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# Cytogenetic and Mutagenic Effects of Fondaparinux on Albino Male Mice

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**Abstract:** Fondaparinux was the first of a new class of antithrombolic agents developed for the preventation and treatment of venous and arterial thrombosis, blocking thrombin generation by selectively inhibiting factor Xa. The safety use of fondaparinux has not been adequately studied.Therefore, this study aimed to evaluate the cytogenetic and mutagenic effects of fondaparinux. Male adult albino mice were administrated subcutaneously with a doses of 0.1 and 0.2 mg/kg/day which approximately equal to the lowest and the highest recommended doses for treatment of thrombosis for 21 consecutive days. The treated groups and control were sacrificed 24h after the last treatment, the incidences of sperm head abnormalities, chromosomal aberrations in both somatic and germ cells and the percentages of DNA damage were determined. Fondaparinux at the two recommended doses did not produce any significant adverse effects on the incidences of sperm abnormalities and in the chromosomal aberrations of both somatic and germ cells compared with the control. In addition, fondaparinux was found to be non-mutagenic in the comet assay and did not produce DNA damage in the liver cells of male mice compared with the control. Therefore, fondapraniux did not exhibit cytotoxic or mutagenic effects on male mice.

**Key words:** Fondaparinux · Antithrombosis · Chromosomal Aberrations · DNA Damage · Sperm Abnormalities · Mice

## INTRODUCTION

Thromboembolic diseases is a major public health problem worldwide, contributing to an estimated 500,000 death in Europe and up to 300,000 death in the united states each year [1].

Thrombosis is the formation of a blood clots called a thrombus, that blocks part or all of a blood vessels such as artery or vein.

Blood clotting is a natural protective mechanism that is triggered by the body in response a cut or wound. It is essential to prevent the wounds from bleeding excessively. The blood-clotting process is a complex sequence of chemical reactions. The blood contains blood clotting proteins, anticlotting proteins and minute cell fragment called platelets, all of which are important in this process. However, blood clotting can malfunction and the result can be thrombosis, (the formation of a harmful blood cloth or thrombus). This can be caused by a marrow, blocked or damaged blood vessel (an artery or vein) as a result of poor circulation, inactivity (e.g. prolonged bed rest), severe infection and cancers blood clots can either partially or completely block the flow of blood. Some people are born with factors that make clots more likely to form in their blood vessels and others may be born with defects in natural, vital blood thinning substances called anticlothing proteins and are therefore more likely to develop blood clots [1].

There are two types of blood thrombosis, arterial blood thrombi which made up mainly of platelets and are the major cause of heart attack and stroke and venous blood thrombi which made up mainly of sticky threads of fibrin, venous blood thrombi such as deep-vein thrombosis (DVT) and pulmonary embolism (PE).

Deep vein thrombosis occur when a blood clots develops in vein deep in the body, these clots most often develop in the lower legs or thighs they may appear in the upper body such as the arms or other locations in the body. It is estimated that there are more than 2.5 million persons in the U.S.A. who develop deep vein thrombosis each year.

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Deep vein thrombosis can pose a serious threat to health if not pieces of a clot can break off and travel through the blood stream to the lung this is called pulmonary embolism and can be fatal soon after it occurs, these clots can be obstruct the blood vessels and reduce or prevent the flow of blood to the lungs leading to a sudden death.

Deep vein thrombosis can also block blood flow in the vein of the leg, causing swelling, pain and permanent damage to the lung this is called a pulmonary embolism and can be fatal soon after it occurs, these clots can be obstruct the blood vessels and reduce or prevent the flow of blood to the lungs leading to a sudden death.

Deep vein thrombosis can also block blood flow in the vein of the leg, causing swelling, pain and permanent damage to the leg called post thrombolic syndrome [2].

There are a variety of factors that contribute to the development of deep vein thrombosis such as the surgery of leg and hip, long period of be drat, birth control pills or hormones, cancer, heart failure arterial disease, spinal cord injury pregnancy and a disease of immune system.

