

Cytogenetic and Biochemical effects of Metenix on Albino Male Mice

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ABSTRACT: Background: Metenix (metolazone) is a new antihypertensive, it is effective in the treatment of patients with high blood pressure, heart failure and edema. **The aim of the study** is to evaluate the cytogenetic and biochemical effects of metenix. **Materials and methods:** Study sample (Male adult albino mice) was divided into two major groups; treated group and matched control. Treated group administered orally doses of (0.01, 0.02) and 0.04mg (kg) of metenix for 10 consecutive days. Control did not receive any treatment. All treated groups and control were sacrificed 24h after the last treatment the incidences of sperm head abnormalities and chromosomal aberrations in (bone marrow and spermatocytes, were determined in all treated groups. **Results** showed that (metenix) caused a significant increase in the frequencies of abnormal sperms and chromosomal aberrations in (somatic and germ cells, in all treated groups compared with control and these increases were dose dependent. The biochemical analysis (nucleic acids and total protein) were examined in the livers of treated animals. The results showed that there was significant decrease in the nucleic acids and total protein content in the livers of treated animals compared with control; these decreases were dose dependent. The data obtained in this study suggested that (metenix) should be used under medical control due to its cytogenetic and biochemical toxic effects on male albino mice. [New York Science Journal 2010; 3(6):127-132]. (ISSN 1554 – 0200).

Keywords: metenix, chromosomal analysis, sperm head abnormalities, DNA, RNA, total protein, male mice.

1-Introduction

Hypertension is a common disorder of the circulatory system, affecting around one in seven adult and becoming more common with age.

Blood pressure is the amount of force exerted on the artery walls by the pumping blood. High blood pressure means that the blood is pumping with more force than normal through the arteries, this added stress on the arteries caused many illnesses, such as heart attack **Spiendiani and Condo,(2006)** and stroke ,in addition, over time, may cause damage to many organs in the body such as, kidneys (**Zuccala et al., 2005**), brain (**Dong et al., 2009**), eyes, **Ling and Sun, (2008)** and liver (**Dursun, et al., 2010**).

Hypertension can be classified as essential (primary) and secondary, about 90 to 95% of hypertension cases called primary which have no known cause and may be influenced by genetic factor. On the other hand, secondary hypertension is hypertension that is caused by an underlying medical condition such as kidney disease, thyroid dysfunction and adrenal gland disorder.

Secondary hypertension is much less common than essential hypertension and tends to develop more rapidly than primary hypertension. The most common way for treating hypertension is administering antihypertensive drugs such as diuretics. (**Puschett, 2000; Supuran, 2008**). Metolazone (metenix) is known as athiazide diuretic drug (**Sica, 2003**) that act by increasing the removal of salts (potassium and sodium), from the blood, these salts draw water out of the blood causing decrease in fluid volume circulating through the blood vessels resulting in a drop of the fluid pressure which helps to lowering high blood pressure. (**Supuran, 2008; Pridjian, et al., 2006**).

The safety use of metolazone (metenix) has not been adequately studied. So, in the present study we examined the cytogenetics and biochemical effects of (metenix) metolazone on male mice if given orally for 10 consecutive days.

2-Materials and Methods

2.1. Materials:

2.1.1. Test drug:

A Metolazone (metenix) tablet (provided by Aventis pharmaceutical company) is considered as athiazide-like diuretic which is used for the treatment of acute and chronic hypertension. Metenix tablets for oral administration contain 5mg metolazone, the molecular formula is (C₁₆H₁₆ClN₃O₃S), the molecular weight is 365.83454 (g/mol). It is very soluble in water. The recommended initial dose in mild and moderate hypertension is 5 mg/daily

2.1.2. Treatments:

Different concentrations were prepared by dissolving the tablets in distilled water. Metenix were administered orally at doses of (0.01, 0.02 and 0.04) mg/kg/day once daily for 10 consecutive days for albino mice (30 gm), according to **pagat and Barnes. (1964)**.

2.1.3. Animals:

Adult males Swiss albino mice each weighting (30 g) 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 *ad libitum*. Mice were divided into two major groups; (control) and

treated groups. The treated groups were redivided into three groups as following. Animals of the first group were administered orally with a single dose (0.01 mg/kg/day) of (metenix) once daily.

Animals of the second group were administered orally with a single dose (0.02 mg/kg/day) of (metenix) once daily.

