Study of the Effect of Moringa Oleifera on Sperm Abnormalities in White Mice

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Abstract
This study was conducted in the Cellular Genetics Laboratory - Department of Life Sciences / Faculty of Education / University of Qadisiyah from 16 February 2021 to 1/6/2021 and designed to assess the cellular genetic effects aqueous extract of *Moringa oleifera* on reducing the side effects of carboplatin in male laboratory mice through a study sperm aberration. Three concentrations of extract were selected to study genetic effects and these concentrations were (1500, 500, 250) mg/kg of body weight and the white mice Mus musculus were dosed for 35 days and after reaching the best dose (optimal) of extract (1500) mg/kg, which led to decreased sperm deformities, The effect of Carboplatin was studied in the same time period and at a dose of (90) mg/kg. The interaction of the optimal concentration of the aqueous extract of Moringa oleifera leaves 1500 mg/kg with carboplatin was performed on three interactions (before, together, after). Carboplatin in case of sperm abnormalities. Each of the overlap factors had a significant reduction in sperm abnormalities This results in the ability of the aqueous extract of Moringa oleifera leaves to counteract the damage caused by carboplatin due to sperm abnormalities. The treatment together was less than the interference treatments (before, after) in the rate of sperm abnormalities. In addition, the results showed that the aqueous extract of Moringa oleifera leaves has a protective role because it contains powerful antioxidants in protecting sperm from abnormalities when given (before the drug, after the drug or simultaneously with the drug.
Keywords: *Moringa oleifera*, White mice, Sperm abnormalities

1. Introduction

Herbal medicine has been known since ancient times as the oldest friend of mankind and herbs are the primary source of health care for the earth's most populous population because of the belief that they are reliable and more effective than traditional medicines (Alsarhan et al., 2014).
The Plant Kingdom contains many natural compounds that have therapeutic, parasitic or anti-virulent properties, so studies aimed at highlighting natural compounds with anti-mutagenesis traits have expanded.
Moringa oleifera leaves are used to treat various diseases: circulatory tonic, anti-tumor, anti-heat, anti-epileptic, anti-inflammatory, diuretic, hypotension, anti-cholesterol, anti-anxiety, anti-anxiety, anti-muscular contractions, diabetes treatment, antibacterial and fungal (Meireles et al., 2020), and also protects the body's *Moringa oleifera* leaves from toxins, pollutants and free radicals. (Jimenez et al., 2017).
It belongs to *Moringa oleifera* sex within the family of pink plants Moringaceae *Moringa oleifera* is mainly grown in tropical and subtropical regions and it is one of the most popular plant in Southeast Asia, particularly in the Philippines. Native to the foothills of the southern Himalayas in north-west India (Premi and Sharma, 2013; Radovich, 2010).
Moringa oleifera contains alkaloids, dragons, phenolic compounds, amino acids, steroids and carbohydrates and is rich in compounds containing simple sugars Rhamnose, rich in a unique range of compounds called isothiocynate and Rahman (Ganatra M et al., 2009; Tejas H. et al., 2012) .
Chemotherapy is one of the most common interventions for cancer treatment but causes patients discomfort due to its harmful side effects, limiting its full use in cancer treatment (Saxena et al., 2016). Chemotherapy works by attacking rapidly divided cells that include cancer cells (Smith et al., 2014). In the 1950s and 1960s, the development of chemotherapy led to therapeutic methods for patients with malignant blood tumors, but the effects of chemotherapy were the toxicity they caused to healthy tissue of the body and the development of resistance to cellular drugs, as well as the development and application of molecular techniques and analysis of the gene expression of healthy cells at the level of RNA, DNA, or protein to facilitate the identification of some mechanics of the effect of (chan et al., 2019).
Cis-diammine1,1-cyclobutanedi-carboxylate (II) platinum is a second-generation platinum compound also known as CBDCA, whose commercial name Paraplatin is a symmetrical platinum latinu analog, representing hydrocarbon complexes of izumi-binary platinum. The XS engineering of the nitrogen amino group, platinum drugs are among the most widely used drugs for the treatment of cancer, carboplatin, a counterpart to szplatin, was introduced in clinical trials in 1981 to help avoid some of the toxicity of sizplatin (Tiseo and Ardizzoni, 2007).

