Cytogenetic and Biochemical effects of anti depression drug (wellbutrin) on male mice

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ABSTRACT: Background:Wellbutrin (bupropion hydrochloride) is a new highly selective norepinephrine and dopamine reuptake inhibitor, it is effective in the treatment of patients with major depression. **Aim**: Evaluating the cytogenetics and biochemical effects of wellbutrin .**Material and methods**: The sample of this study is male albino mice, divided into control group (did not administrate any treatment) and adult male albino mice group administrated orally doses of (0.2 and 0.4 mg kg/day) wellbutrin for 14 consecutive days and after one day from the last treatment the treated males were sacrificed and examined for sperm head abnormalities, cytogenetic analysis in (spermatocyte and bone marrow cells) and biochemical analysis (DNA, RNA and protein brain content). **Results** showed that in the group of males treated with (0.2 mg/kg/day) wellbutrin, the frequencies of sperm head abnormalities and chromosomal aberrations in spermatocyte and bone marrow cells were increased significantly while the DNA, RNA and total protein brain content were decreased significantly as compared with the control. On the other hand in the group of males treated with (0.4 mg/kg/day) wellbutrin there was a highly significant increase in the frequencies of sperm (head abnormalities and chromosomal aberrations (in spermatocyte and Bone marrow cells and a highly significant decrease in the DNA, RNA and total protein content in the brain of treated males as compared with the control group. Thus, we **concluded** that wellbutrin should be taken under extreme medical care because it is considered a mutagenic drug. [New York Science Journal 2009; 2(4):85-90]. (ISSN 1554 – 0200).
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Key words: Wellbutrin – chromosomal aberrations - sperm abnormalities -DNA-RNA- total protein - mice - bupropion hydrochloride.

1-Introduction

Depression is a mental health disorder that can affect the way we eat, sleep, the way we feel about our self and the way we think about things. A depressive disorder involves the body mood and thoughts. People who are depressed cannot "snap out of it" and get better, without treatment, symptoms can last for months or years. Treatments such as antidepressant drugs and psychotherapy can reduce and sometimes eliminate the symptoms of depression.

Depressive disorders come in different forms. Three of the most common are Major depression, Dysthymia and Bipolar disorder. Even within these types of depression there are variations in the number of symptoms, their severity, and persistence.

Major depression is manifested by a combination of symptoms that interfere with the ability of work, study, sleep, eat and enjoy once pleasurable activities. While dysthymia is a less severe type of depression that lasts along time but involves less sever symptoms. The suffering from dysthymia lead to a normal life, but one may not be function well or feeling good.

Biopolar disorder (also called manic-depression) is thought to be less common than other depressive disorders.

Researchers believe that it is possible to inherit a tendency to get depression. This seems to be especially true for bipolar disorder (manic depression) Studies of families with several generations of bipolar disorder (Bpo) found that those who develop the disorder have differences in their genes from most who dont develop (BPO). Major depression also seems to run in families, but it can also develop in people who have no family history of depression. Either way major depressive disorder is often associated with changes in brain structure or brain function.

There are several types of antidepressant medications used to treat depressive disorders. These include newer medications, the selective serotonin reuptake inhibitors (SSRIS), the tricycles and the older monoamine oxidase inhibitor (Ma OIS).

The (SSRIS) and other newer medications that affect neurotransmitters such as dopamine or norepinephrine generally have lower side effects than tricycles.

Wellbutrin Sr (bupropion hydrochloride) is a newer antidepresent drug which is efficacious in the

treatment of depression in adults, and is among the agents recommended for the first-line treatment of major depressive disorder.

Wellbutrin (bupropion hydrochloride) is a selective catacholamine (norepinephrine and dopamine) reuptake inhibitor, it has only a small effect on serotonin reuptake. The antidepresent effect of bupropion is considered to be mediated by its dopaminergic and noradrenergic action. Bupropion has also been shown to act as a smoking – cessation aid. Ferris et al ., (1983).

For wellbutrin (bupropion hydrochloride) no adequate data is available to illustrates the safety use of wellbutrin in human and animals.

In the present study we discussed the cytogenetic (in somatic and germ cell) and biochemical (DNA and RNA and total protein in brain) effects of wellbutrin on male mice if given orally for (14) consecutive day.