In fact, the goal of treatment is to prevent to clot from growing, to ensure that it dose not break off and travel through the veins to the lungs and to help reduce the possibility of another blood clot formation.

Various types of medications may be used in the treatment of thrombosis especially deep vein thrombosis.

The most common medications are the coagulants (blood thinners), they do not destroy the clots, they keep the clots from growing and other clots from forming such as warfarin (coumadin) and heparin [3].

Another type of medication called inhibitor of activated factor X are used recently in the treatment and preventation of thrombosis.

Activated coagulation factor X (factor Xa) is a major target for designing anticoagulant drugs. It is located at the convergence of the intriunsic and extrinsic coagulation cascade and activation of one molecular of factor X results in the generation of 1000 molecules of thrombin. Therefore, inhibition of factor Xa should theoretically be more effective for reducing fibrin formation than inactivation of thrombin.

Fondaparinux (Arixtra) is the first and the only selective inhibitor of factor Xa which as been approved for use in the treatment and preventation of thrombosis worldwide [4].

Fondaparinux is a synthetic molecule composed of five saccharides, which selectively inhibits factor Xa. The inhibition is indirect, mediated by plasma antithrombin. By inhibiting factor Xa, fondaparinux attenuates thrombin generation and fibrin formation. Furthermore, it has a favorable and predictable pharmacokinetic profile when administered subcutaneously and has along half-life, allowing once-daily dosing [5].

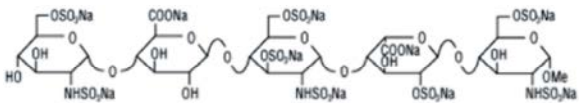
In fact fondaparinux has not been tested for its carcinogenic and mutagenic potential in duration long than 11 days the usual duration time for the treatment of thrombosis and also there are no data on the effects of fondaparinux on male fertility. So, in the present study we examined the cytogenetic and mutagenic effects of fondaparinux on male mice if given subcutaneously at two recommended doses (low and high) for 21 consecutive days.

## MATERIALS AND METHODS

**Drug:** Arixtra (fondaparinux sodium) injection provided by (GlaxoSmithKline) is a sterile solution containing fondaparinux sodium. It is a synthetic and specific inhibitor of activated factor X (Xa).

Fondaparinux sodium is methyl O-2-deoxy-6-O-sulfo-2-(sulfoamino)-α-D-glucopyranosyl-(1→4)-O-β-D-glucopyranuronosyl-(1→4)-O-2-deoxy-3,6-di-O-sulfo-2-(sulfoamino)-α-D-glucopyranosyl-(1→4)-O-2-Osulfo-α-L-idopyranuronosyl-(1→4)-2-deoxy-6-O-sulfo-2-(sulfoamino)-α-D-glucopyranoside, decasodium salt.

The molecular formula of fondaparinux sodium is $C_{37}H_{43}N_3Na_{10}O_{49}S_8$ and its molecular weight is 1728 and the structural formula is:



Arixtra (fondaparinux) is used to prevent the formation of blood clots especially blood clot called deep vein thrombosis and pulmonary thrombosis.

Arixtra (fondaparinux sodium) is supplied as a sterile preservative free injectable solution for subcutaneous used. The recommended dose of arixtra is 2.5mg administered by subcutaneous injection once daily and this dose may be increased up to 5 mg once daily. The usual duration of the therapy is 9-11 consecutive days. In some severe cases treatment can be extended up to 21 days.

Dilutions of different concentrations were prepared by increasing the sterile solution with distilled water or sodium chloride (NaCl) 0.9%.

Arixtra were injected subcutaneously at two dose levels (0.1 and 0.2) mg/kg/day once daily for 21 consecutive days. These doses corresponding to the low and high recommended doses for human after modified to suit the small weight of albino mice (25gm) according to Pagat and Barnes [6].

