

Research Article



Cytogenetic, Biochemical and Histopathological Effects of Ropinirole on Albino Male Mice

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ABSTRACT

Ropinirole is a synthetic, nonergot derivative receptor agonist that has selective activity for the D₂ class of dopamine receptors. Ropinirole is approved for the therapy of symptomatic Parkinson's disease and restless legs syndrome. The effects of ropinirole in doses equal and above the maximum recommended doses for human has not been adequately studied. Therefore, to evaluate the cytogenetic, biochemical and pathological effects of ropinirole on male mice ropinirole administered orally to male mice at doses 50, 75 and 100 mg/kg approximately equal (10, 15, 20) times the MRD for 21 consecutive days after one day from the last treatment the treated and control males were sacrificed and examined for sperm head abnormalities, cytogenetic analysis (somatic and germ cells), micronuclei (bone marrow cells), DNA damage (liver cells), biochemical analysis (liver and kidney functions) and histopathological analysis in (liver and renal tissues). The results showed that ropinirole caused significant increases in the frequencies of sperm head abnormality chromosomal aberrations in (somatic and germ cells), in the percentages of DNA damage and in the frequencies of micro nucleated cells in all dose levels and these increases were dose dependent. Also, the biochemical analysis were examined in the liver and kidney of treated males. The results showed that there were significant increases in the frequencies of MDA, ALT, AST, urea, uric acid and creatinine levels in all treated groups compared with the control males and these increase were dose-dependent. Moreover, the pathological analysis examined in the liver and kidney tissues of treated and control males showed that there were vacuolar degeneration of hepatocytes reduction of cellular in filtration and dilation of main blood vessels in liver tissue and intestinal hemorrhage and thickening of the parietal wall of Bowman capsule was observed in renal tissue and these effects were dose-dependent. Thus, it could be concluded that, ropinirole induced toxic effects in the chromosomes of germ and somatic cell, micro nuclei in bone marrow cells, DNA damage in liver cells (comet assay), increase in the levels of MDA, ALT, AST (liver function), urea, uric acid, creatinine levels (kidney function) and increase in the degeneration and hemorrhage of blood vessels in the liver and renal tissues in all dose levels (50, 75 and 100 mg/kg/day) in male mice and there increases were more frequent in the high dose 100 mg/kg/day.

Keywords: Ropinirole, chromosomal aberrations, Sperm abnormality, micronuclei, DNA damage (comet assay), liver and kidney functions, histopathology, mice.

INTRODUCTION

Parkinson's disease is the second most common neurodegenerative disorder and the most common movement disorder.

It is characterized by progressive loss of muscle control, which leads to trembling of the limbs and head while at rest, stiffness, slowness, and impaired balance. As symptoms worsen, it may become difficult to walk and complete simple tasks.

The progression of Parkinson's disease and the degree of impairment vary from individual to individual.

Many people with Parkinson's disease live long productive lives, whereas others become disabled much more quickly.

Parkinson's disease occurs in approximately 1% of individuals aged 60 years and in about 4% of those aged 80 years.

The most common cause of Parkinson's disease a substance called dopamine acts as a messenger between two brain areas: The substantia nigra and the controlled movements¹.

Most of the movement related symptoms of Parkinson's disease are caused by a lack of dopamine due to the loss of dopamine producing cells in the substantia nigra. When the amount of dopamine is too low communication between the substantia nigra and corpus striatum becomes ineffective, and movement becomes impaired the greater the loss of dopamine, the worse the movement related symptoms.

In fact, it is not clear why the dopamine producing brain cells deteriorate. Genetic and pathological studies have revealed that various dysfunctional cellular processes, inflammation, and stress can all contribute to cell damage. In addition, by studying families with hereditary Parkinson's disease, scientists have identified several genes that are associated with the disorder. In general scientists suspect that Parkinson's disease is due to a combination of genetic and environmental factors².

There is currently no treatment to cure Parkinson's disease; several therapies are available to delay the onset of motor symptoms and to ameliorate motor symptoms.

All of these therapies are designed to increase the amount of dopamine in the brain either by replacing



dopamine, mimicking dopamine, or prolonging the effect of dopamine by inhibiting its breakdown.

The most effective therapy for Parkinson's disease is (Levodopa) which is converted to dopamine in the brain.

However, because long-term treatment with levodopa can lead to unpleasant side effects, its use is often delayed until motor impairment is more severe.

In earlier stages of Parkinson's disease, substances that mimic the action of dopamine (dopamine agonists), and the substances that reduce the breakdown of dopamine (monoamine oxidase inhibitors) can be very efficacious in releasing motor symptoms³.

Ropinirole is a newly oral medication called a nonergoline dopamine agonist, used in the treatment of early and advanced Parkinson's disease. It is available in most countries by different trade names.