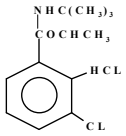
2-Materials and Methods:

2.1. Materials:

2.1.1.Test drug:

Wellbutrin (bupropion hydrochloride) provided by (Galxo Smith Kline. U.S.A) is an antidepressant of the aminoketone class, is chemically unrelated to tricydics tetracyclic selective serotonin re-uptake inhibitor or other known antidepressant agents. Its structure closely resembles that of diethylpropion, it is related to phenylethyl amines. It is designated as (±)-1-(3-chlorophenyl)1-2-[(1,1-dimethyl ethyl) amino]-1-propanone hydrochloride. The molecular weight is 276.2. The molecular formula is $C_{13}H_{18}CINOHCL$.

The structural formula is



Wellbutrin has a bitter taste and highly soluble in water.

2.1.2. Animals and Treatment:

Dilutions of different concentrations were prepared by dissolving the tablets of wellbutrin in distilled water.

Wellbutrin were administered orally at two dose levels (0.2) and (0.4) mg/kg once daily .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 (1964).

2.1.3. Animals:

Adult male albino mice weighting about (25mg) obtained from National Research Center were used. Animals were kept in 12h light /dark cycle and temperature controlled room (25-27°C) for one week prior to starting experiment and they were provided with food and water available add libitum.

Animals were divided into three groups. The first group were administered orally with a single dose of (0.2 mg/kg /day) once daily.

Animals of the second group were administered orally with a single dose of (0.4 mg/kg/day) once daily.

Animals of the third group served as controls and were administered orally with distilled water.

All animals were administered orally for (14) consecutive days and after 24h from the last treatments animals were sacrificed by cerficial dislocation for studying cytogenetic analysis, sperm head abnormalities and chromosomal aberrations in (bone marrow and spermatocytes) and Biochemical analysis (DNA, RNA and total protein) in brain.

2.2.Methods

2.2.1. Cytogenetic analysis:

2.2.1.1 Sperm head abnormality assay:

The treated males were sacrificed by decapitation the caudaepididymis was removed and placed in physiological saline then 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. Three hundreds sperms from each animal were examined for the abnormalities in sperm head shapes following the method recommended by Wyrobek and Bruce (1975).

2.2.1.2 Chromosomal preparation (in bone marrow cells)

Chromosomes from bone marrow cells were prepared according to the method of Hsu and Poctton (1969). Mice were injected with colchicines (2.5mg/kg/b.w i-p). After 3hours animals were killed by cervical dislocation. The bone marrow cells were aspirated in pros phosphate buffer solution (PH7.2) and centrifuged at 1000 r.p.m for 2min. The pellets obtained were mixed in aqueous solution of KCL (0.56%) and left for 30 min at 37°C .

The prepared cells were re-centrifuged fixed in (3:1) methyl: glacial acetic acid. Finally slides were air-dried and stained with 10% Giemsa stain for 20 minutes.

2.2.1.3 Chromosomal preparation (in Spermatocytes)

Testis were obtained from the same animals to study the abnormalities in Spermatocytes (germ cells)

according to (Brewen and Preston 1978) with some modifications. Mice were injected with colchicine (2.5mg/kg/b.w i.p) .After 3h animals were killed by cervical dislocation. The testis were collected in (2.2%) sodium citrate solution and citrate solution , minced into pieces with scissors and then centrifuged at 1000 r.p.m for 2min. The pellets obtained were mixed in aqueous solution of sodium citrate (1.1%) and left for 25 min at 37^{o}C. The prepared cells were re-centrifuged, fixed in (3:1) 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 in bone marrow and Spermatocytes.

2.2.2. For biochemical analysis:

The brain of each animal, fresh or frozen was taken, blotted using filter paper, weighted and homogenized in 0.9%. Sodium chloride solution for 5 minutes at 0^{o}C for the determination of nucleic acids and total proteins. Nucleic acids were extracted from brain by using trichloracetic acid (TCA) and ethanol according to the methods of Schneider (1945) and Sambrook et al (1980).

