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Genetic Toxicity of Silodosin and Prostacure on Male Mice and Their Embryos.

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ABSTRACT

Silodosin and prostacure are two novel medications used in the treatment of men who have symptoms of an enlarged prostate gland, which is also known as benign enlargement prostate (benign prostatic hyperplasia BPH). The mutagenic and the effects of silodosin and prostacure on the fertility of human and animals have not been adequately studied. Therefore, the present study was performed to evaluate the effects of silodosin and prostacure on sperm-head abnormalities and genetic effects including micronuclei, DNA damage, chromosomal aberrations and embryonic toxicity in the male mice and embryos. Adult male mice were administrated orally with different dose levels as follow, the first and the second groups were administrated orally with two dose levels (8 and 16 mg/kg/day) once daily at approximately equal to IX and 2X the therapeutic dose of silodosin, the third and fourth groups were administrated orally with two dose levels (100 and 200 mg/kg/day) once daily approximately equal to 1X and 2X the therapeutic dose of prostacure and the fifth group was considered as a control group and administrated orally with distilled water. After 21 days of the treatments treated males were mated with untreated females, on day 18 of gestation the pregnant females and treated males were sacrificed and examined for sperm-abnormality, genetic and embryonic abnormalities. It was observed that silodosin at the two dose levels (8 and 16 mg/kg/day) induced slight increases in the frequencies of sperm-abnormalities, micronuclei formation, DNA damage chromosomal aberrations and embryonic toxicity compared with the control group but these increases were not statistically significant. On the other hand, in the males treated with the two dose levels (100 and 200 mg/kg/day) of prostacure there were decreases in the frequencies of sperm head abnormalities, micronuclei formation, DNA damage, chromosomal aberrations and embryonic toxicity including (absorbed and dead embryos) but these decreases were statistically significant only in the males treated with the double dose (200 mg/kg/day) compared with the controls. Thus, from the above results we can say that silodosin in the two dose levels was unable to induce statistically significant increases in the sperm-abnormalities, micronuclei formation, DNA damage, chromosomal aberrations and embryonic toxicity over the controls so; it is considered to be safe. While, prostacure induced significant decreases under the control level in the frequencies of sperm head abnormalities, micronuclei formation, DNA-damage, chromosomal aberrations and embryonic toxicity. So, prostacure is considered to be very safe to the males and their embryos and can improve their fertility.

Keywords: Silodosin, prostacure, sperm-abnormality, micronuclei, DNA damage-embryonic toxicity-mice-embryos.

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INTRODUCTION

Benign prostatic hyperplasia (BPH), also called benign enlargement of the prostate, is a benign increase in size of the prostate.

BPH involves hyperplasia of prostatic stoma and epithelial cells, resulting in the formation of large, fairly discrete in the per urethral region of the prostate [1]. When sufficiently large, the nodules impinge on the urethra and increase resistance to flow of urine from the bladder. Resistance to urine flow requires the bladder to work harder during voiding, possibly leading to progressive hypertrophy, instability or weakness of the bladder muscle. Although prostate specific antigen levels these may be elevated in these patients because of increased in the organ volume and inflammation due to urinary tract infections, BPH does not lead to cancer but it can be a progressive disease, especially if left untreated results in stasis of bacteria in the bladder residue and an increased risk of urinary tract infection which lead to renal failure [2].

Most experts consider androgens (testosterone and related hormones) play an important role in the development of BPH, this means that the increase in the levels of androgens associated with the presences of BPH [3].

Di hydro testosterone (DHT), metabolite of testosterone, is a critical mediator of prostatic growth; DHT is synthesized in the prostate from circulating testosterone by the action of the enzyme Sα-reductase, type 2. This enzyme is localized in the stoma cells and epithelial cells, in both of these cell types, DHT binds to the nuclear androgen receptors and signals the transcription of growth factors that are mutagenic to the epithelial and stoma cells. The importance of DHT in causing nodular hyperplasia is supported by clinical observations in which therapy with a 5α-reductase inhibitor markedly reduces the DHT content of the prostate, in turn reduces prostate volume and in many cases, BPH symptoms [4].

In 2008, Yoga Gat *et al* [5], published evidence that BPH is caused by failure in the spermatic venous drainage system resulting in increased hydrostatic pressure and local testosterone levels elevated more than 100 fold above serum levels.

On a microscopic level, BPH can be seen in the vast majority of men as they age, in particular over the age of 70 years, around the world. However, rates of clinically significantly, symptomatic BPH vary dramatically depending on lifestyle. Men living in rural areas have very slow rates of BPH than men living in cities. It also seems that there is some connection between prostate cancer and BPH, as is demonstrated in 50-75% of men over 50 years.