The mechanism of action by which ropinirole works is by stimulating the dopamine receptors in the brain.

Dopamine is a chemical neurotransmitter which carries messages between the nerves and stimulates the dopamine receptors. Patients with Parkinson's disease have mineral to no dopamine, so the dopamine receptors are left un-stimulated. By stimulating the dopamine receptors, ropinirole may reduce the symptoms associated with parkinson's disease. This action is different than adding dopamine to the body, it is something like substitute for dopamine that can fool dopamine receptors into working even when the body lacks a good supply of this neurotransmitter⁴.

Ropinirole can also used in the treatment of restless leg syndrome and extrapyramidal symptoms. It can also reduce the side effects caused by selective serotonin reuptake inhibitors, including Parkinsonism- syndrome as well as sexual dysfunction and erectile dysfunction.

In all clinical studies, the recommended starting dose for mild Parkinson's disease is 0.25mg/kg/day and for sever Parkinson's disease the daily dosage may be increased to achieve a maximum therapeutic dosage 24 mg/kg/day⁵.

Moreover, many studies examined the mutagenic effects of ropinirole on mice and rats they found that ropinirole has no mutagenic effects in mice and rats at doses 5, 15, 50 mg/kg/day and did not affect the fertility of male mice and rats at doses 5, 10, and 25 mg/kg/day which equivalent to 10 and 20 times the maximum recommended dose for human 24 mg/kg/day.

So, in the present study we examined the cytogenetic, biochemical and histo-pathological effects of ropinirole on male mice if taken orally at doses 50, 75 and 100mg/kg/day.

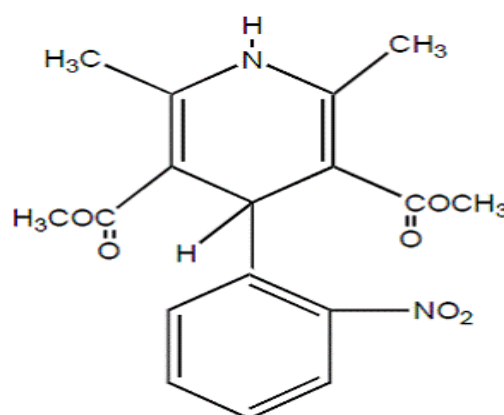
MATERIALS AND METHODS

Test Drug

Tremodect (Ropinirole) provided by (Eva pharm) is an orally administered non-ergoline dopamine agonist. It is

supplied as ropinirole hydrochloride salt 4-[2-(dipropylamino)ethyl]-1,3-dihydro-2H-indol-2-one and has a molecular formula of $C_{16}H_{24}NO_2 \cdot HCl$.

The molecular weight is 296.84 and the structural formula is



Ropinirole hydrochloride is a white to yellow solid and a solubility of 133mg/ml in water.

Animals and Treatments

Dilutions of different concentrations were prepared by dissolving the tablets in distilled water. Ropinirole were administrated orally at doses of (50, 75 and 100) mg/kg/day once daily for (21) consecutive days, these doses of ropinirole approximately equivalent to 10, 15 and 20 the maximum recommended dose for the treatment of Parkinson's disease for human after modified it to suit the small weight of albino mice (30gm) according to pagat and Barnes⁷.

Animals

Adult males albino mice each weighting (30gm) served as experimental animal.

The mice were housed in plastic cages at an environmentally controlled room (constant temperature 25-27 °C) with 12h light/dark cycle for one week prior to starting the experiment, and they were provided with water and libitum. Mice were divided into four groups (group/10 animals) as following

- The first group were administered orally with a single dose of 50 mg/kg/day once daily.
- Animals of the second group were administered orally with a single dose of 75 mg/kg/day once daily.
- Animals of the third group were administered orally with a single dose of (100 mg/kg/day) once daily.
- Animals of the fourth group served as controls and were administered orally with a distilled water.

All animals were administered orally for (21) consecutive days and after 24h from the last treatment animals were sacrificed by cerficial dislocation for studying cytogenetic analysis including Sperm head abnormalities, chromosomal aberrations in bone marrow cells and germ cells, micronuclei in bone marrow cells and comet assay

(liver cells), biochemical analysis (liver and kidney functions) and lipid peroxidation and pathological analysis in liver and kidney tissues.

Methods

For Cytogenetic Analysis

Sperm Head Abnormality Assay

The treated males were sacrificed by decapitation the cadava epididymis was removed and placed in physiological saline then it was minced into pieces with scissors and then left undisturbed for 20 minutes for the diffusion of spermatozoa.

The spermatozoa were spread on microscopic slides, air-dried, fixed in absolute methanol for 15 minutes and stained with 1% aqueous eosin-y on the following day. Three hundred sperms from each animal were examined for the abnormalities in sperm head shaped following the method recommended by Wyrobek and Bruce⁸.