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I proposed and completed the current study to highlight the genetic effects of carboplatin and *Moringa oleifera*, and for the purpose of reaching the target, the white laboratory mouse was selected as a test system by studying a set of genetic criteria available within our potential at present.
Study of the inhibitive role of *Moringa oleifera* plant in the direction of cellular and genetic and effects caused by carboplatin in sexual cells based on sperm deformity testing.

2. Materials and Methods

Eosin stain

was prepared with a dissolved solution (1 g) of yellow eocin dye (Eosin Yellowish) in (100 ml) of distilled water (Wyrobek and Bruce, 1975).

Phosphate buffer saline (PBS)

melted The ingredients below were into one liter of D.W. distilled water Potassium chloride (KCl .) 0.2 g
Sodium chloride (NaCl.) 8 g
Sodium phosphate hydrate (Na2HPO4 1.15 g
Potassium phosphate dehydrate (KHPO4 0.2 g
(PH) to (2.7), then sterilize the solution with pressurized steam and store in the refrigerator at 4 celsius (Allen et al., 1977).

Carboplatin dose

The carboplatin dose of 90 mg/kg was selected on the basis of body weight and equivalent to the therapeutic human dose of 400 mg/kg (Basit et al., 2021). Carboplatin was dissolved in distilled water and injected into the peritoneal cavity of mice as a single 0.1 ml dose.

Laboratory animals

were used in this study 60 male white mice, their weights ranged between (30-25) grams and their ages ranged between (7-14) days. They were obtained from the animal house of the Department of Life Sciences, College of Science, University of Kufa. The animals were bred in the animal house belongs to the College of Science, University of Al-Qadisiyah. The animals were left for two weeks to acclimatize under laboratory conditions of suitable ventilation and at a temperature of (25) Celsius. That's what animals used in experiments. Design of experimental study Choosing a dose of *Moringa oleifera* extract
The experiment assigns (30) males to six mice per group. This stage included studying the genetic effects of the Moringa extract using three doses according to (Olufunsho et al., 2012) and the dose of carboplatin according to (Basit et al., 2021).
The first group, negative control: the animals were left without treatment other than distilled water for 35 days.
The second group, the positive control: the rats were injected daily with the carboplatinum mutagen in the peritoneal cavity At a concentration of 90 mg kg for 35 days.
The third group: Orally administered using a modified syringe for this purpose, a plant extract Moringa at a concentration of 250 mg kg for 35 days.
Fourth group: Orally administered using a syringe modified for this purpose plant extract Moringa at a concentration of 500 mg/kg for 35 days.
Fifth group: Orally using a modified syringe for this purpose plant extract Moringa at a concentration of 1500 mg kg for 35 days.
After the end of the period, the animals were explained for the purpose of conducting genetic tests, and in light of the results of these groups, the optimum dose of the extract was chosen for the purpose of conducting an interaction with one of the genetically mutagenic drugs (Mutagenic) and this drug is Carbopltin, and the body weight of the experimental animals was calculated before and after treatment with the extract for mice.
The study of the interaction between the optimal dose (mg/kg) of the aqueous extract of Moringa and the drug carboplatin to study the mechanism by which the extract of Moringa works. 6) mice for each group, i.e. a total of (30) proximal mice:
The first group, negative control: the animals were left without treatment except for distilled water for 35 days.
The second group, the positive control: the rats are injected daily with the carbo-platinum in the peritoneal cavity a concentration of 90 mg kg for 35 days.
The third group: Treatment with the plant extract optimal concentration three weeks before the mutagenic drug for 35 days of treatment.
Fourth group: Treatment with the plant extract with the mutagenic drug periodically, the plant extract is dosed at the optimum concentration, and then it is injected with the mutagen for a period of 35 days from the treatment.
Fifth group - treatment with the plant extract after the mutagenic drug for the three weeks.