BPH can be managed by change life style alterations include decreasing fluid intake before bedtime, moderating the consumption of alcohol and caffeine products, and following a timed voiding schedule.

Also BPH can be managed by using the two main medications 5α-reductase and alpha blockers inhibitors.

The 5 α-reeducates inhibitors are medications inhibit 5α-reductase enzyme, which in turn inhibits production of DHT, a hormone responsible for enlarging the prostate, their effects may take longer to appear than alpha blockers medications [6].

On the other hand, alpha blockers (technically α_1-adrenergic receptor antagonist), are the most common therapy used in the treatment of BPH. Alpha blockers relax smooth muscle in the prostate and the bladder neck, thus decreasing the blockage of urine flow, such as alfuzosin, tamsulosin and silodosin.

Rapallo contains the medical ingredient silodosin. Silodosin is highly selective antagonist of post-synaptic alpha-1-adrenoreceptors, which are located in the prostate, bladder base, bladder neck, prostatic capsule and prostatic urethra, silodosin is a newly medication use for the treatment of BPH [7]. Recently herbal medications were approved, for the treatment of signs and symptoms of benign prostate hyperplasia (BPH) and several and approved in the European countries and also available all over the world. Saw palmetto extract from serene ripens is one of the most commonly used and studied, having showed some promise in

early studies. Later trials of higher methodological quality have shown it to be no better than placebo in treatment of BPH.

Other herbal medications include pigeon Africana have shown to be more effective than the other herbal medications in the treatment of BPH and maintain healthy prostate, from these herbal medications is prostacure. Prostacure is a pure 100% natural medicine used for the treatment of benign prostate hyperplasia (BPH) or enlarged prostate gland. Prostacure appears to be a reasonable first line natural alternative for men with BPH. It contains pigeon Africana and doxazosin. Pigeon Africana contains phytosterols including docosanol. Docosanol has been found to reduce prolactin levels in the body. Prolactin increases the uptake of testosterone and increases the conversion of testosterone to DHT in the body which associated with both prostate enlargement and cancer, Pigeon has also been found to increase prostatic secretions and improve the composition of the seminal fluid.

Prostacure contains also doxazosin-through its adrenergic blockade-is effective in producing an additive relief of obstructive and irrigative symptoms of BPH [9].

At present no adequate studies are available that illustrates the genetic and embryo toxic effects of silodosin and prostacure. Therefore, the present study was undertaken to determine the genetic effects of silodosin and prostacure and their effects on the fertility of male mice and on embryos if female mice mated with male mice treated with the two medications.

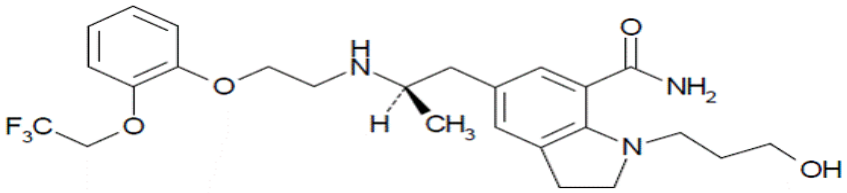
MATERIALS AND METHODS

Test drugs

Rapallo

Rapallo from Watson laboratories, Inc. Is the brand name for silodosin, a selective antagonist of alpha-1- adrenoreceptors is indicated for the treatment of benign prostatic hyperplasia (BPH).

The chemical name of silodosin is 1(3-Hydroxypropy L)-5-[(2R)-2([2[2-(2, 2, 2-trifluoroethoxy) peony] ethyl) amino) propel]] (2, 3-dihydro-1H-indole-7-carboxamide). The molecular formula is ($C_{25}H_{32}F_3N_8OH$). With a molecular weight of 495.53. The structural formula is:



Prostacure

Prostacure from October pharmacy natural substances indicated for the treatment of benign prostatic hyperplasia (BPH), maintain healthy prostate as well as promote comfort urinary men. Prostacure contains: pigeon Africana and doxazosin.

Animals and treatments

Dilutions of different concentrations prepared by dissolving the oral tablets of silodosin in ethyl alcohol and prostacure in distilled water.

Animals

Adult males and virgin females Swiss mice each weighting 25g served as experimental animals were divided into five groups (5 mice/group).

Males of the first group were administered orally with a single dose of silodosin (8 mg/kg/day) per day.

Males of the second groups were administered orally with a single dose of silodosin (16 mg/kg/day) per day.

Males of the third group were administered orally with a single dose of prostacure (100 mg/kg/day) per day.

Males of the fourth group were administered orally with a single dose of prostacure (200 mg/kg/day) per day.

Males of the fifth group served as a control group were administered with the same volume of distilled water the males were administered three weeks and after that the treated males were housed with untreated females by ratio 1:1 respectively per cage. The females which exhibited a vaginal plug this day was considered as day one of pregnancy.