**Animals:** Adult male albino mice weighting about 25 gm were obtained from National Research Center Animal House. Animals were kept in 12h light/dark room at 25-27°C for one week prior to starting the experiment and they were provided with food and water available add libitum. Animals were divided into three groups. The first group of five male mice were administered subcutaneous with a single dose of 0.1 mg/kg/day of foundaparinux one daily.

The second group of five male mice were administrated subcutaneous with a single dose (0.2 mg/ kg/day) of fondaparinux once daily.

The third group of five male mice served as controls and were administered subcutaneous with distilled water.

Animals were administered subcutaneously for 21 consecutive days and after one day from the last treatment, animals were sacrificed by cervical dislocation for analysis of sperm head abnormalities, comet assay and chromosomal aberrations in somatic and germ cells.

**Methods**

**The International Normalized Ratio (INR) Measurement:** The (INR) is a laboratory measurement of how long it takes blood to form a clot. It used to determine the effects of anticoagulants on the clotting system. The treated males were sacrificed bode capitation blood is drown into a test tube containing liquid sodium citrate. The mixed blood then centrifuged to separate blood cells from plasma. The plasma is analyzed by a laboratory technician on an outomateq instrumental 37°C and the international normalized ratio is measured by the method of Horsti *et al.* [7].

**Cytogenetic Analysis**

**Sperm Head Abnormality Assay:** The cauda epididymis was removed and placed in 0.9% sodium chloride solution and then minced into pieces with scissors and left undisturbed for 20 minute for the diffusion of spermatozoa. The spermatozoa were spread in microscopic slides, air dried, fixed in absolute methanol for 15 minute and stained with 1% aqueous eosin-y.

Three hundred sperms from each animal were examined for the abnormalities in sperm head shapes following the method recommended by Wyrobeck and Bruce [8].

**Spermatocytes (Germ Cells) Assay:** Testes were obtained from the animals to study the abnormalities in spermatocytes (germ cells) according to Brewen and Preston [9] with some modifications. Mice were injected with colchicines (2.5 mg/kg/b.w.i.p), after 3h animals were killed by cervical dislocation, the testes were collected in 2.2% sodium citrate solution and minced into pieces and then centrifuged at 1000 r.p.m. for 2 min. The pellets 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 of methyl: glacial acetic acid. Finally two or three drops of cell suspension were dropped on a clean slide, air-dried and stained with 10% Giemsa stain for 25 minutes. 50 metaphases were studied per animal scoring different types of aberrations.

**Bone Marrow (Somatic Cells) Assay:** Chromosomes from bone marrow cells were prepared according to the method of Agarwal *et al*. [10]. Mice were injected with colchicines (2.5 mg/kg/b.w. i-p) 3 hours prior animals were killed by cervical dislocation. The bone marrow cells were aspirated in phosphate buffer at P.H 7.2and centrifuged at 1000 r.p.m for 2 min. The pellets obtained were mixed in aqueous solution of KCL (0.56%) and left for 30 min at 37°C, then cells were re centrifuged, fixed in (3:1) methyl glacial acetic acid. Finally, slides were air-dried and stained with 10% Giemsa stain for 20 minutes. 50 metaphase spreads were examined for each male, scoring the different types of chromosomal aberrations.

**Comet Assay:** Isolated hepatic cells of all groups of mice were subjected to the modified single-cell electrophoreses or comet assay according to Singh *et al*. [11]. To obtain the cells, a small piece of the liver was washed with an excess of ice-cold Hank's balanced salt solution (HBSS) and minced quickly into approximately $1mm^3$ pieces while immersed in HBSS, with a pair of stainless steel scissors. After several washings with cold phosphate buffered saline (to remove red blood cells), the minced liver was dispersed into single cells using a pipette according to Lai and Singh [12]. The protocol for electrophoresis involved embedding of the isolated cells in agarose gel on microscopic slides and lysing them with detergent at high salt concentrations overnight (in the cold). The cells were treated with alkali for 20 min to denature the DNA and electrophoresis under alkaline conditions (30 min) at 300 mA, 25V. The slides were stained with ethidium bromide and examined using a fluorescence microscope (Olympus BX60 F-3) with a green filter at x 40 magnification.