Cytogenetic study

Sperm head anomaly test test explained mice and extracted sperm from epididymis using the method (Wyrobek and Bruce, 1975) and according to the following steps:
1- Cut the epididymis and place in a petri plate containing (5 ml) of phosphate-filled solution and using a sharp blade and fine tongs the epididymis was cut into very small parts and the solution containing those parts was placed in a clean test tube.
2- Clean glass slices were prepared and a drop of the solution in the tube was brushed on the glass slide, and the slices were left on a hot plate (50 m) to dry.
3- Dry glass slides were dyed with Eosin dye (1% Eosin) for (3-1 minutes) after which the excess dye was removed by washing the slides with distilled water.

Sperm head anomalies examined

Check the slides after they dried with the optical microscope and using the oil lens, as the percentage of sperm head deformities was calculated by

examining (1,000 sperm) and comparing the forms of those sperm with the natural form.

3. Statistical Analysis

The results of the data were analyzed statistically by using the statistical analysis program (SPSS 23) discovery version 2015 by finding the mean + standard error (meanom) and using the SE M Least significant difference (LSD) test. Statistical data between experimental groups on animals was analyzed using the method one way ANOVA and - two sample T-Test for analysis of control groups). The results are considered significant if the p-value is less than 0.05 (Sorlic, 2014).

4. The result

From table (6-4) to a moral rise (0.05 >p) in the percentage rate of folded tail sperm abnormalities, Sperm without tail, Sperm without head, sperm hook) in positive control treatment at (37.4) compared to negative control (13.8), and there is a moral decrease (0.05 >p) in the percentage of sperm abnormalities of the three compositions of the moringa oliveira leaf water extract (1500, 500, 250 mg/kg body weight if the ratio (11,11.8, 13) compared to positive control, as the concentrations (1500, 500) mg/kg showed a moral decrease (0.05 >p) from negative control, The concentration (250) mg/kg did not show a moral difference from negative control. A moral decrease (0.05 >p) in the percentage of moving sperm was also observed in the table, at (64.6) in positive control compared to negative control (90.2), and a moral rise (0.05 >p) among the three trakes water extract coefficients.1500. 500. 250) mg/kg as it reached (94, 93, 92) respectively compared to positive control. There is also a moral rise (0.05 >p) in the treatment with a concentration of (1500) mg/kg compared to negative control, There are no moral differences for the two concentrations (500,250) mg/kg, reaching (93,92.4) respectively with negative control. A moral rise (0.05 >p) was also observed in the percentage of dead sperm in positive control 30.2 compared to negative control of 12.8, A moral decrease (0.05 >p) was also observed in the water extract coefficients of the three moringa oliveira leaves (1500, 500, 250) mg/kg, amounting to (10.4, 10.8, 11.2) respectively compared to positive control, and there are no moral differences between them and the treatment of negative control. A moral rise (0.05 >p) was also observed in the number of sperm, reaching 21.4 in negative control compared to positive control 12.8, and a moral increase (0.05 >p) In the number of sperm for the three compositions of the water extract of the Moringa Oliveira leaves (1500, 500, 250) mg/kg, with a value of 28.8, 23.2, 23) respectively compared to positive control, and there are no moral differences in (500, 250) mg/kg of negative control treatment, a moral rise (0.05 >p) was observed between concentration (1500) mg/kg at 28.8 and negative treatment.

Table 1: Effect of carboplatin, which causes mutations and various compositions of the water extract of Moringa Olivera leaves, on the percentage of sperm deformities in white mice.