The pregnant females were caged individually and on day 18 of gestation the treated males and the pregnant females were sacrificed by cervical dislocation for studying sperm head abnormalities, comet assay (DNA damage), micronuclei formation and chromosomal aberrations on males and their embryos.

Methods

Developmental toxicity

On day 18 of gestation, the pregnant females were sacrificed by cervical dislocation. The uterus contents were evaluated for the number of implantation sites, absorbed, dead and live embryos.

Sperm head abnormality assay

The treated males were sacrificed by decapitation; the caudal epididymis was removed and placed in physiological saline. It was minced into pieces with scissors and then left undisturbed for 20 minute for the diffusion of spermatozoa. The spermatozoa were spread-on microscopic slides, air-dried, fixed in absolute methanol for 15 minute and stained with 1% aqueous eosin-y on the following day. Five hundred sperms from each animal were examined for the abnormalities in sperm head shapes following the method recommended by Wyrobek and Bruce 1975 [9].

Comet assay for DNA strand break determination

Liver tissues collect from males and their embryos were isolated by homogenization and centrifugation (15 min, 280g) in a density gradient of Grad sol L (aqua medical, Lodz) Poland's. The concentration of the cells was adjusted to (1-3) x 10^5 cells/ml by adding RPM 1640 without glutamine to the single cell suspension. A freshly prepared suspension of cells in 0.75% low melting point agars (sigma chemicals) dissolved in phosphate buffer saline (PBS; sigma chemicals), was cast onto, microscope slides are coated with 0.5% normal melting agars. The cells then lyses for 1h at 4°C in a buffer consisting of 2.5M NaCl, 100mM EDTA, 1% Triton X-100, 10mMtris, pH10. After the lyses, DNA was allowed to unwind for 40 min in electrophoresis solution consisting of 300 mom NaOH 1mM EDTA, PH>13. Electrophoresis was conducted at 4°C for 30 min at electric field strength 0.73v/Cm (30mA). The slides were then neutralized with 0.4M tries, pH 7.5 stained with 2 us/ml ethidium bromide (sigma chemicals) and covered with cover slips. The slides were examined at 200 x magnification fluorescence microscope (Nikon Tokyo, Japan) to a CoHU4910 video camera (Chou, Inc, San Diego, CA, USA) equipped with a UV filter block consists an excitation filter (359nm.), and barrier filter (461nm) and connected to a computer based images analysis system, Lucia-comet V-4.51. Hundred images were randomly selected from each sample and the comet tail DNA was measured [10]. Endogenous DNA damage measured as the mean. Comet tail DNA of liver tissues of male and embryo groups. The number of cells scored for each animal was 100 [10].

Micronucleus assay

In male mice

The male mice were sacrificed by cervical dislocation after 3 weeks from the last treatment. For each treatment 5 animals were used in different groups. Both the femora were removed and the bone marrow was flushed out into a centrifuge tube with 1% sodium citrate solution (20°C) from a syringe –pupating and collected by centrifugation at 1000 rpm for 5min a 4°C. The cell pellet was re suspended in a small volume of 5% fetal calf serum. A drop of this suspension was smeared on a clean slide, air-dried fixed in absolute methanol for 25 min and stained the following day with Gyms stain. One thousand each of polychromatic erythrocytes and norm chromatic erythrocytes were analyzed for the presence of micronuclei [11].

In embryos

Liver tissues of embryos re suspended in a small volume of fetal calf serum on a glass slide were used for smear preparations the smear of liver cells was prepared from each embryo. After air-drying the slides were fixed in methyl alcohol for 10min and stained with 5% Gyms stain for 10 min. Three slides were prepared for each animal and were coded before observation and one was selected for scoring. From each slide coded, 1000 polychromatic erythrocytes (PCEs) were scored for the presence of micronuclei under oil immersion at power magnification. In addition, the percentage of micro nucleated polychromatic erythrocytes (% Man PCES) was calculated on the basis of the ratio of Mince, to PCEs [12].

Chromosomal aberrations assay in embryos

Chromosomal aberrations from embryonic cells were prepared according to Romagna no *et al* 1985 [13]. Embryos were collected from each group and placed in 5mL T.C.M. 199 Media, 12mL of 0.05 colchicines was added for each tube and incubated at 37°C for 90 minutes, then an amount of 5mL of hypotonic solution of 0.56% HCl was added to the pellet and the cells were incubated at 37°C for 20 minutes. 5mL of fresh fixative (3 methyl alcohol: 1 glacial acetic acid) were added to the cells. After that two or three drops of the cells were dropped on the surface of clans slides, air dried and stained with 5% Gyms stain and examined for chromosomal aberrations. 50 metaphase spreads were examined for each embryo scoring the different types of chromosomal abnormalities.

