg/Kg *i.p*) treated group, the neuroapoptotic process was only marginally reduced indicating the the above mentioned dose was of sub-therapeutic benefit (Fig. 8c) In ANDRO (3 mg/Kg *i.p*) treated groups, the neuroapoptotic process had been dampened relatively (Fig. 8d). In ANDRO (4 mg/Kg *i.p*) treated groups striatal tissue showed significantly less regions of striatal cell damage, diminished vacuolization and significantly less degeneration indicating prevention against neuro-apoptotic process (Fig. 8e).

The present study aimed to evaluate the effect of ANDRO; which is a potent NF-kB inhibitor, on 3-NP induced neurodegeneration. Systemically administered 3-NP (mitochondrial enzyme inhibitor) is implicated in producing HD-like symptoms by causing biochemical and morphological changes in human and animal brain [23]. The compound 3-NP induces motor impairment and striatal toxicity by causing the degeneration of GABAergic medium spiny neurons in the striatum in a pattern that is similar to the neuronal cell death seen in HD patients [24]. Recent studies have highlighted the role of oxidative stress and resultant reactive oxygen species (ROS) in 3-NP induced neuroinflamation and neurodegeneration. Moreover, oxidative stress has been associated with the activation of NF-kB, a factor whose activation has been directly linked with progressive neuroinflamation and resultant neurodegeneration [25].

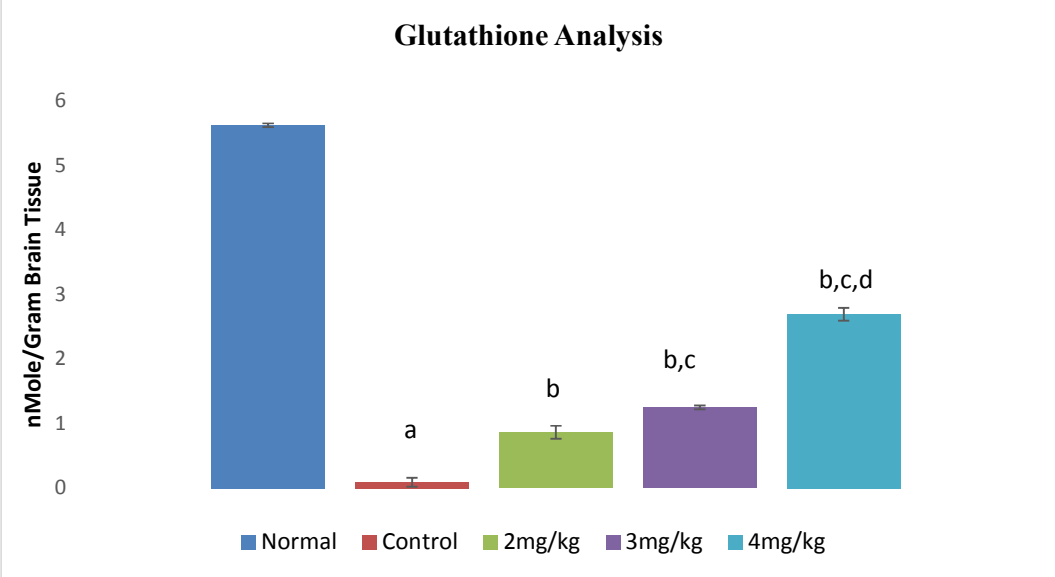
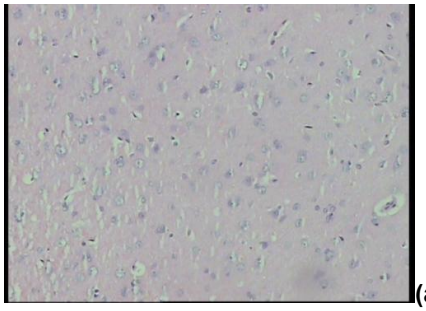


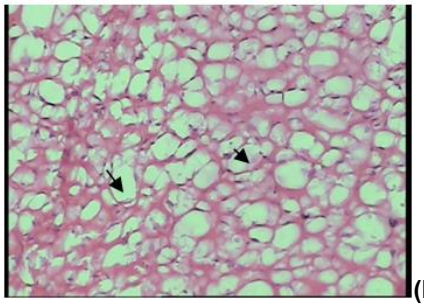
Fig. 7. Changes in brain tissue GSH levels of normal, control and ANDRO treated groups

Values are represented as the Mean ± S.D., n = 5 in each group, [a] p=.05, as compared to the Normal group; [b] p=.05, as compared to Control group; [c] p=.05, as compared to 3-NP + ANDRO Low dose group (2mg/Kg i.p); [d]p=.05, as compared to 3-NP + ANDRO Middle dose group (3mg/Kg i.p)



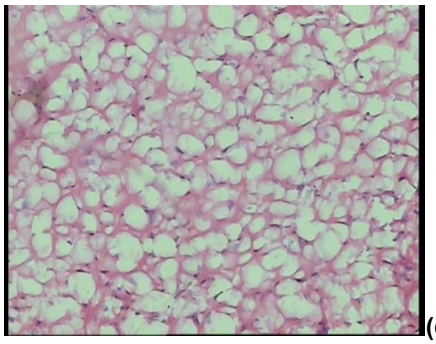
(a)

Normal Striatal Tissue



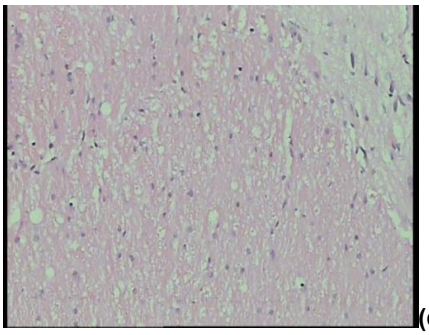
(b)