Animals of the third group were administered orally with a single dose (0.04 mg/kg/day) of (metenix) once daily.

Control group administered distilled water orally. After administering the drug orally for (10) consecutive days, they were scarified after one day from the last treatment .Perform cytogenetic analysis (sperm head abnormalities, and chromosomal aberrations in bone marrow cells and spermatocyte) and biochemical analysis include (DNA, RNA and total protein) in the animals liver.

2.2.Methods

2.2.1. Cytogenetic analysis:

2.2.1.1. Sperm head abnormality:

The treated males were sacrificed by decapitation. The Cauda epididymis was removed and placed in physiological saline (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 on microscopic slides, air-dried fixed in absolute methanol for 15 minute and stained with 1% aqueous eosin-y on the following day. Five hundreds sperms from each animal were examined for the abnormalities in sperm head shapes following the method recommended by **Wyrobeck and Bruce. (1975)**.

2.2.1.2. Spermatocytes Assay:

Spermatocytes were obtained through cell separation according to **Brewen and Preston, (1978)**. In summary, both mice testis were de capsulated, after dissociation tubules were filtered through a nylon mesh with phosphate buffer then the cells were centrifuged at 1000r.p.m. for 5 min and supernatants were decanted. The pellet was re-suspended and washed twice with 1ml of sodium citrate solution (2.2%), added drop by drop to the suspension, followed by centrifugation for 5min at 1000r.p.m. Subsequently, cells were left in 1ml hypotonic solution (1.1% sodium citrate) for 12 min. Cells were then fixed twice in 1ml of 3:1 methanol and glacial acetic acid. Fixed cells were dropped on slides pre-warmed at 45°C. Slides were \pm per animal, for each treatment group, were scored to identify the different aberrations.

2.2.1.3. Bone marrow assay:

Chromosome preparations made by the method of (**Agarwal el a., 1994**) mice were injected with colchicine (2.5 mg/kg/b.w.p) 3 hours prior animals were scarified by cervical dislocation. The bone marrow cells were aspirated in

phosphate buffer (pH 7.2) and centrifuged at 1000r.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 animal, scoring the different types of chromosomal abnormalities.

2.2.2. Biochemical analysis:

After one day from the last treatment, mice were sacrificed and the liver of each animal, fresh or frozen were taken, blotted using filter paper, weighted and homogenized in 0.9% sodium chloride solution for 5 minutes at 0°C for the determination of nucleic acids and proteins. Nucleic acids were extracted from liver homogenates by using trichloroacetic acid (TCA) and ethanol according to the methods of **Schneider, (1945)** and **Sambrook et al., (1989)**, respectively. Deoxy-ribonucleic acid, ribonucleic acid and total proteins were determined according to the methods of **Burton, (1956)**, **Khafagy et al., (1980)** and **Lowry et al., (1951)**, respectively.

2.2.3. 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, (1990)**. Least significant differences were used to compare between means according to **Waller and Duncan. (1969)** at probability 5%.

The data of biochemical analysis (DNA, RNA and total protein) was carried out using the student's test according to the method described by **Murray, (1982)**.

3. Results

Means \pm S.D. values and the results of sperm head abnormality are given in Table (1) and Fig. (1). Various forms of sperm heads i.e. banana shaped, dwarf. Amorphous, hooked, etc was recognized in all treated groups of metenix. Analysis of these abnormal sperm showed that amorphous head, dwarf, No hook, were more frequent than banana shaped, two tails and Hook at wrong angle.

Our results showed that the treatment of male mice with doses 0.01, 0.02 and 0.04 mg/kg/day of metenix for 10 consecutive days resulted a significant increase in sperm head abnormalities (abnormal sperms) compared with control group and this increase was dose dependent.

The total number of abnormal sperms in the (0.04, 0.02 and 0.01 mg/kg/day) metenix groups, were (149, 123 and 92) respectively compared with control (65, 67).

Table (1): The effect of oral administration of metenix on sperm head abnormalities in male mice.