Statistical analysis

The experiment of sperm-head abnormalities, micronuclei and chromosomal aberrations followed complete randomized design. The obtained data were subjected to analysis of variance (ANOVA) according to seducer and Cochran 1990[14]. Duncan's multiple range test were used to compare between means of treatments according to Walter and Duncan 1969[15] at probability 5%.

The frequencies of comet assay in males and embryos between experimental and control values were calculated as percentage.

The incidences of absorbed, dead and live embryos between experimental and control values were calculated non- parametrically using Wilcoxon's rank sum test (Siegel, 1956) [16].

RESULTS

Developmental toxicity

The percentage of living, dead and absorbed embryos resulted from untreated females mated with treated males with the therapeutic and double doses of silodosin and prostacure for 21 consecutive days are summarized in Table (1).

In the groups of embryos resulted from males treated with therapeutic and double doses (8 and 10 mg/kg/days) of silodosin there were a slight increase in the percentage of absorbed and dead embryos and a

slight decrease in the percentage of live embryos in the two groups of silodosin but these treatment effects close to the limit of control group.

On the other hand in the groups of embryos resulted from treated males with the therapeutic dose and double dose (100 and 200 mg/kg/day) of prostacure there were decreases in the percentage of dead and absorbed embryos and increases in the percentage of live embryos in the two groups but these treatment effects were more frequent in the group of embryos resulted from males treated with double dose (200 mg/kg/day) compared with the control.

Sperm head abnormality

Means ± S.D. values and the results are given in table (2). Various forms of sperm heads, i.e. banana shaped, triangular, amorphous, dwarf etc were recognized in all the treated groups and the control. Analysis of these abnormal sperm showed that overall amorphous types triangular and dwarf heads were more prevalent in different groups than the other types of abnormalities and the other all types occurred with different frequencies in both treated and control groups.

The results showed that the treatment of male mice with the two doses (8 and 16 mg/kg/day) of silodosin for 21 consecutive days resulted increases in the sperm abnormalities but these increases were not significant compared with the control. While the treatment of male mice with two doses (100 and 200 mg/kg/day) of prostacure resulted decreases in the total number of sperm abnormalities and these decreases were not significant and in the limit of control group in the group treated with (100 mg/kg/day) and these decreases were significantly different in the group of males treated with (200 mg/kg/day) compared with the control.

DNA strand break determination using comet assay

In male mice

Results of the comet assay for DNA strand break in individual liver cells of male mice administrated with therapeutic and double doses of silodosin and prostacure for 21 consecutive days are summarized in table (3). The results showed that in comparison to the control male mice DNA damaged cells were observed in the cells of male mice administrated with the therapeutic and double dose of silodosin in a dose-dependent manner but these increases were not significant.

Also, in the group of male mice administrated with the therapeutic dose of prostacure (100 mg/kg/day) DNA damaged cells were observed but this damage in the limit of control group.

While in the group of male mice administrated with double (200 mg/kg/day) dose of prostacure there were significant decreases in the damaged cells compared with the other groups and with the control group.

In the embryos

Results of the comet assay for DNA strand break in individual liver cells of embryos resulted from treated males for 21 consecutive days are summarized in table (4). The results showed that in comparison to the control group DNA damaged cells were observed in the two groups of embryos resulted from treated males with the dose and double therapeutic dose of silodosin (8 and 16 mg/kg/day) but this damaged is not significant.

Also, in the embryos resulted from males treated with therapeutic dose (100 mg/kg/day) of prostacure there were DNA damaged cells but this damage was not significant and within the control limit while, in the group of embryos resulted from males treated with (200 mg/kg/day) of prostacure there were significant decreased in the DNA damaged cells compared with the other groups and with the control.

Additionally, the DNA damaged cells categorized in class 2 defined with medium tail and class 3 defined with long tail of damage were higher in males and embryos treated with therapeutic and double

therapeutic dose (8 and 16 mg/kg/day) of silodosin compared with prostacure groups and compared with the control.

Micronucleus (MN) formation

In male mice

the effect of oral treatments of silodosin and prostacure with the two tested doses for 21 consecutive days on Man PCEs formation in the bone marrow cells of male mice is summarized in table (5) Fig (1). In comparison to the controls, the frequency of micro-nucleated in the bone marrow cells of males treated with the two doses of silodosin (8 and 16 mg/kg/day) slightly increased as compared with the controls but these increases were not significant. On the other hand the treatments with the two dose levels of prostacure (100 and 200 mg/kg/day) induced a decrease in the frequency of micro-nucleated cells in the bone marrow cells of male mice but these decreases were significantly in the male treated with the double dose of prostacure (200 mg/kg/day) as compared with the controls.