Chromosomal Preparation (in bone marrow cells)

Chromosomes from bone marrow cells were prepared according to the method of HSU and patton⁹. Mice were injected with colchicines (2.5 mg/kg/b.w.i.p) after 3 hours animals were killed by cervical dislocation.

The bone marrow cells were a spirited in phosphate buffer solution (pH 7.2) and centrifuged at 1000 r.p.m. for 2min. The pellets obtained were mixed in aqueous solution of KCL (0.56%) and left for 30 min at 37 °C.

The prepared cells were re-centrifuged, and fixed in (3:1) methyl:glacial acetic acid.

Finally slides were air-dried and stained with 10% Giemsa stain for 20 minutes.

Chromosomal Preparation (in spermatocytes)

Testes were obtained from the same animals to study the abnormalities in spermatocytes (germ cell) according to Brewen and preston¹⁰ with some modifications.

Mice were injected with colchicines (2.5 mg/kg/b.w i.p) after 3 hours animals were killed by cervical dislocation the testes were collected in 2.2% sodium citrate solution and minced into pieces with scissors and then centrifuged at 1000 r.p.m. for 2 min.

The pellets obtained were mixed in aqueous solution of Na citrate 1.1% and left for 25 min at 37 °C. The prepared cells were re-centrifuged, fixed in (3:1) methyl:glacial acetic acid, finally two on three drops of cell suspension were dropped on a clean slide, air-dried and stained with 10% Giemsa stain for 25minutes.

50 metaphases were studied per animal scoring different types of aberrations in bone marrow and spermatocytes.

Comet Assay

The comet assay was performed under alkaline conditions essentially according to the procedure of Singh¹¹ with modification Klaude.¹² A freshly prepared

suspension of liver cells in 0.75% low melting point agarose (Sigma chemicals) dissolved in phosphate buffered saline (PBS; Sigma chemicals) was cast onto microscope slides pre coated with 0.5% normal melting agarose. The cells then lysed for 4h at 4 °C in a buffer consisting of 2.5M NaCl, 100m MEDTA, 1% triton x 100, 10mM Tris, PH10. After lysis, DNA was allowed to Unwind for 40 min in electrophoretic solution consisting of 300mM NaOH, 1mM EDTA, PH>15. Electrophoresis was conducted at 4 °C for 30 min at electric field strength 0.73 V/cm (30mA).

The slides were then neutralized with 0.4M tris, pH 7.5, stained with 2Mg/ml ethidium bromide and covered with cover slips.

The slides were examined at 200x magnification fluorescence microscope. Fifty images were randomly selected from each sample and the comet DNA was measured. Two parallel tests with aliquots of the same sample of cells were performed for a total of 100 cells. Each experiment was repeated two times. Percentage of DNA was analyzed. The mean value of % DNA in a particular sample was taken as an index of DNA damage in this sample.

Micronucleus Test

Bone marrow slides were prepared according to the method described by Krishina and Hayashi¹³. The bone marrow were washed with 1ml of fetal calf serum and then smeared on clean slides. The slides were left to air dry and then fixed in methanol for 5 minutes, followed by staining in 5% Giemsa stain for 5 minutes then washed in distilled water and mounted. For each animal 1000 polychromatic erythrocytes (PCEs) were examined for the presence of micronuclei.

For Biochemical Analysis

Determination of Lipid Peroxidation

Lipid peroxidation was assayed by measuring the level of malondialdehyde (MDA). Malondialdehyde forms a 1:2 a duct with thiobarbituric acid which can be measured by spectrophotometry. Malondialdehyde was determined by measuring thiobarbituric reactive species using the method of Ruiz-Zarra.¹⁴ in which the thiobarbituric acid reactive substances react with thiobarbituric acid to produce a red colored complex having peak absorbance at 532nm.

Liver Function

Blood samples were collected from each animal in a clear sterile centrifuge tube without anticoagulant obtaining serum for determination of serum Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and ALP Alkaline phosphatase. The tubes were left at room temperature. After 1 hour the tubes centrifuged at 2500 rpm for 10 min the supernatant which is serum, was assayed for (ALT and AST) using ranox kit following Standard method of Reitman and Frankel¹⁵ and the



activity of alkaline phosphatase was measured according to the method of Bowers and McCombs¹⁶.

Kidney Function

Serum was obtained from control and treated mice of each group by centrifugation of blood at 900 rpm for 15 min.

The urea and uric acid concentration was determined using modified Berthelot method Walton¹⁷ and creatinine concentration by alkaline picture method Standard method of using Owen¹⁸.