For reach experimental condition, about 100 cells were examined to determine the percentage of cells with DNA damage that appear like comets. The non over lapping cells were randomly selected and were visually assigned a score on an arbitrary scale of 0-3 (i.e., class 0 = no detectable DNA damage and no tail; class 1 = tail with a length less than the diameter of the nucleus; class 2 = tail with length between 1 x and 2 x the nuclear diameter; and class 3 = tail longer than 2 x the diameter of the nucleus) based on perceived comet tail length migration and relative proportion of DNA in the nucleus as reported by Kobayashi *et al*. [13].

A total damage score for each slide was derived by multiplying the number of cells assigned to each class of damage by numeric value of the class and summing up the values.

Slides were analyzed by one observer to minimize the scoring variability.

**Statistical Analysis:** The data of sperm head abnormalities, chromosomal aberrations in sperm cells and bone marrow cells were subjected to analysis of variance (ANOVA) according to Snedecor and Cochran [14]. Duncan's multiple range test were used to compare between means of treatments according to Waller and Duncan [15] at probability 5%. The data of comet assay for DNA damage were expressed as percentage.

## RESULTS

**The International Normalised Ratio:** The data presented in Table (1) showed that the subcutaneous administration of fondaparinux at dose levels of 0.1 and 0.2 mg/kg/day for 21 consecutive days caused at significant increased in the INR than the control group and these increases were dose dependent.

**Sperm Head Abnormality:** Means + S.D. values and the results are given in Table (2). Various formed of sperm heads, i.e. amorphous head, banana shaped, dwarf, triangle, double headed, two tails etc were recognized in all treated groups of fondaparinux and in the control group. Analysis of these abnormal sperms showed that amorphous head, banana, dwarf, triangle were more frequent in different groups than double headed, no hook, wrong angle and two tails.

The comparative analysis of sperm abnormalities in male mice treated with the two recommended doses of fondaparinux for 21 consecutive days showed no significant increase in all types of sperm head abnormalities as compared with control group and also there was no significant difference in sperm head abnormalities between the two dose levels of fondaparinux groups. The total number of abnormal sperms in the (0.1 and 0.2 mg/kg/day) fondaparinux groups were 78.67 and 80.33 respectively compared with control group (77.00). There is no indication that fondaparinux affect the fertility of male mice.

Table 1: The international normalized ratio.

| Treatments | INR |
|---|---|
| Control | $1.0767^{a}\pm0.0404$ |
| 0.1 mg/kg | $1.1767^{b}\pm0.02517$ |
| 0.2 mg/kg | $1.2567^{c}\pm0.02082$ |

Means of different letters (A,B,C) in the same column are significantly different ($P < 0.05$)

**The Effect of Fondaparinux on Spermatocytes (Germ Cells) of Male Mice:** Means + S.D. values and the results are given in Table (3). Cytogenetic examination showed that the groups of males mice treated with fondaparinux with the two dose levels (low and high) had no significant increase in the total numbers of structural and numerical aberrations compared with the control group.

Also, there was no significant difference in the total number of structural and numerical aberrations between the two treated groups of fondaparinux.

The most frequent structural aberrations were x-y univalent and outosomal univalent and the most frequent numerical aberrations were periploidy including hypoploidy and hyperploidy, there was no indication that fondaparinux induced chromosomal aberrations in spermatocytes.

**The Effect of Fondaparinux on Bone Marrow of Male Mice:** Means + S.D. values and results are given in Table (4), cytogenetic examination in the bone marrow cells of male treated with fondaparinux for 21 consecutive days showed that the groups of males treated with fondaparinux (0.1 and 0.2 mg/kg/day) had no significant increase in the total number of structural and numerical aberrations than the control group. Also, the frequencies of total structural and numerical aberrations in the two treated groups (0.1 and 0.2 g/kg/day) had no significant increases between them.