In the embryos

The effect of oral treatments of silodosin and prostacure with the two doses for 21 consecutive days on Minces formation in embryos is summarized n table (6) fig (2).

The treatments of males with the two tested doses of silodosin induced increases in the micro nucleated cells of resulted embryos in a dose-dependent manner but these increases were not significant compared with the control.

While, In comparison to the controls, the frequency of micro nucleated cells in the embryos treated with the two doses of prostacure (100 and 200 mg/kg/day) were decreased significantly as compared to the controls and to the other treated groups and these decreases were more significant in the group of embryos treated with (200 mg/kg/day).

Chromosomal aberration in embryos

Table (7) presents the results of chromosomal aberrations analysis in embryonic cells 18 day resulted from untreated females mated with males treated with therapeutic and double the therapeutic dose of silodosin and prostacure for 21 consecutive days. The data showed that embryos resulted from the therapeutic and double therapeutic dose of silodosin both induced slight increases in the total number of structural and numerical aberrations but these increases were not significant compared with the control group. On the other hand the embryos resulted from males treated with the therapeutic and double doses of prostacure induced decreases in the total number of structural and numerical aberrations and these decreases were significant in the double dose of (200 mg/kg/day) prostacure compared with the therapeutic dose of prostacure and compared with the control

Table 1: The effects of silodosin and prostacure on the development of embryos from treated males on day 18 of gestations:

Treatment groups	Control	Silodosin		Prostacure	
		8 mg/kg/day	16 mg/kg/day	100 mg/kg/day	200 mg/kg/day
Females on study	5	5	5	5	5
Pregnant females	5	4	4	5	5
Fertility index %	100%	80%	80%	100%	100%
Total no of implantations	42	40	40	42	44
Total no of absorption	1	1	2	0	0
Total no of live	39	37	36	40	42
%	92.8	92.5	90	95	95.5
Total no of dead	2	2	2	2	2
%	4.76	5	5	4.76	4.54

Numbers given are the absolute numbers of embryos in that group with the indicated abnormality.

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Table 2: Incidence of sperm head abnormality in male mice

Treatment	Abnormal sperms	Amorphous	Banana	No-hook	Triangular	Dwarf	Double headed
Control	74.00^{b} ± 0.577	29.00^{b} ± 0.577	5.00^{b} ± 0.000	10.00^{b} ± 0.00	15.00^{c} ± 0.000	15.000^{b} ± 0.000	0.00^{a} ± 0.000
Silodosin 8mg/kg/day	76.33^{b} ± 0.662	3.100^{b} ± 0.00	6.00^{c} ± 0.000	10.67^{b} ± 0.333	15.00^{c} ± 0.000	14.000^{c} ± 0.000	0.33^{a} ± 0.333
Silodosin 16mg/kg/day	77.67^{b} ± 0.882	31.33 ± 0.333	7.67^{d} ± 0.333	11.00^{b} ± 0.577	14.00^{b} ± 0.000	13.33^{d} ± 0.333	0.33^{a} ± 0.333
Posta cure 100 mg/kg/day	75.00^{b} ± 0.577	29.67^{a} ± 0.333	7.00^{d} ± 0.000	10.67^{b} ± 0.333	14.67^{bc} ± 0.333	22.67^{a} ± 0.333	0.33^{a} ± 0.333
Posta cure 200 mg/kg/day	71.00^{b} ± 0.577	18.00^{a} ± 0.577	6.33^{b} ± 0.333	10.00^{b} ± 0.577	14.00^{b} ± 0.577	12.67^{a} ± 0.333	0.00^{a} ± 0.000

Means ± S.D. the different letters (a, b, c, d) are significant at $P < 0.05$.

Table 3: Visual score of DNA damage in male mice treated with silodosin and prostacure

Treatment	No of cells		Class of comet				DNA damaged cells %
	Analyzed	Total comet	0	1	2	3	
Control	100	16	84	14	2	0	16^{a}
Silodosin 8mg/kg/day	100	17	83	13	3	1	17^{a}
Silodosin 16mg/kg/day	100	19	81	12	4	3	19^{a}
Prostacure 100 mg/kg/day	100	15	85	14	1	0	15^{a}
Prostacure 200 mg/kg/day	100	10	90	10	0	0	10^{b}

Class 0 = no tail; 1= tail length < diameter of nucleus, 2 = tail length between 1X and 2X diameter of nucleus, and 3= tail length > 2 X the diameter of nucleus. Means are significant at $P < 0.05$.