3-NP induced Neuroapoptosis in Striatum (Arrows Show Striatal Cell Pyknosis due to apoptosis)



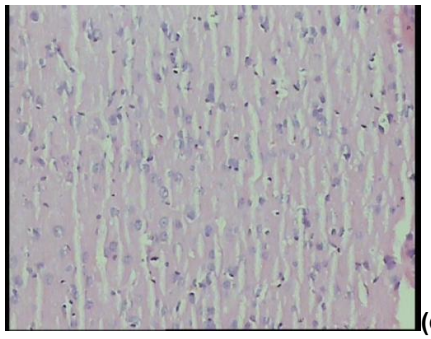
(c)

3-NP + 2mg/Kg *i.p* ANDRO



(d)

3-NP + 3mg/Kg *i.p* ANDRO



(e)

3-NP + 4mg/Kg *i.p* ANDRO

Fig. 8. The microscopic photographs of striatal tissues of normal, control and ANDRO treated groups. Slides of normal group showed well defined striatal cell morphology with no signs of degeneration or striatal cell margination (Fig. 8a). 3-NP treated group showed significant degenerative changes (Fig. 8b) with widespread pyknosis (arrows) of the striatal tissue multifocal vacuolization (a hallmark of neuronal apoptosis), indicating severe neuronal apoptosis (Fig. 8b). In ANDRO (2 mg/Kg *i.p*) treated group, the neuro-apoptotic process was only marginally reduced indicating the above-mentioned dose was of sub-therapeutic benefit (Fig. 8c) In ANDRO (3mg/Kg *i.p.*) treated groups, the neuro-apoptotic process had been dampened relatively (Fig. 8d). In ANDRO (4 mg/Kg *i.p.*) treated groups striatal tissue showed significantly less regions of striatal cell damage, diminished vacuolization and significantly less degeneration indicating prevention against neuro-apoptotic process (Fig. 8e)

In the present study, systemic administration of 3-NP for 07 days significantly reduced locomotor activity (hypokinetic movements) and grip strength performance in animals, suggesting that 3- NP more likely mimics either the juvenile onset or late stages of HD-like behavior. Earlier reports also confirm that 3-NP caused impairment in motor and cognitive functions, oxidative defense and mitochondrial enzyme complex activities in rats [26,27]. Our findings are therefore, in concordance with previously documented effect of 3-NP induced neurodegeneration, as in HD.

Administration of ANDRO at a dose of 2mg/Kg *i.p*, 3 mg/Kg *i.p* and 4 mg/Kg *i.p* was able to significantly improve the mental alertness, muscle coordination, grip strength, muscle strength, motor activity and other functional abnormalities in the animal suggesting its therapeutic potential against HD-like behavior. The models used to evaluate mental alertness, muscle coordination; grip strength, muscle strength, motor activity and other functional abnormalities have been widely acclaimed as excellent models for evaluation of progressive neuronal degeneration [28].

It has been hypothesized number of times that ROS may act directly by accumulating in brain and causing derangement and demolition of antioxidant defense system of the brain [29] or may act indirectly through various pathological pathways such as NF-kB. ROS mediated activation of NF-kB has been implicated in neurodegeneration.In the present investigation, 3-NP treatment (25 mg/kg *i.p*) resulted in marked increase in oxidative stress as was indicated by significant increase in lipid peroxidation (TBARS) and a subsequent decrease in antioxidant defense situation as was evident by a significant decline in level of brain tissue GSH and dopamine levels. Moreover, there was increased neurodegeneration.

However, administration of ANDRO at doses of 2 mg/Kg *i.p.*, 3mg/Kg *i.p.* and 4mg/Kg *i.p.*significantly decreased TBARS and restored GSH and dopamine levels. Furthermost, there was significant decrease in neurodegeneration.It is interesting to know that ANDRO is a potent inhibitor of NF-kB activation [30] and is widely aclaimed antioxidant. From the results we hypothesize that 3-NP can be associated with HD like symptoms that are neurodegeneration and dementia by two known mechanisms involving potentiation of oxidative stress as well as NF-kB activation which culminates in progressive neurodegeneration and dementia. As per the results of our investigation, ANDRO induced protection against 3-NP mediated HD like neurodegeneration could be attributed to antioxidant as well as NF-kB inhibitory action of ANDRO. However, further studies are still warranted to elucidate the molecular mechanism of observed effect of Andrographolide in 3-NP induced HD and other associated models.

4. CONCLUSION

We conclude that administration of ANDRO resulted in protection against 3-NPA induced HD like neurodegeneration. The administration of 3-NP to the control and treatment groups of rats resulted in significant development of symptoms of HD. The symptoms were characterised as movement and coordination abnormilities indicating possible striatal neuron damage by administration of mitochondrial toxin, 3-NP.Moreover, administration of 3-NP resulted in oxidative stress and disruption of free radical scavenging mechanisms in rat brains.Finally, the histopathological evaluation of 3-NPA treated animal showed marked degeneration of the striatal neurons. Majority of the striatal cells revealed apoptotic changes with significant neuronal loss.In the treatment groups there was significant decrease in the severity of neurodegeneration with majority of the striatal neurons still intact. Conclusively, we hypothesize that the antioxidant as well as NF-kB inhibitory activity of ANDRO could be possibly involved in the observed beneficial effects of ANDRO in the present study.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed. The care of laboratory animals was done as per the guidelines of CPCSEA, Ministry of Forests & Environment; Government of India. The research protocol of this study was approved by Institutional Animal Ethics Committee (IAEC) of Khalsa College of Pharmacy, Amritsar.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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