Histopathological Analysis

Liver and kidney specimens from all animals were dissected immediately after death, and fixed in 10% neutral-buffered formal saline for 72 hours at least. All the specimens were washed in tap water for half an hour and then dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin, serial sections of 6Mm thick were cut and stained with haematoxylin and eosin Drury and Wallington¹⁹ for histopathological investigation.

Statistical Analysis

The data of sperm head abnormalities; chromosomal aberrations, micronucleus, and biochemical analysis (liver and kidney function and MDA) were subjected to analysis of variance (ANOVA) according to Snedecor and Cochran²⁰. Least significant differences were used to compare between means according to Waller and Duncan²¹ at probability 5%. The data of comet assay were expressed as percentage.

RESULTS

Sperm Head Abnormality

The results are given in Table 1. The analysis of sperm head abnormalities were examined in all treated groups and control showed that overall amorphous head, Dwarf and banana head were more prevalent in different groups than the other types of abnormalities which occurred with different frequencies in both treated and control groups.

Table 1 showed that the frequencies of sperm abnormality in all treated mice with ropinirole were increased significantly than the control mice and these increases were dose-dependent.

Chromosomal Aberrations

In bone marrow cells (somatic cells)

The results are given in Table 2. Cytogenetic analysis in the present study showed that there were structural and numerical chromosomal aberrations in all treated groups and control.

Structural aberrations consisted of gaps, breaks, deletion, fragments, centromeric attenuations and endometosis numerical aberrations consisted of preploidy and polyploidy.

The results shown in Table 2 revealed that there was a significant difference between ropinirole treated mice and control mice in all types of chromosomal aberrations (structural and numerical) and these increases were dose-dependent.

In sperm cells (germ cells)

The results are given in Table 3 showed that there were increased in the frequencies of structural aberrations (x-y univalent, autosomal, fragments and total structural aberration) as well as numerical aberrations (hypo, hyper and polyploidy) in all ropinirole treated groups (50, 75, 100 mg/kg/day) compared with the control group and these increases were dose-dependent.

Detection of DNA Damage using Comet Assay

The results are given in Table 4. It is clear that there was a significant increase in the tail length in blood cells of male mice treated with the three different doses of ropinirole compared with the control mice and these increases were more frequent in the high dose of ropinirole (100mg/kg/day) that clearly indicates ageno-toxic effect of ropinirole in the high doses.

Micronucleus (MN) Assay

The effect of ropinirole on micro nucleated polychromatic erythrocytes (MNPE) in the bone marrow cells of male mice are given in Table 5.

The present results in the control group of male mice showed a homogeneous low amount of damaged cells all through the assay.

In contrast, the administration of ropinirole in three different doses (50, 75 and 100 mg/kg/day) caused a significant increase in the frequencies of micronuclei in the cells of mice bone marrow and this increase was highly significant in the mice treated with a dose of 100mg/kg/day compared with the control group.

The frequencies of micronuclei in the bone marrow cells treated with (50, 75 and 100 mg/kg) were (8.80, 13.20 and 18.60) respectively compared with (5.60) for the control group.

Biochemical Analysis

The Percentage of Malondialdehyde (MDA)

The data in Table 6 represented the effect of ropinirole on serum malondialdehyde (MDA) in liver tissues in oxidative stress model.

The administration of male mice with three different doses (50, 75 and 100 mg/kg) of ropinirole for 21 consecutive days caused a significant increase in the serum MDA and this increase were more frequent in the group of (100 mg/kg/day) compared with the control group. The frequencies of (MDA) in the liver cells treated with three doses of ropinirole (50, 75 and 100 mg/kg) were (1.90, 3.20 and 7.20) respectively compared with (0.70) for the control group.



Liver and Kidney Functions

The results in Table 7 represented the effect of ropinirole on serum ALT, AST and ALP (liver function) and creatinine level, urea and uric acid (kidney function) in male mice.

The results of high dose of ropinirole 100 mg/kg showed highly significant increases in liver and kidney functions compared with the control group, while the treatments of the other low doses 50 and 75 mg/kg showed only a significant increase in the kidney and liver function compared with the control group.

Histopathological Analysis

Images were captured and processed using Adobe Photoshop version 8.0.