Table 4: Visual of DNA damage in embryos at day (18) of gestation resulted from treated males with silodosin a prostacure

Treatment	No of cells		Class of comet				DNA damaged cells
	Analyzed	Total comet	0	1	2	3	
Control	100	12	88	9	2	0	12^{a}
Silodosin 8mg/kg/day	100	13	87	8	3	2	13^{a}
Silodosin 16mg/kg/day	100	15	85	8	4	3	16^{a}
Prostacure 100 mg/kg/day	100	11	89	10	1	0	11^{a}
Prostacure 200 mg/kg/day	100	9	91	9	0	0	9^{b}

Class 0 = no tail; 1= tail length < diameter of nucleus, 2 = tail length between 1X and 2X diameter of nucleus, and 3= tail length > 2 X the diameter of nucleus. Means are significant at $P < 0.05$.

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Table 5: Results of micronucleus test in male bone marrow cells treated with silodosin and prostacure.

Treatment groups	Mean number of ± S.D. MN
Control	20.33^{b}±0.577
Silodosin 8mg/kg/day	21.00^{bc}±1.00
Silodosin 16mg/kg/day	22.00^{bc}±1.00
Prostacure 100 mg/kg/day	19.67^{b}±0.577
Prostacure 200 mg/kg/day	18.00^{a}±1.000

Means of different letters (a, b, c, d) are significant at P < 0.05

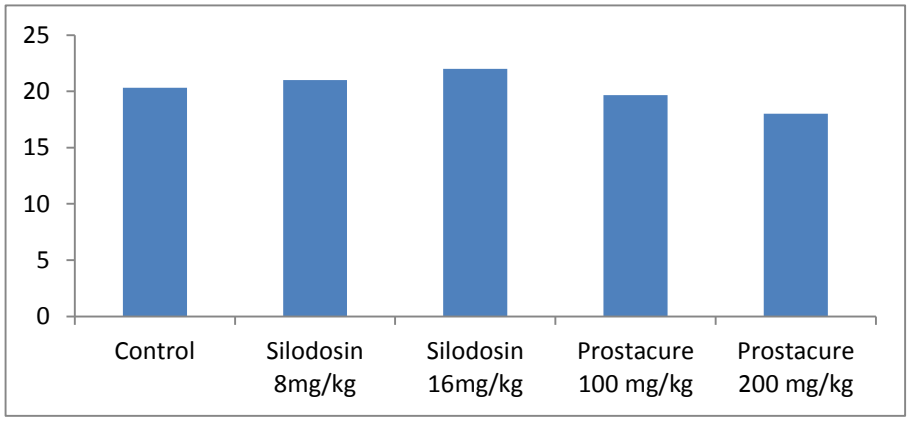

Figure 1: Micro nucleated poly chromatic erythrocytes (Minces) of male mice treated with silodosin and prostacure.

Table 6: Results of micronucleus test in embryos resulted from treated male mice with silodosin and prostacure.

Treatment groups	Mean number of ± S.D. MN
Control	14.00^{b}±1.000
Silodosin 8mg/kg/day	14.67^{b}±1.000
Silodosin 16mg/kg/day	15.00^{b}±1.00
Prostacure 100 mg/kg/day	13.67^{b}±1.000
Prostacure 200 mg/kg/day	11.67^{a}±0.577

Means of different letters (a, b, c, d) are significant at P < 0.05

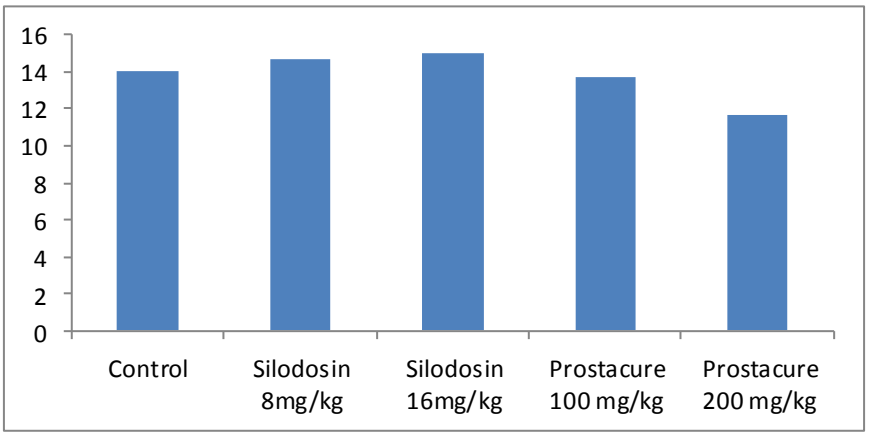

Figure 2: Micro nucleated poly chromatic erythrocytes (Minces) of embryos resulted from treated males with silodosin and prostacure.

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Table 7: Effect of oral administration of male mice with silodosin and prostacure on embryonic cells.