The total structural and numerical aberrations for (0.1 and 0.2 mg/kg/day) fondaparinux groups were 34.33, 15.67 and 36, 17.53 respectively compared with control (34 and 15). There was no indication that fondaparinux induced chromosomal damage in somatic cells.

Table 2: The effect of fondaparinux on sperm head abnormalities in male mice

| Treatments | Abnormal sperms | Amorphous head | Banana shaped head | Dwarf | Triangle | Double headed | Hook at wrong angle | No hook | Two tails |
|---|---|---|---|---|---|---|---|---|---|
| Control | 77.00$^{a}$ ± 1.000 | 41.67$^{a}$±1.528 | 6.33$^{a}$±0.577 | 13.00$^{a}$±1.000 | 6.67$^{a}$±1.155 | 1.67$^{a}$±0.577 | 4.00$^{a}$±1.000 | 3.00$^{a}$±1.732 | 0.67$^{a}$±0.577 |
| 0.1 mg/kg/day | 78.67$^{a}$±1.528 | 43.67$^{b}$±0.577 | 7.67$^{b}$±0.577 | 12.33$^{a}$±0.577 | 6.67$^{a}$±0.577 | 0.33$^{a}$±0.577 | 3.67$^{a}$±0.577 | 4.00$^{a}$±1.00 | 0.33$^{a}$±0.577 |
| 0.2 mg/kg/day | 80.33$^{a}$±1.155 | 44.67$^{b}$±0.577 | 8.33$^{b}$±0.577 | 12.67$^{a}$±0.577 | 7.67$^{a}$±0.577 | 0.33$^{a}$±0.577 | 3.33$^{a}$±0.577 | 3.00$^{a}$±0.00 | 0.33$^{a}$±0.577 |

Means of different letters (A,B,C) in the same column are significantly different (P < 0.05), the column with the same letter is not significant. 50 metaphase cells were examined from each animal.

Table 3: The effect of fondaparinux on spermatocytes of male mice

| | Structural aberrations | | | | Numerical aberrations | | | |
|---|---|---|---|---|---|---|---|---|
| Treatments | x-y univalent | Autosomal Univalent | Fragments | Total structural Aberration | Hypo <20 | Hyper >20 | Poly-ploidy | Tot al numerical aberration |
| Control | 4.67$^{a}$±0.516 | 6.00$^{a}$±0.632 | 2.50$^{a}$±0.837 | 13.17$^{a}$±7.472 | 3.67$^{a}$±0.516 | 2.83$^{a}$±0.753 | 2.00$^{a}$±0.632 | 8.50$^{a}$±1.517 |
| 0.1 mg/kg/day | 5.00$^{a}$±1.00 | 7.00$^{a}$±0.00 | 2.67$^{a}$±0.577 | 14.67$^{a}$±0.577 | 4.00$^{a}$±0.00 | 3.33$^{a}$±0.577 | 2.00$^{a}$±0.00 | 9.00$^{a}$±1.00 |
| 0.2 mg/kg/day | 6.00$^{a}$±1.00 | 7.00$^{a}$±1.00 | 3.00$^{a}$±1.00 | 16.00$^{ab}$±1.00 | 4.67$^{b}$±0.577 | 3.00$^{a}$±0.00 | 2.33$^{a}$±0.577 | 10.00$^{a}$±0.00 |

Means of different letters (A,B,C) in the same column are significantly different (P < 0.05), the column with the same letter is not significant. 50 metaphase cells were examined from each animal.