Deoxy-ribonucleic acid, Ribonucleic acid and total protein were determined according to the methods of Burton (1956), Khafagy et al (1990) and Lowery et at (1951) respectively .

2.2.3. Statistical analysis:

The experiment of sperm head abnormalities and chromosomal aberrations followed complete randomized design. The obtained data were subjected to analysis of variance (ANOVA) according to Snedecor and Cochran (1980).

Duncon's multiple range tests were used to compare between means of treatments according to Walter and Duncan (1969) at probability 5%.

The experiment of biochemical analysis (DNA, RNA and total protein) was carried out using the students (t) test According to the method described by Murray (1982)

3-Results

3.1. Cytogenetic Analysis:

3.1.1. Sperm head abnormality:

Means ± S.D. values and the results are given in the table (1) and Fig. (1). various forms of sperm heads, i.e., banana shaped, dwarf, triangular, amorphous, hooked…etc were recognized in all treated groups of wellbutrin. Analysis of these abnormal sperm shapes showed that overall amorphous types, hooked, dwarf and banana were more prevalent, in different groups than double headed, triangular, two tails and Hook at Wrong angle.

The comparative analysis of sperm abnormalities in mice treated with the two recommended doses of wellbutrin showed highly significant increase in all sperm head abnormalities as compared with control group and these increases were dose dependent. Also the total number of abnormal sperms for the two dose levels of wellbutrin was more frequent than the control group.

3.1. 2. Chromosomal aberrations:

The results of the cytogenetical examination in bone marrow and spermatocyte cells of male mice administrated orally with two tested doses of well butrin (0.2 and 0.4 mg/kg/day) are listed in Table (2) and (3).

In somatic cells, chromosomal aberrations consisted of structural and numerical aberrations. Structural aberrations included gaps, breaks, and deletions, as well as chromosomal gaps, endomitosis and centromeric attenuations,. Numerical aberrations were perideploidy (hypo and hyper) and polyploidy.

In germinal cells, structural aberrations were observed and represented by x-y univalent, autosomal univalent and fragments and numerical aberrations were observed and represented by perideploidy and polyploidy.

3.1. 2. 1. In (Spermatocytes):

Cytogenetic examination (Table 2) showed that the groups of males treated with wellbutrin (0.2 and 0.4 mg/kg/day) had more frequent of chromosomal aberrations (structural and numerical) than that of the control group and these increases were dose – related. The most frequent structural aberrations were (x-y univalent and autosomal univalent) and the most frequent numerical aberrations were periploidy (hypoploidy and hyperploid.

3.1. 2.2.In Bone marrow cells:

Cytogenetic examination (Table 3) showed that the groups of males treated with wellbutrin (0.4 and 0.8 mg/kg/day) had significantly increased in the total number of structural and numerical aberrations than the control group and these increases were dose related. The most frequent structural aberrations were (chromatid gaps, breaks, deletions, centromeric attenuation and endometosis) and the most frequent numerical aberrations were peripolidy (hypoploidy and hyperploidy).

3.2. In Biochemical analysis:

The data presented in Table (4) clearly show that orally administration of wellbutrin at a dose of (0.2 mg/kg)for 14 days had a slight effect on the

DNA content of the brain. While animals given the higher dose (0.4 mg/kg/b.w.) showed a highly significant decrease in brain DNA content. Also animals given wellbutrin at the levels of 0.2 and 0.4 mg/kg body weight showed a significant decrease in RNA content compared with the controls. The total protein content of the brain decreased significantly at the different dose levels and this decreased was dose-dependent.

Table (1): Distribution of different types of abnormal sperms in male mice treated with wellbutrin.