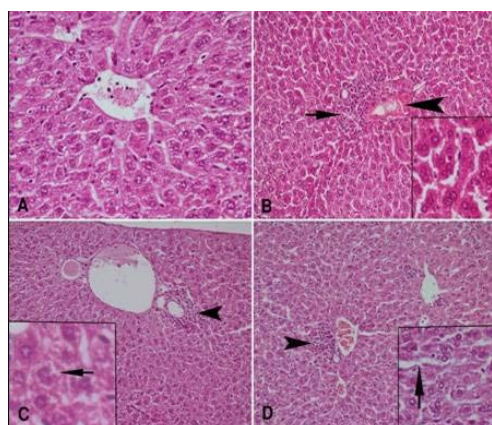


Figure 1: **A)** is a photomicrograph of a section of liver tissue shows the normal structure of this tissue. **B)** shows the effect of the drug in a dose of 50 mg/kg/day in the form of small focal aggregation of cellular infiltrates (arrow) and mild dilatation and congestion of blood vessels (arrowhead). The lower right part of the figure shows normal hepatocytes. **C)** shows the effect of the drug in a dose of 75 mg/kg/day, where the same results of the previous group are still observed in addition of mild vacuolar degeneration of hepatocytes (arrow) as seen in the lower left part of the figure. **D)** shows the effect of the drug in a dose of 100 mg/kg/day in the form of reduction of cellular infiltration (arrowhead) and dilatation of main blood vessels. The lower right part of the figure shows slight dilatation of blood sinusoids (arrow) and normal hepatocytes.

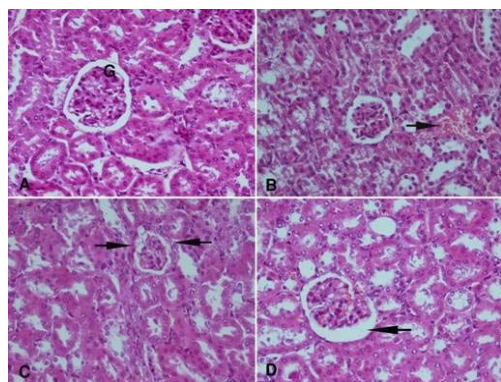


Figure 2: **A)** is a photomicrograph of a section of normal kidney tissue. **B)** shows the effect of the drug in a dose of

50 mg/kg/day, where mild interstitial hemorrhage (arrow) is observed. **C)** shows the effect of the drug in a dose of 75 mg/kg/day, where thickening of the parietal layer of glomeruli (arrow) is observed. The tubules are looked normal. **D)** shows the effect of the drug in a dose of 100 mg/kg/day. The renal tubules appear normal, while the glomeruli show widened urinary space (arrow) denoting increased volume of filtrate.

DISCUSSION

Ropinirole is a non-ergoline dopamine agonist with high relative in vitro specificity and full intrinsic activity at the D₂ and D₃ dopamine receptor subtypes binding with higher affinity to D₃ than to D₂ or D₄ receptor subtypes. Ropinirole has some of the same effects as a chemical called dopamine, which occurs naturally in the body low levels of dopamine in the brain are associated with Parkinson's disease.

The precise mechanism of action of ropinirole as a treatment for Parkinson's disease is unknown, although it is believed to be due to stimulation of postsynaptic dopamine D₂ type receptors within the caudate-putamen in the brain. This conclusion is supported by studies that show that ropinirole improves motor function in various animal models of Parkinson's disease.

Many studies in animals²² have shown that ropinirole has no mutagenic or carcinogenic effects and did not affect the fertility and had no toxic effects on liver and kidney in animals treated with ropinirole at the recommended dose and 10 times MRHD (50 mg/kg/day)⁶. So, in the present study we evaluated the mutagenic, biochemical and pathological effects of ropinirole if administered orally at doses over the maximum recommended dose for human (24mg/day) for 21 consecutive days.

In the present study we found that when male mice administered with ropinirole at different three dose levels of (50, 75, 100 mg/kg/day) which are approximately equivalent to 10, 15 and 20 times respectively the MRHD of 24mg/kg/day, there were significant increases in the frequencies of sperm abnormality and chromosomal aberrations in somatic and germ cells compared with the control and these increases were dose-dependent this finding is agreement with Roberts.²³ who found that the administration of male rat with ropinirole at doses of 1.5, 15 and 50mg/kg there was a significant increase in testicular leydig cell adenomas and increase in the number of sperm abnormality at all level doses.

Also, this finding is agreement with McNeilly²⁴ who found that when female mice were administered with a dose of 50 mg/kg (10 times the MRHD on a mg/m² basis) there was an increase in benign uterine endometrial polyps.

Also, positive results were observed by Dostal²⁵ who found that when ropinirole given to pregnant rats during organogenesis (gestation day 8 through 15) resulted decreased in the fetal body weight at 60 mg/kg/day

increased fetal death at 90 mg/kg/day and digital malformations at 150 mg/kg/day.

Moreover, Robotom²⁶ reported that in a perinatal-postnatal study in rats, 10 mg/kg/day of ropinirole impaired growth and development of nursing offspring and altered neurological development of female offspring.

Also, positive results were obtained by Prentice²⁷ who found that the treatment of male rats with a dose of 100 mg/kg/day, clinical signs, including tremors, stereotypy convulsions and deaths have occurred, and the incidence of pregnancy was slightly decreased 72% versus 80% in the control group.