Table 4: The effect of fondaparinux on bone marrow cells of male mice

| | Structural aberrations | | | | | Numerical aberrations | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatments | Chromatid gaps | Chromosomal gaps | Chromatid breaks | Deletions | Fragments | Endo-metosis | Centro- meric attenuation | T.S.A | <40 | >40 | Poly- ploidy | T.N.A. |
| Control | 5.00$^{a}$±1.000 | 3.67$^{a}$±0.577 | 4.33$^{a}$±0.577 | 4.67$^{a}$±0.577 | 4.67$^{a}$±0.577 | 6.33$^{a}$±0.577 | 5.33$^{a}$±0.577 | 34.00$^{a}$±0.00 | 7.00$^{a}$±0.00 | 5.00$^{a}$±0.00 | 3.00$^{a}$±0.00 | 15.00$^{a}$±0.00 |
| 0.1 mg/kg/day | 5.33$^{a}$±0.577 | 4.00$^{a}$±0.00 | 4.00$^{a}$±1.00 | 4.67$^{a}$±0.577 | 4.67$^{a}$±0.577 | 6.67$^{a}$±0.577 | 5.00$^{a}$±1.00 | 34.33$^{a}$±1.528 | 7.33$^{a}$±0.577 | 5.33$^{a}$±0.53 | 3.00$^{a}$±1.00 | 15.67$^{a}$±0.577 |
| 0.2 mg/kg/day | 5.67$^{a}$±0.577 | 4.33$^{a}$±0.577 | 4.00$^{a}$±0.00 | 5.00$^{a}$±1.00 | 5.00$^{a}$±0.00 | 6.67$^{a}$±0.577 | 5.33$^{a}$±0.577 | 36.00$^{a}$±0.00 | 8.00$^{a}$±0.00 | 6.00$^{a}$±1.00 | 3.33$^{a}$±1.153 | 17.33$^{a}$±3.215 |

Means of different letters (A,B,C) in the same column are significantly different (P < 0.05), the column with the same letter is not significant. 50 metaphase cells were examined from each animal.

Table 5: Visual score of DNA damage in mice exposed to fondaparinux

| | No. of cells | | Class | | | | |
|---|---|---|---|---|---|---|---|
| Treatments | Analyzed | Comet | 0 | 1 | 2 | 3 | DNA damaged % |
| Control | 100 | 12 | 88 | 9 | 2 | 1 | 12$^{a}$ |
| Low dose | | | | | | | |
| 0.1 mg/kg | 100 | 14 | 86 | 10 | 2 | 2 | 14$^{a}$ |
| High dose | | | | | | | |
| 0.2 mg/kg | 100 | 16 | 84 | 8 | 3 | 5 | 16$^{a}$ |

Class 0 = no tail; class 1 = Trail length > diameter 04 nucleus;
Class 2 = tail length between 1x and 2x t he diameter.
Of nucleus; and class 3 = tail length > 2x t he diameter of nucleus
% values within columns were significantly different (P < 0.05)

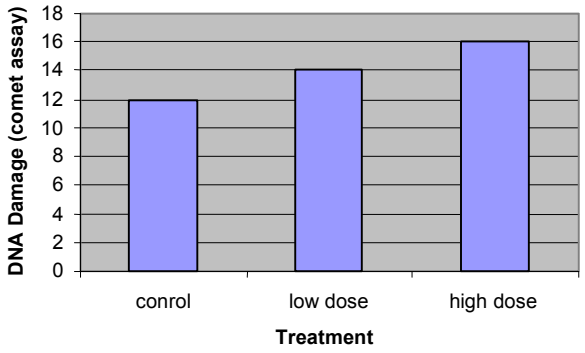



Fig. 1: DNA damage in liver cells of male mice treated with low and high dose of fondaparniux analyzed by comet assay. Results are expressed as mean + SEM of data from at least five samples, P < 0.05

**The Effect of Fondaparinux on DNA Damage in Male Mice (Comet Assay):** The data presented in Table (5) Fig. (1) showed that the treatment of male mice with fondaparinux for 21 days at dose levels of 0.1 and 0.2 mg/kg/day showed no significant increase in the percentage of DNA damage in the cells of male mice compared with the control group. Also there was no significant increase in the percentage of DNA damage between the two treated groups of fondaparinux. The distribution of DNA damaged in the two treated groups and control were as follows, the majority of the cells containing nucleus of class (1) tail and the other cells containing nucleus of class (2) and (3) tail. There was no indication that fondaparinux induced DNA damage in hepatocyte cells.