Parameters Groups	Motility % (mean+SD)	Dead % (mean+SD)	Sperm Abnormalities % (mean+SD)					Count ×107 (mean+SD)
			folded tail sperm	sperm without tail	sperm without Head	Blunt head hook sperm	Abnormalities % (mean+SD)	
Control	B 90.2±3.25	B 12.8±1.72	B 4.8±0.837	B 4±1.225	B 3.2±1.095	B 1.8±0.837	B 13.8±1.72	B 21.4±2.059
Carboplatin	C 64.6±5.12	A 30.2±3.43	A 17.4±2.3	A 9.4±1.14	A 6.4±1.517	A 4.2±0.837	A 37.4±3.01	C 12.8±1.72
Morenga 250mg	AB 92.4±2.154	B 11.2±1.72	B 4.8±0.837	B 3.6±0.894	B 2.4±0.548	B 2.2±0.837	BC 13±1.414	B 23±1.414
Morenga 500mg	AB 93±2	B 10.8±1.72	B 4±0.707	B 3.8±0.837	B 2±0.707	B 2±0.707	BC 11.8±1.327	B 23.2±1.72
Morenga 1500mg	A 94±2.366	B 10.4±1.02	B 4.2±0.837	B 3.4±0.548	B 2±0.707	B 1.4±0.548	C 11±1.414	A 28.8±2.79
Sign.	Sign.	Sign.	Sign.	Sign.	Sign.	Sign.	Sign.	Sign.
P-value	0.00002	0.0001	0.0002	0.0000	0.0005	0.00017	0.00005	0.00000

The results in the table 2 showed a moral rise (0.05 >p) in the percentage of sperm abnormalities folded tail sperm, sperm with out tail, sperm with out head, sperm head (Blunt head) at (39.4) in positive control compared to negative control (14). A moral decrease (0.05 >p) in interference coefficients (before, together, after) was also observed by (22.6, 17.4, 23) compared to positive control, and the interference treatment (together) was closer to negative control as there were no moral differences between them. A moral rise (0.05 >p) was also observed in the percentage of sperm movement in the negative control treatment of 89.4 compared to positive control 60.8, and there was a moral rise (0.05 >p) in the percentage of sperm movement in interference transactions (before, together, After) it reached (70(80.73, respectively, compared to positive control and the interference treatment (together) was closer to control and there are no moral differences between them. There is also a moral rise (0.05 >p) in the percentage of dead sperm in the positive control treatment of 30.6 compared to negative control 13, A moral decrease (0.05 >p) was also observed in the percentage of dead sperm in interference coefficients (before, together, after) to 16.6, 14.8,

19.2) respectively compared to positive control, and there is no moral difference in the treatment of interference (together) with negative control. A moral rise (0.05 > p in the number of sperm in negative control was also observed in the table, compared to positive control 13, There is also a moral rise (0.05 >p) in the number of sperm in interference transactions (before, together, after) at 18.4, 20.8,16 compared to positive control and the number of sperm in the interference transaction (together) was closer to negative control.

Table 2: Effect of carboplatein, which causes mutations, and water extract for moringa olivera leaves in interferences (before, together, after) on the percentage of sperm deformities in white mice.

Parameters Groups	Motility % mean+SD)	Dead % (mean+SD)	Sperm Abnormalities % (mean+SD)					Count ×107 (mean+SD)
			folded tail sperm	sperm without tail	sperm without Head	Blunt head hook sperm	Abnormalities % (mean+SD)	
Control	A 89.4±3.44	D 13±1.414	C 5.6±0.548	C 4.2±0.837	C 2.4±1.342	B 1.8±0.837	D 14±1.414	A 22.8±2.135
Carboplatin	D 60.8±3.87	A 30.6±2.58	A 19.2±2.28	A 10.2±1.924	A 5.4±1.14	A 4.6±1.14	A 39.4±2.154	D 13±1.414
Morenga 1500mg±Car	C 70.6±3.44	B 19.2±1.939	B 10.6±1.517	B 6±1.581	B 3.8±0.837	B 2.6±0.894	B 23±3.16	C 16±1.414
Car±Morenga 1500mg	C 73±3.35	C 16.6±2.154	B 11.2±1.924	BC 5.4±1.14	BC 3.2±0.837	B 2.8±1.095	B 22.6±2.154	B 18.4±1.855
Carb. =Morenga 1500mg	B 80±3.03	CD 14.8±1.166	C 7.4±1.14	BC 4.8±0.837	BC 2.6±0.548	B 2.6±0.548	C 17.4±1.855	A 20.8±1.72
Sign.	Sign.	Sign.	Sign.	Sign.	Sign.	Sign.	Sign.	Sign.
P-value	0.00004	0.00006	0.0003	0.0005	0.001	0.002	0.0002	0.0000