While, this result is in agreement with Huhtaniemi²⁸ who found that ropinirole did not affect female fertility at a dosage up to 100 mg/day and also no effect on male fertility was observed in rats at dosages up to 125 mg/kg/day.

Also, in the present study we found that the administration of male mice with doses of (50, 75 and 100 mg/kg/day) caused increase in the frequencies of DNA damage (comet assay) and increase in the frequencies of micro-nucleated cells and these increases were more significant in the high dose (100 mg/kg/day).

This finding in agreement with McMartin²⁹ who found that ropinirole was not mutagenic or clastogenic in the *in vitro* Ames test, the *in vitro* chromosomal aberration test in human lymphocytes, the *in vitro* mouse lymphoma, and the *in vivo* mouse micronucleus test.

In contrast this finding is in agreement with Rascol³⁰ who observed that patients which treated with ropinirole specially with high doses have a risk for developing melanoma (skin cancer) than the untreated patients this finding suggested that ropinirole had a mutagenic action in the high doses.

Also this finding is in agreement with Tompson and Vearer³¹ who found that in rats ropinirole binds to melanin-containing tissues to a greater degree than non-pigmented tissues. Therefore ropinirole might have accumulated in these tissues over time and caused toxicity.

In the present study the administration of male mice with ropinirole at different doses of (50, 75 and 100 mg/kg/day) caused statistically significant increases in the levels, of AST, ALT and MDA (liver function) and also increases in the frequencies of urea, uric acid and creatinine levels (kidney function) compared with control mice and these increases were more frequent in the group of males treated with 100 mg/kg/day. These findings were in agreement with McBurney³² who found that in *in vitro* studies ropinirole is extensively metabolized by the liver and the major cytochrome P45B iso-enzyme involved in the metabolism of ropinirole is CYP1A2, in the repeated and higher doses of ropinirole this enzyme could not metabolize this large amount and

as a result ropinirole accumulated in the liver and excreted by the kidney as unchanged drug in the urine this may cause toxicity and increase in the levels of ALT, AST and MDA.

Also positive results were reported by Zimmerman³³ who found that in the liver of mice and rats there was increased incidence of centrilobular hepatocellular hypertrophy in mid and high dose animals, but there was decrease in incidence of vacuolation and biliary hyperplasia.

Moreover, positive results were obtained by Acuma³⁴ who found that in some cases the treatment with ropinirole may cause abnormal hepatic function and increase in the frequencies of AST and ALT.

Also studies were carried out in mice. Lorrey³⁵ observed that the maximal no effect dose was 25 mg/kg and in higher dose there were toxic effect in the liver and kidney and few animals death were observed.

Moreover, positive results were obtained by Navacerrada³⁶ who found that there were a liver toxicity related with ropinirole use in the treatment of restless legs syndrome.

Also, in the present study, using ropinirole drug in different doses affected the liver tissue in many ways as with 50 mg/kg it caused small focal aggregation of cellular infiltrates and mild dilatation and congestion of blood vessels, this effect was increased with increasing the dose to 75 mg/kg as mild vacuolar degeneration was added to the previous findings. These results can be explained by Eden³⁷ who stated that Ropinirole acts as a D₂, D₃, and D₄ dopamine receptor agonist with highest affinity for D₂, it is extensively metabolized by the liver to inactive metabolites via N-despropylation and hydroxylation pathways, largely by the P450 iso enzyme CYP1A2. Lida³⁸, reported that there are *in vitro* free radical scavenging and antioxidant activities of ropinirole, a non-ergot DA agonist, as well as its glutathione (GSH), catalase and superoxide dismutase (SOD) activating effects and neuroprotective effect *in vivo*. Ropinirole scavenges free radicals and suppresses lipid peroxidation *in vitro*, but these activities are very weak, suggesting that the antioxidant effect of ropinirole observed *in vitro* may be a minor component of its neuroprotective effect *in vivo*. Administration of ropinirole for 7 days increased GSH, catalase and SOD activities in the striatum and protected striatal dopaminergic neurons against 6-hydroxydopamine (6-OHDA) in mice. This is in coincidence with the results obtained from the last group received 100mg/kg that revealed reduction of cellular infiltrates and blood vessels dilatation as well as normalization of hepatocytes.

In renal tissue, a dose of 50 mg/kg/day resulted in mild interstitial hemorrhage, while thickening of the parietal wall of Bowman's capsule was observed with the use of 75 mg/kg/day and widening of the urinary space was noticed by using 100 mg/kg/day ropinirole. These findings



can be explained by statements of Debra³⁹ that Ropinirole is metabolized primarily by cytochrome P450 CYP1A2 to form two metabolites; SK&F-104557 and SK&F-89124, both of which are renally excreted. Kvernmo⁴⁰ stated that N-despropyl ropinirole is the predominant metabolite found in urine (40%), followed by the carboxylic acid metabolite (10%), and the glucuronide of the hydroxy metabolite (10%), and less than 10% of the administered dose is excreted as unchanged drug in urine.