## DISCUSSION

Thrombosis is the formation of a blood clot, called a thrombus that blocks part or all of a blood vessel, such as a vein or an artery. There are many possible causes. In activity, such as sitting for long periods of time or resting in bed, is a major cause. Surgery, tumors and injuries to the leg also may cause thrombosis. Certain infections and cancers may alter the clothing substances in the blood and cause thrombosis. Women are especially at risk, because the female hormone estrogen is linked to thrombosis. The biggest danger is when a clot forms in

the large veins that are deep within the body. If the blood clot grows, it may break off. The clot may then travel toward and then through the heart and block the pulmonary artery, which is a major blood vessel, causing a pulmonary embolism. This serious complication of thrombosis may cause death if not treated rapidly and effectively. For people at high risk for developing thrombosis doctors sometimes recommend preventive measures such as the use of drugs that interfere with blood clotting that help keep blood from pooling in the deep veins.

Recently, fondaparinux a noval selective factor Xa inhibitor, has been evaluated for the preventation and treatment of venous and arterial thrombosis. Fondaparinux is a synthetic pentasaccharide that binds exclusively to the activation site of anti-thrombin, thereby increasing its activity toward factor Xa inactivation 300-fold [16].

In contrast to the other anti-thrombin-dependent anticoagulants, i.e. unfractionated heparin and low-molecular-weight heparin, fondaparinux selectively inactivates factors Xa without thrombin inhibition. Fondaparinux was superior to low-molecular-weight heparin in the preventation of venous thrombosis. In fact many studies reported the safety use of fondaparinux when administrated subcutaneously for 9 consecutive days, but in severe cases of disease the duration of the treatment extends up to 21 days [17].

So in our study we examined the cytogenetic effects of fondaparinux if administrated subcutaneously at a two recommended dose levels (low and high) for 21 consecutive days.

In the present study, the administration of adult male mice with fondaparinux for 21 consecutive days by subcutaneous injection has not produced any significant increase in the percentage of sperm head abnormalities and in the frequencies of chromosomal aberrations (somatic and germ cells) in the two tested doses (0.1 and 0.2 g/kg/day) as compared with the control, also, there was no significant differences between the two dose levels of fondaparinux.

However, positive results were obtained by Schumacher *et al*.[18] who found that the treatment with fondaparinux for 9 consecutive days with a dose equal to the recommended dose did not affect the fertility of male mice.

Also, positive results were obtained by Dongluzhang *et al*. [19] who reported that fondaparinux was not mutagenic in the Ames test and in the rat lymphocyte chromosomal aberration test.

Also, Bijsterveld *et al*. [20] reported that fondaparinux is safe when administrated in healthy male volunteers at subcutaneous dose of long for 9 connective days.

However, negative results were obtained by Turpie *et al*. [21] who stated that the administration of fondaparinux above the recommended doses caused a very serious blood clot around the brain and the spinal cord this clot cause long-term paralysis and may be caused a genetic spinal defect.

Also, negative results were obtained by Bates *et al*. [22] who found that the treatment with fondaparinux in the pregnant rats and rabbits during pregnancy with a dose up to 10mg/kg/day caused a fetal abnormalities to the embryos. Moreover, Alison and Lee Garvin [23] found that the treatment with fondaparinux in the patients their body weight less than 50kg may be subjected to sever side effects such as renal gastrointestinal disorders.Also, in our study, the administration of fondaparinux at two dose levels of 0.1 mg and 0.2 mg did not cause DNA damaged in the cells of male mice compared with the control and also this damage were not significant between the two tested groups.

These finding was agreement with Turpie [24] who found that, there was no damaged in the DNA synthesis test or in the rat micronucleus test when fondaparinux administrated up to 11 days.

## CONCLUSION

Our results indicated that fondaparinux has no cytotoxic effects on male albino mice and did not cause an increase in the frequencies of sperm abnormalities and chromosomal aberrations in germ and somatic cells and also did not cause any significant increase in the DNA damage.

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