Treatments	Sperm head abnormalities						
	Abnormal sperms	Amorphous head	Banana shaped head	Dwarf	No hook	Hook at wrong angle	Two tails
Control	65.67d ± 3.06	43.00 c ± 1.00	4.00 b ± 1.00	11.00 b ± 1.00	5.33 c ± 0.58	2.00 b ± 1.00	0.00 b ± 0.00
Low 0.01 mg/kg	92.33 c ± 2.52	52.33 b ± 4.04	9.00 a ± 1.00	21.00 c ± 1.00	10.33 b ± 1.53	5.33 b ± 1.53	1.00 b ± 1.00
Medium 0.02 mg/kg	123.00 b ± 1.73	69.67 a 1.53	9.67 a ± 1.15	27.67 b ± 1.15	10.33 ± 1.53	4.00 b ± 1.00	1.67 ab ± 1.53
High 0.04 mg/kg	149.00 a ± 1.00	74.00 a 2.00	10.00 a ± 2.00	33.00 a ± 2.00	14.67 a ± 1.15	13.33 a ± 2.89	4.00a ± 1.73
LSD at α 0.05 level	3.86	4.58	2.55	3.03	2.37	3.35	2.37

Means of different letters (a, b, c and d) in the same column are significantly different.

The column without letters is not significant.

50 metaphase were examined from each animals.

Means ± L.S.D values and results of the effect of metenix on Spermatocytes (germ cells) of male mice are illustrated in Table (2). An increase in chromosomal aberrations was observed in germ cell (spermatocyte) in all treated groups of metenix (0.01, 0.02 and 0.04 mg/kg/day) and these increases were dose-dependent. The total structural aberration was represented by (x-y univalent, Autosomal and fragments) and the total numerical

aberration represented by (hypo, hyper and polyploidy). The total aberrations at the level of 0.01 mg/kg/day increased significantly (8.33, 3.00) compared with the control (4.00, 1.67) while a highly significant increase in the chromosomal aberrations were observed in treated animals with the higher doses of metenix (0.02 and 0.04 mg/kg/day) (11.33, 5.00) and (14.00, 6.67) respectively compared with control (4.00, 1.67).

Table (2): The effect of oral administration of metenix on spermatocytes of male mice.

Treatments-A3	Structural aberration				Numerical aberration			
	X-Y Univalent	Autosomal	Fragments	TSA	Hypo<20	Hyper>20	Polyploidy	TNA
Control	1.00 d ± 0.00	3.00 d ± 0.00	0.00 c ± 0.00	4.00 d ± 0.00	1.00c ± 0.00	0.67 a ± 0.58	0.00 b ± 0.00	1.67 d ± 0.58
Low 0.01 mg/kg	2.00 c ± 0.00	4.67 c ± 0.58	1.67 b ± 0.58	8.33c ± 0.58	1.67c ± 0.58	1.00 a ± 0.00	0.33 b ± 0.58	3.00 c ± 0.00
Medium 0.02 mg/kg	3.33 b ± 0.58	5.67 b ± 0.58	2.33 ab ± 0.58	11.33 b ± 0.58	2.67b ± 0.58	1.67 a ± 0.58	0.67 b ± 0.58	5.00 b ± 1.00
High 0.04 mg/kg	4.33 a ± 0.58	6.67 a ± 0.58	3.00 a ± 0.00	14.00 a ± 1.00	3.67a ± 0.58	1.00 a ± 1.00	2.00 a ± 0.00	6.67 a ± 0.58
LSD at α 0.05 level	0.769	0.941	0.769	1.216	0.941	1.216	0.769	1.216

Means of different letters (a, b, c and d) in the same column are significantly different.

The column without letters is not significant.

50 metaphase were examined from each animals.

Means \pm L.S.D. values and results of the effect of metenix on bone marrow (somatic cells) in male mice are given in Table (3). The cytogenetic effect of metenix on bone marrow cells was studied. The results showed that the orally administration of metenix for 10 days at the level of (0.01) mg/kg/day showed a significant increase in the chromosome aberrations (25.67 and 12) compared with control (7.67 and 4.67). While a

highly significant increase in the chromosomal aberrations (34.67, 18.33) and (45, 22.67) respectively were observed in treated animals with higher doses of metenix (0.02 and 0.04 mg/kg/day) compared with control (7.67, 4.67). The structural type of aberration was represented by gaps, breaks, deletions, endometosis and centric attenuation and the numerical aberration represented by (periploidy and polyploidy).