	Chromatic gaps	Chromosomal gaps	Breaks	Deletion	Fragments	Endometosis	Centromeric attenuation	T.S.A	<40	>40	Poly policy	T.N.A.
Control	5.67^{b} ± 0.333	2.67^{b} ± 0.333	1.67^{b} ± 0.333	1.67^{b} ± 0.333	2.33^{b} ± 3.333	2.33^{b} ± 0.332	2.67^{b} ± 0.333	19.00^{b} ± 0.577	3.00^{b} ± 0.00	1.77^{b} ± 0.333	0.00^{b} ± 0.00	4.67^{b} ± 0.333
Silodosin 8mg/kg	6.00^{b} ± 0.77	2.33^{b} ± 0.337	1.33^{b} ± 0.33	2.00^{b} ± 0.000	2.67^{b} ± 0.333	3.00^{b} ± 0.000	3.00^{b} ± 0.000	20.00^{cb} ± 0.333	3.00^{b} ± 0.577	2.33^{b} ± 0.337	0.00^{b} ± 0.00	5.33^{b} ± 0.333
Silodosin 16mg/kg	6.33^{b} ± 0.33	3.00^{b} ± 0.000	2.00^{b} ± 0.00	2.00^{b} ± 0.577	1.67^{b} ± 0.333	3.000^{b} ± 0.57	3.00^{b} ± 0.577	21.00^{cb} ± 0.000	3.33^{b} ± 0.666	2.67^{b} ± 0.33	0.00^{b} ± 0.00	6.00^{b} ± 0.577
Prostacure 100 mg/kg	6.00^{b} ± 0.000	2.00^{abs} ± 0.000	1.7^{abs} ± 0.570	1.67^{b} ± 0.333	3.00^{b} ± 0.00	2.00^{a} ± 0.00	1.67^{a} ± 0.333	18.00^{b} 0.577	2.67^{b} ± 0.333	1.67^{a} ± 0.333	0.00^{a} ± 0.00	4.33^{b} ± 0.333
Prostacure 200 mg/kg	4.67^{b} ± 0.333	1.33^{a} ± 0.577	1.00^{a} ± 1.000	0.67^{a} ± 0.333	2.67^{ab} ± 0.335	3.33^{b} ± 0.333	2.000^{ab} ± 0.000	15.57^{a} ± 0.667	1.67^{a} ± 0.667	1.67^{a} ± 0.333	0.00^{a} ± 0.00	3.33^{a} ± 0.333

Means of different letters (a, b, c, d) in the some column are significant different ($P < 0.05$).

DISCUSSION

The prostate gland forms part of the male reproductive system. It is a gland responsible for male sexual function and reproduction. In many men, the prostate gland may continue to grow when they are in their 40S. This continued growth of the prostate is the disease referred to as benign prostatic Hyperplasia (BPH), also known as enlarged prostate. Studies showed that 50% of the men over the age of 40 have an enlarged prostate. As the prostate grows, it puts pressure on the urethra- the tube that carries urine and semen. This increasing pressure on the urethra may cause bother some urinary symptoms and in rare cases may even lead to prostate surgery. BPH may develop from the growth of cells from the relatively increased levels of estrogen that occur in men as they age. Another line of thought states that the testosterone-derivative DHT is involved with the increased cellular growth associated with BPH [17].

The two main medications for the treatment of BPH are alpha-blockers and 5α-reducats inhibitors. Alpha-blockers are the most common choice for the treatment of BPH.

The newest alpha blockers used for the treatment of BPH is silodosin. In vitro studies have shown that silodosin is highly selective for α_{1A}-adrenoreceptors are primary located in the human prostate, bladder base, and bladder neck, prostatic capsule and prostatic urethra. Silodosin blockade the α_{1A}-adrenoreceptors cause's smooth muscle in these tissues to relax, thus decreasing bladder outlet resistance and reducing the symptoms of BPH [18].

Other medications which used in the treatment of BPH are herbal medications such as prostacure appears to be a reasonable first line natural alternative for men with BPH. Prostacure contains pigeon Africana and doxazosin this combination act synergistically in counteracting the biochemical, morphological and functional complex associated with BPH [19].

In fact the safety use of silodosin and prostacure, the mutagenic and their effects on fertility has not been adequately studied.

The present study was carried out in order to evaluate the mutagenic and embryo toxic effects of silodosin and prostacure in adult male mice and their embryos.

In the present study, the administrating of the adult male mice with silodosin for 21 consecutive days with doses (8 and 16 mg/kg/day) by oral injection has produced increases in the number of absorbed and dead embryos and decreases in the number of live embryos and in the fertility index and these results were more frequent in the double dose (16 mg/kg/day) compared with the control. However, negative results were

obtained by Villa *et al*, [20] who found that silodosin did not affect the fertility and mating rates in male and female rats at doses of 20 mg/kg/day. Also Muto *et al*, [21] reported that silodosin was not teratogenic in rats at doses up to (1000 mg/kg/day and in rabbits at doses up to 60 mg/kg/day.