CONCLUSION

In contrast to its benefit effect in the treatment of Parkinson's disease, Ropinirole induces a mutagenic and

cytotoxic effects in male albino mice, increases in the sperm abnormality and in the micronuclei in bone marrow cells, increase in the chromosomal abnormalities in somatic and germ cells, DNA damage in liver cells, Adverse effect on liver and kidney function and pathological toxicity in the liver and renal tissues if taken orally at doses 50, 75, 100 mg/kg/day (approximately 10, 15, 20 times the maximum recommended dose for human).

Therefore, the present study suggested that ropinirole should not be taken in doses over the maximum recommended dose.

Table 1: The Effect of Ropinirole on Sperm Head Abnormalities in male Mice

Treated Groups	Abnormal Sperms	Amorphous head	Banana Shaped Head	Dwarf	Hook at wrong angle	No Hook	Triangle	Two tails
Control	73.00 ^a ± 2.65	37.67 ^a ± 2.52	5.33 ^a ± 0.57	15.00 ^a ± 0.00	5.00 ^a ± 0.00	4.33 ^a ± 4.53	5.00 ^a ± 7.00	0.67 ^a ± 0.57
50 mg/kg/day	83.00 ^b ± 2.65	42.33 ^b ± 2.08	6.33 ^b ± 0.57	16.67 ^b ± 0.57	6.33 ^b ± 0.57	4.33 ^a ± 1.76	6.00 ^a ± 1.00	1.00 ^a ± 0.00
75 mg/kg/day	88.00 ^c ± 1.00	44.67 ^{bc} ± 0.57	7.33 ^c ± 1.00	17.00 ^c ± 7.00	7.33 ^c ± 0.57	5.33 ^a ± 1.16	5.33 ^a ± 1.53	1.00 ^a ± 0.00 ^c
100 mg/kg/day	92.33 ^d ± 0.57	45.67 ^c ± 0.57	8.00 ^c ± 0.00	17.67 ^b ± 0.57	7.67 ^c ± 0.57	6.00 ^a ± 0.00	6.33 ^a ± 0.57	1.00 ^a ± 0.00

Data were expressed as Means ± S.D.

Means with different letters (a, b, c, d) in the some column are significantly different (P < 0.05)

50 metaphase were examined from each animal

Table 2: The Effect of Ropinirole on Bone marrow cells of male Mice

Treatment	Structural aberrations								Numerical aberrations			
	Chromatid gaps	Chromosomal gaps	Chromatid break	Deletion	Fragments	Centro meric Attenuation	Endo-mitosis	T.S A	< 40	> 40	Polyploidy	T.N A
Control	5.00 ^a ±1.00	3.67 ^a ±0.57	2.33 ^a ±0.57	2.00 ^a ±0.00	1.67 ^a ±0.57	3.67 ^a ±0.57	4.33 ^a ±0.57	22.67 ^a ±2.52	3.67 ^a ±0.57	1.67 ^a ±0.57	0.33 ^a ±0.57	5.67 ^a ±0.57
50mg/k/day	6.67 ^b ±0.57	4.67 ^b ±0.57	4.00 ^b ±1.00	3.67 ^b ±0.58	4.00 ^b ±1.00	5.67 ^b ±0.57	6.00 ^b ±1.00	34.67 ^b ±1.53	5.00 ^b ±0.00	3.33 ^b ±0.57	1.33 ^b ±0.57	9.67 ^b ±0.57
75mg/k/day	8.00 ^c ±0.00	5.67 ^{bc} ±0.57	5.33 ^b ±0.53	5.67 ^c ±0.58	4.67 ^b ±0.57	6.67 ^{bc} ±0.57	6.67 ^{bc} ±0.57	42.67 ^c ±2.08	5.67 ^b ±0.57	4.67 ^{bc} ±1.15	2.67 ^b ±0.57	13.00 ^c ±1.00
100mg/k/day	8.67 ^c ±0.57	6.67 ^c ±0.57	7.00 ^c ±1.00	6.67 ^c ±0.58	5.67 ^c ±0.57	7.33 ^c ±0.57	7.67 ^c ±0.57	49.67 ^d ±0.57	7.00 ^c ±0.00	5.67 ^c ±1.15	4.00 ^c ±0.00	16.67 ^d ±1.15

Data were expressed as Means ± S.D.