Table (3): The effect of oral administration of metenix on Bone marrow cells of male mice

Treatments	Structural Aberration						Numerical aberration				
	Chromotid gaps	Chromosomal gaps	Chromotid break	Deletion	Endometosis	Centric attenuation	TSA	<40	>40	Polyploidy	TNA
Control	3.00 c \pm 1.00	0.33 c \pm 0.58	0.00 d \pm 0.00	1.67 d \pm 0.58	1.67 c \pm 0.58	1.00 d \pm 1.00	7.67 d \pm 0.50	2.00 d \pm 0.00	2.67 d \pm 0.58	0.00 b \pm 0.00	4.67 d \pm 0.58
Low 0.01 mg/kg	5.67 b \pm 0.58	1.67 b \pm 0.58	5.00 c \pm 1.00	4.00 C \pm 0.00	4.67 b \pm 1.15	4.67 c \pm 0.58	25.67 c \pm 1.53	5.33 c \pm 0.58	6.00 c \pm 0.00	0.67 b \pm 0.58	12.00 c \pm 0.00
Medium 0.02 mg/kg	7.67 a \pm 0.58	3.00 a \pm 0.00	6.67 b \pm 0.58	5.00 b \pm 0.00	5.67 b \pm 0.58	6.67 b \pm 0.58	34.67 b \pm 1.15	6.67 b \pm 0.58	9.00 b \pm 1.00	2.67 a \pm 0.58	18.33 b \pm 1.53
High 0.04 mg/kg	8.67 a \pm 0.58	3.67 a \pm 0.58	8.67 a \pm 0.58	6.67 a \pm 0.58	8.67 a \pm 0.58	8.67 a \pm 0.58	45.00 a \pm 1.00	8.67 a \pm 0.58	10.67 a \pm 0.58	3.33 a \pm 0.58	22.67 a \pm 0.58
LSD at α 0.05 level	1.331	0.941	1.216	0.769	1.438	1.331	2.105	0.941	1.216	0.941	1.631

Means of different letters (a, b, c and d) in the same column are significantly different. The column without letters is not significant. 50 metaphase were examined from each animals.

The data presented in table (4) showed the biochemical results. Orally administration of metenix for 10 days at the dose level of 0.01 mg/kg/day showed a slightly significant decrease in the liver DNA content (2.86 \pm 0.11 mg/whole liver), while a highly significant decrease was observed in DNA content of the liver of animals to which the high doses (0.02 and 0.04 mg/kg/day) was administered. (2.01 \pm 0.35 and 1.9 \pm 0.27mg/ whole liver), respectively compared with control (3.39 \pm 0.24).

Table (4): Effect of Metenix - 5 at different dose levels on DNA, RNA and total protein content (mg / whole liver) of mice.

Groups	DNA	RNA	Protein
	mg/whole liver		
Control	3.39 \pm 0.24	6.89 \pm 0.23	1014.21 \pm 15.53
Low 0.01 mg/kg	2.86 \pm 0.11	5.12 \pm 0.46	831.42 \pm 28.05
Medium 0.02 mg/kg	2.01 \pm 0.35	4.37 \pm 0.26	800.30 \pm 45.61
high 0.04 mg/kg	1.99 \pm 0.27	3.63 \pm 0.18	793.34 \pm 62.14

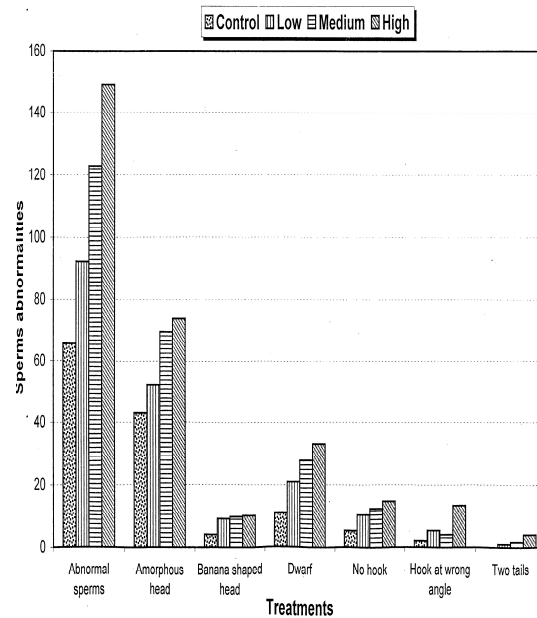


Fig. (1). Incidence of sperm head abnormality in male mice treated with metenix.