Parameters Groups	Motility % (mean±SD)	Dead % (mean±SD)	Sperm Abnormalities % (mean±SD)				Count ×10^7 (mean±SD)
			folded tail sperm	sperm without tail	sperm without Head	Blunt head hook sperm	
Control	A 89.4±3.44	D 13±1.414	C 5.6±0.548	C 4.2±0.837	C 2.4±1.342	B 1.8±0.837	A 22.8±2.135
Carboplatin	D 60.8±3.87	A 30.6±2.58	A 19.2±2.28	A 10.2±1.924	A 5.4±1.14	A 4.6±1.14	D 13±1.414
Morenga 1500mg±Car	C 70.6±3.44	B 19.2±1.939	B 10.6±1.517	B 6±1.581	B 3.8±0.837	B 2.6±0.894	C 16±1.414
Car±Morenga 1500mg	C 73±3.35	C 16.6±2.154	B 11.2±1.924	BC 5.4±1.14	BC 3.2±0.837	B 2.8±1.095	B 18.4±1.855
Carb. =Morenga 1500mg	B 80±3.03	CD 14.8±1.166	C 7.4±1.14	BC 4.8±0.837	BC 2.6±0.548	B 2.6±0.548	A20.8±1.72
Sign.	Sign.	Sign.	Sign.	Sign.	Sign.	Sign.	Sign.
P-value	0.00004	0.00006	0.0003	0.0005	0.001	0.002	0.0000

5. Discussion

Sperm anomalies showed current results that animals treated with plant extract led to a significant reduction in sperm abnormalities, indicating the preventive and therapeutic role of the water extract of moringa oleifera leaves in the body's cells against the effect of observed mutations of carboplatin. The mechanism of action of many phytochemicals may be inhibition of cytochrome activation - by hydroxylN enzymes with the resulting reduction in genetic material (DNA or chromosomes) and sperm deformities (Aitken and Krausz, 2000; Farag et al., 2002). This is consistent with Zade and his group (2013), who stated that the water extract of moringa oliveira seeds with a concentration of 300 mg/kg of body weight for 21 days has a positive effect on the genesis of sperm in rats, which may be due to the presence of alkaloid compounds, flavonoids, phenolates and taninates that can reduce testicular weakness associated with oxidative stress in animal tissues. As well as It also increases antioxidants, restores their defensive effectiveness and their ability to prevent lipid peroxide and protect mitochondria and cell membranes from oxidative stress (Mansour et al., 2020).

Studied cellular tests of carboplatin effect Studied cellular tests for the effect of carboplatin

Sperm-generating cells give large numbers of sexual cells that are at different stages of the cell cycle, and those cells vary in sensitivity towards mutational substances, and the substance that induces sperm abnormalities has the potential to trigger mutation (Zdienicka et al., 1982). This provides a better chance of studying the effect of the mutational substances and the extent to which the appearance changes take place. The results in the table indicated a higher rate of abnormalities and different types in male mice treated with carpoplatin at a dose of 10 mg/kg of body weight compared to negative control. There was significant induction of sperm abnormalities in mice treated in carboplatin, indicating the toxic effects of carboplatin.

Studies and reports are available on the effects of

bacterial cells caused by cisplatin, where cisplatin showed clastogenic effects in differentiated sperm in mice (Adler and El-Tarras, 1990), according to their reports, sperm cells are less sensitive than bone marrow cells. However, in other studies, sysplatin has caused significant stimulant effects in sperm, and a sexually related recessive killer mutation has also been identified in male germ cells (Burke and Woodruf, 1980), cloning in sperm cells and sperm (Brodberg et al., 1983) in the melano gaster fruit fly by cisplatin causes sysplatin in large abnormal sperm cells and monovalent physical and sexual chromosomes, Quadruple parity etc., in male mice (Adler and El-Tarras, 1990; Choudhary et al., 2000). Platinum compounds also reduced sperm movement in humans (Mann and Stelner, 2012).