Means with different letters (a,b,c,d) in the column are significantly different P< 0.05

50 metaphase were examined from each animal

Table 3: The Effect of Ropinirole on Spermatoocytes of male Mice

Treatment	Structural Aberrations				Numerical Aberrations			
	x-y univalent	Auto somal	Fragments	Total structural aberrations	Hypo < 20	Hyper > 20	Polyploidy	Total numerical aberrations
Control	1.67 ^a ± 0.57	8.00 ^a ± 0.00	1.67 ^a ± 0.57	6.33 ^a ± 1.15	1.33 ^a ± 0.57	0.33 ^a ± 0.57	0.00 ^a ± 0.00	1.67 ^a ± 0.57
50 mg/k/day	2.67 ^{bc} ± 0.57	4.67 ^b ± 0.57	2.67 ^b ± 0.57	10.00 ^b ± 1.00	2.33 ^b ± 0.57	1.33 ^b ± 0.57	1.00 ^{ab} ± 0.00	4.67 ^b ± 0.57
75 mg/k/day	3.67 ^c ± 0.57	5.00 ^b ± 1.00	3.00 ^b ± 0.00	11.67 ^c ± 0.57	3.33 ^c ± 0.57	2.00 ^b ± 0.00	1.67 ^{bc} ± 0.57	7.00 ^c ± 0.00
100 mg/k/day	4.33 ^c ± 0.57	6.33 ^a ± 0.57	3.33 ^b ± 0.57	14.00 ^d ± 0.00	4.33 ^c ± 0.57	3.00 ^c ± 0.00	2.33 ^c ± 0.57	9.67 ^d ± 0.57

Data were expressed as Means ± S.D.

Means with different letters (a, b, c, d) in the same column are significantly different P< 0.05

50 metaphase were examined from each animal

Table 4: Visual score of DNA damage in male mice treated with Ropinirole.

Treatment	No. of cells		Class (Range)				DNA damaged cells (%)
	Analyzed	Comet	0	1	2	3	
Control	100	4	96	4	0	0	4 ^a
50	100	13	87	6	4	3	13 ^b
75	100	17	83	8	3	6	17 ^c
100	100	23	77	8	6	9	23 ^d

Class 0= no tails 1 = tail length < diameter of nucleus; 2 = tail length between 1 x and 2 x the diameter of nucleus; and 3 = tail length > 2 x the diameter of nucleus.



Table 7: Liver and kidney functions in male mice treated with ropinirole

Treated Groups	Liver functions			Kidney functions		
	Alanine Transaminase ALT	Aspartate Transaminase AST	Alkaline Phosphatase ALP	Creatinine	Urea	Uric acid
Control	22.10 ± 0.71 ^a	36.40 ± 1.07 ^a	140.20 ± 0.58 ^a	0.47 ± 0.03 ^a	18.00 ± 0.057 ^a	2.40 ± 0.24 ^a
50 mg/kg/day	22.10 ± 0.71 ^b	45.20 ± 0.58 ^b	167.40 ± 0.87 ^b	0.63 ± 0.00 ^b	25.40 ± 0.50 ^b	3.20 ± 0.48 ^b
75 mg/kg/day	41.20 ± 1.39 ^c	52.20 ± 0.058 ^c	177.60 ± 0.92 ^c	1.40 ± 0.24 ^c	34.20 ± 0.66 ^c	4.20 ± 0.20 ^c
100 mg/kg/day	53.70 ± 0.48 ^d	64.50 ± 3.73 ^d	205.40 ± 2.44 ^d	2.20 ± 0.37 ^d	47.60 ± 1.07 ^d	5.80 ± 0.37 ^d

Data were expressed as Means ± S.E.

Means with different letters (a, b, c, d) are significantly different P < 0.05.

Table 5: The effect of Ropinrole on micronuclei formation in bone marrow cells of male mice.

Treatment mg/kg	Number of cells analyzed	Animals examined	Mean ± S.D. number of (MN)
Control	5000	5	5.60 ± 2.61 ^a
50 mg/k/day	5000	5	8.80 ± 0.83 ^b
75 mg/k/day	5000	5	13.20 ± 2.44 ^c
100 mg/k/day	5000	5	18.60 ± 1.14 ^d

Data are expressed as Means ± S.D.

Means with different letters (a, b, c, d) are significantly different P < 0.05

Table 6: Percentage of Malondialdehyde (MDA) in mice treated with Ropinrole

Treated Groups	Number of Animals	Malondialdehyde (MDA) nmol/gm tissue
Control	10	0.70 ± 0.30 ^a
50 mg/k/day	10	1.90 ± 0.33 ^b
75 mg/k/day	10	3.20 ± 0.37 ^c
100 mg/k/day	10	7.20 ± 0.58 ^d

Data are expressed as Means ± S.E. means with different letters (a, b, c, d) are significantly P < 0.05.

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