Also, the results as recorded in the same table (4) show that the liver RNA content of animals given metenix at all tested doses (0.01, 0.02, 0.04 mg/kg/day) caused a significant decrease in liver RNA content (5.12 ± 0.46 , 4.37 ± 0.26 and 3.63 ± 0.18 mg/ whole liver) as compared with control (6.89 ± 0.23 mg/ whole liver).

The total protein content of the liver of mice decreased significantly at the three different dose levels (831.42 ± 28.05 , 800.30 ± 45.61 and 793.34 ± 62.14 mg/ whole liver) compared with control (1014.21 ± 15.53 mg/ whole liver) and this decrease was dose dependent.

4-Discussion

Metenix 5 (metolazone) is a newly diuretic antihypertensive drug used for the treatment of hyper tension, and edema. The cytogenetic and biochemical effects of metenix have not been adequately studied. This study was performed to evaluate the cytogenetic effects (sperm abnormalities, chromosomal aberrations in spermatocyte and bone marrow) and biochemical effects (DNA, RNA and total protein) in male albino mice treated with different dose levels of metenix.

In the present study we found that the frequencies of chromosomal aberrations (spermatocyte and bone marrow cells) and the percentage of abnormal sperms were increased significantly as the dose level increased compared with the control group.

Similar cytogenetic results were obtained by (Pridjian et al., (2006); Andrianopoulos et al., 2006) who observed that when female rats were administered orally with a high dose of metenix (metolazone) caused an increase in the cytotoxic effects in female rats chromosomes.

Also, another finding obtained by Nakajima et al. (1978) and (1985) observed that in a study in which rat males were treated orally with metolazone at doses of 2, 10 and 50 mg/kg for 127 days prior to mating with untreated females, an increased number of sperm abnormalities and an increased of resorption sites was observed in dams mated with males from the 50 mg/kg group.

Also, Hashim, (1985) and Chahoud et al. (1985) observed that when male mice treated with 10 and 50 mg/kg of metenix and mated with untreated females the birth weight of offspring was decreased and the pregnancy rate was reduced in dams mated with males from the 10 and 50 mg/kg groups.

While, negative results were obtained by Matsuo et al. (1983). Mice and rats administered with metolazone 5 days respectively at daily doses of 2, 10 and 50 mg/kg, exhibited no evidence of a tumor-genic effect of the drug .

Also, negative results obtained by Puschett, (1988) who observed that metolazone was not mutagenic in vitro in the Ames test using salmonella tryhimurium strains TA-97, TA -98, TA-100, TA-120 and TA-1535.

Also, negative results obtained by Cangiano et al, (1974) who found that the long term animal studies with metolazone have not shown any evidence of carcinogenicity. Mice and rats given metolazone for 2 months at doses of 2, 10 and 50 mg/kg by stomach tube, showed no evidence that metolazone caused an increased in the number of tumors.

In our study we found that the frequencies of DNA, R.NA and total protein in the livers were decreased significantly in all treated groups of metolazone and these decreased were dose dependant, these finding were in agreement with Chang et al. (1996) who observed that the administration of single high doses (100 to 200 mg/kg) of metolazone intra-peritoneal to rats caused a decrease in liver DNA, RNA and total protein .

Also, positive results were obtained by Hassan, (1985) who found that the daily doses of metenix up to 50 mg/kg given orally for one month caused a slight decrease in the DNA and total protein content in the liver. While, negative results were obtained by Giovanni, and Antonietta. (2006) who observed that metolazone had no genotoxic or carcinotoxic effects on animal culture (in vitro) nor human lymphocytes (in vivo) and also metolazone did not cause DNA or RNA damage in the animal cells.

In the present study, male albino mice were treated with different dose levels of metenix for (10) consecutive days and the animals were subjected to cytogenetic and biochemical genetic analysis. It was found that metenix had mutagenic and cytotoxic effects on male albino mice and there were an increased frequencies of sperm head abnormalities and chromosomal aberrations in germ and somatic cells. Also, we found that the frequencies of DNA, RNA and total protein were decreased significantly in all treated groups of metenix suggesting a molecular changes as deletion and break in one or more loci which affect gene expression and interruption the nucleotide chain of DNA and protein (Batkai and Thumt, 2009; Han et al., 2009).

In Conclusion, Metenix (metolazone) which is a newly antihypertensive drug used for the treatment of high blood pressure stroke, heart failure and edema should be used only after careful consideration of the risk/benefit.

5-References

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