In this study, a significant moral rise in sperm count was observed indicating the toxic effects of carboplatin on sperm formation. According to Topham (1980 a and b). The properties that control the sperm head are made in the subjective particles and the test of sperm abnormalities determines those factors that cause small changes in the DNA of the testicle.

The treatment of carboplatin has shown oxidized kidneys, enhanced lipid peroxide, platinum content, plasma creatinine and urea nitrogen levels in the blood in mice, androgens play a vital role in the initiation and maintenance of reproductive function or testicular function that includes sperm production, the main androgen in the testicular i.e. testosterone is produced by ledc cells under the stimulation of the pituitary gland LH, a hormone necessary for sperm formation and fertility and the maintenance of the male phenotype.

Sperm formation depends on the action of testosterone, the androgen prevalent in sperm formation produced by LEDC cells in the testicle. The two main enzymes involved in the biosynthesis pathway of testosterone are 3- HSD and17- HSD, as 3-HSD and 17-HSD activity levels have been used to study the formation of testicular steroids for mice in experimental conditions, these enzymes have different regulatory functions in maintaining steroid formation and in testosterone synthesis. In mice treated with carboplatin, low levels of activity were observed, indicating poor steroid composition, as the level of steroid enzyme activity indicates a decrease in the composition of androgen production in experimental mice, which in turn leads to a decrease in reproductive activities in male mice.

Carboplatin appears to be working on LEDC cells and prevents the production of testosterone, which was evident through the low activity of 3- HSD-3 and 17- HSD enzymes in experimental rat testicles, as well as many abnormal sperm in carpoplatin-injected mice, many head morphology in animals in the form of a Rod rod rod or Arrowao banana or banana banana or pinpin point instead of its natural hook hook shape.

Carboplatin has a primarily effect on Spermatogonia and rapidly dividing sperm cells. Bacterial dorsal is more sensitive to the effects of cytotoxic drugs and lydk cells, it may directly affect the functions of a lydk cell that causes loss of sperm after damage to bacterial cells and reduced testicular size and testicular blood (Wang et al., 1983). Bacterial epithelium damage and reduced testicular size may also affect the function of Lydk cells by causing structural changes within the testicle or changes in paracarin to the function of LEDC cells (Huhtaniemi and Toppari, 1995; Carreau, 1996). A number of symptoms occur in hypothyrexual fatigue and mood change, especially in patients using cytotoxic chemotherapy (Howell et al., 2000b).

In addition, low bone mineral density associated with testosterone density has been demonstrated in these patients treated with cellular toxins (Holmes et al., 1994; Howell et al.,2000a). Sperm formation is a well-organized process that begins with sperm stem cells and ends with differentiated and mobile sperm, indicating a significant decrease in sperm count and movement and sperm function in carboplatin-injected mice to the effect of carboplatin inhibitor on sperm formation. Current results are consistent with previous reports such as reduced animal movement in mice and in humans treated with platinum-based anti-cancer drugs such as sesplatin (Amin and Hamza, 2006; Pogach et al., 1988). Cellular standards studied after selecting the optimal dose of water extract for moringa oliveira leaves studied cellular parameters after choosing the optimal does of German extract of Moringa Olievera leaves, Sperm deformities Sperm abnormal showed the results shown in the table indicated a decrease in the percentage of sperm abnormalities in interference coefficients (before, together, after) and this decrease was morally in transactions compared to positive control, and the ratio of sperm abnormalities was lower in treatment together and this indicates the efficiency of this treatment in reducing the rate of sperm deformities. Many phytochemicals may be due to association with mutations or inhibition of enzymes N-hydroxylation enzymes activated by the cytochrome system with a subsequent reduction of genetic material (DNA or chromosomes) and sperm abnormalities (Aitken and Krausz, 2000; Farag et al., 2002; Wang et al., 2004; Devaraj et al., 2006). Free radical removal is an important strategy in the anti-mutation and anti-cancer formation (Fiorani and Accorsi, 2005). Moringa contains a rich number of antioxidants (Chumark et al., 2008; Iqbal and Bhanger, 2006). A possible explanation for the preventive or therapeutic effect may be to share its antioxidant properties for protection or treatment by scanning reactive oxidizing species.

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