Also, in the present study, the oral administration of silodosin in doses (8 mg and 16 mg/kg/day) produced increase in the number of sperm head abnormalities but this increase is not significant compared with the control. However, positive results were obtained by Von derhaar [22] who found that the treatment with silodosin leads to a decrease in the amount of semen releases that may temporally affect male fertility and this effect disappeared after discontinuation of silodosin. So, silodosin may be affecting the male fertility.

Also, in the current study, we have found that silodosin did not induce significant DNA damage or increase in the micronucleus formation with the two dose levels and also did not induce significant chromosomal aberrations in the embryonic cells compared with the control.

These results are agreement with Muller and Kasper [23] who reported that silodosin did not appear to be geotaxis in both in vitro and in vivo genotoxicity assays and also did not appear to be mutagenic nor did it induce chromosomal aberrations under conditions tested.

But negative results were obtained by Galloway [24] who found that in mice silodosin was carcinogenic in females at doses of 150 mg/kg/day; however, this is likely due to the increased in the prolactin levels.

In the present study the administration of prostacure in the dose level of (100 mg/kg/day) did not affect the number of absorbed, dead and live embryos and also did not affect the fertility index and all these numbers in the limit of control. On the other hand the administration of prostacure to the adult male mice in a dose double the therapeutic dose (200 mg/kg/day) caused a decrease in the number of dead and absorbed embryos and increase in the fertility index over the control group.

However, positive results were obtained by Caroni *et al*, [25] who found that pigeon Africana has been found to increase prostatic secretions and improve the composition of seminal fluid.

In the present study the oral treatment with prostacure in adult male with the dose (100 mg/kg/day) did not induce a significant increase in the sperm head abnormalities and the number still in the limit of control group but the treatment with a double dose (200 mg/kg/day) caused a significant decrease in the total number of sperm head abnormalities compared with the control.

This result is agreement with Caroni *et al*, [25] who found that in the normal and treated animal's pigeon has been proven effects on the fertility and prostate secretion and the typical dose is 100-200 mg/kg/day for the treatment of BPH.

Also, positive results were reported by Marconi [26] who found that in animals studies pigeon showed an increase in the volume and viability of sperm in the semen.

However, negative results were reported by Latalski *et al*, [27] who found that pigeon may be has a harmful effect to the pregnant female and embryos and should not be taken during pregnancy.

Also, negative results were obtained by Yoblonsky *et al*, [28] who found that pigeon extract (80 mg/kg/day) had no effect on the fertility of male rats and rabbits.

In the present study, we have found that prostacure did not induce DNA damage or micronucleus formation and also did not induce chromosomal aberrations in a dose (100 mg/kg/day) while, in a dose equal to the double therapeutic dose (200 mg/kg/day) prostacure induce significant decreases in the percentage of DNA damage liver cells and in the number of micro nucleated cells of male mice and also significant decreases in the number of chromosomal aberrations in the embryonic cells compared with the control.

These results are agreement with Androl and Riff and [29] who found that in vitro and in vivo studies an absence of mutagenic and clastogenic effects of prostacure (pigeon),

Also, positive and negative results were obtained by Gathumbi *et al*,[30] who found that chloroform extract which found in pigeon did not cause toxicity in rats at oral doses up to (1g/kg/day), however the extract caused marked clinical signs, organ damage and 50% mortality rate at 3.3g/kg/day for 6 days.

However, negative results were reported by Isanti *et al*, [31] who found that in the human trials, a low incidence of toxicity has been demonstrated.

Also, negative results by Gathumbi *et al*, [32] who found that pigeon was administrated repeatedly at doses of (10, 100, 1000 mg/kg/day) to rats caused rises in plasma Alanine amino transferees and creative kinas.

CONCLUSION

In conclusion our results indicated that silodosin in the two dose levels cause a slight increase in the total number of dead and absorbed embryos and also a slight increase in the micro nucleated cells, chromosomal aberrations and DNA damage in both treated males and embryos but these increases did not significant compared with the control.

So, silodosin had no mutagenic or cytotoxic effects in both treated males and their embryos. Also in the present study we found that the treatment of male mice with prostacure (pigeon Africana) with the therapeutic dose induce decreases in the frequencies of embryonic toxicity, in the sperm head abnormalities, in the DNA damage (comet assay), in the number of micro nucleated cells and in the chromosomal aberrations of embryonic cells but these decreases did not significant and in the limit of the control group.

On the other hand the treatment of male mice with a double dose of prostacure induce a significant increase in the male fertility and a significant decrease in the dead and absorbed embryos, in the sperm abnormalities, in the DNA damage and in the mice micro nucleated cells compared with the control.

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