Treatments	Abnormal Sperms	Amorphous head	Banana Shaped head	Dwarf	Double Headed	Triangle	No hook	Two tails	Hook at wrong angle
Control	$77.66^{c} \pm 0.58$	$37.67^{c} \pm 0.58$	$4.67^{c} \pm 1.15$	$21.00^{c} \pm 1.00$	$0.00^{c} \pm 0.00$	$4.33^{c} \pm 1.15$	$4.67^{c} \pm 0.58$	$0.00^{c} \pm 0.00$	$5.00^{c} \pm 1.000$
0.2 mg/kg/day	$131.00^{b} \pm 1.00$	$56.00^{b} \pm 1.00$	$9.67^{b} \pm 0.57$	$37.67^{b} \pm 0.58$	$0.33^{b} \pm 0.58$	$6.67^{b} \pm 0.58$	$8.33^{b} \pm 0.68$	$2.67^{b} \pm 0.58$	$9.67^{b} \pm 0.58$
0.4 mg/kg/day	$187.30^{a} \pm 2.08$	$75.67^{a} \pm 5.13$	$15.33^{a} \pm 1.53$	$46.67^{a} \pm 2.89$	$2.00^{a} \pm 1.00$	$12.00^{a} \pm 2.00$	$15.33^{a} \pm 1.53$	$4.33^{a} \pm 0.58$	$16.00^{a} \pm 1.00$
LSD at α 0.05 level	2.746	6.067	2.307	3.586	1.331	2.746	1.998	0.941	1.762

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

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

Treatments	Structural Aberration				Numerical Aberration			
	x-y Univalent	Autosomal Univalent	Fragments	TSA	Hypo<20	Hyper>20	Polypliody	TNA
0	$1.00^{c} \pm 1.00$	$1.33^{c} \pm 1.00$	$0.00^{c} \pm 0.00$	$2.33^{c} \pm 0.58$	$0.67^{c} \pm 0.58$	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$	$0.67^{c} \pm 0.58$
0.2 Mg/kg/day	$2.67^{b} \pm 0.58$	$4.33^{b} \pm 0.58$	$1.33^{b} \pm 0.58$	$8.33^{b} \pm 1.15$	$2.00^{b} \pm 0.00$	$0.67^{b} \pm 0.58$	$0.67^{b} \pm 0.58$	$3.33^{b} \pm 0.58$
0.4 Mg/kg/day	$4.33^{a} \pm 0.58$	$6.67^{a} \pm 0.58$	$2.33^{a} \pm 0.58$	$13.33^{a} \pm 0.58$	$3.67^{a} \pm 0.58$	$2.33^{a} \pm 0.58$	$1.67^{a} \pm 0.58$	$7.67^{a} \pm 0.58$
LSD at α 0.05 level	1.490	1.632	0.941	1.632	0.941	0.941	0.941	1.153

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

Table (3): The effect of oral administration of wellbutrin on Bone marrow of male mice.

Treatments	Structural Aberration							Numerical Aberration			
	Chromotid Gaps	Chromosomal gaps	Chromotid Break	Deletion	Centromric Attenuation	Endometosis	TSA	<40	>40	Polyploidy	TNA
Control	$2.33^{c} \pm 1.00$	$0.00^{c} \pm 0.00$	$0.00^{c} \pm 0.00$	$0.00^{c} \pm 0.58$	$2.33^{c} \pm 0.58$	$2.67^{b} \pm 0.58$	$7.33^{c} \pm 0.58$	$1.33^{c} \pm 0.58$	$3.00^{c} \pm 1.00$	$0.00^{c} \pm 1.00$	$4.33^{c} \pm 0.58$
0.2 mg/kg/day	$6.00^{b} \pm 0.58$	$2.67^{b} \pm 0.58$	$2.67^{b} \pm 0.58$	$4.33^{b} \pm 0.58$	$6.00^{b} \pm 0.00$	$5.33^{a} \pm 0.58$	$27.00^{b} \pm 1.73$	$5.00^{b} \pm 0.00$	$6.67^{b} \pm 0.58$	$1.67^{b} \pm 0.58$	$13.33^{b} \pm 0.58$
0.4 mg/kg/day	$8.67^{a} \pm 0.58$	$4.33^{a} \pm 0.58$	$5.67^{a} \pm 0.58$	$6.67^{a} \pm 0.58$	$8.33^{a} \pm 0.58$	$7.67^{a} \pm 0.58$	$41.33^{a} \pm 0.58$	$7.33^{a} \pm 0.58$	$9.33^{a} \pm 0.58$	$3.67^{a} \pm 0.58$	$20.33^{a} \pm 0.58$
LSD at α 0.05 level	**1.490**	**0.+941**	**0.941**	**0.941**	**0.941**	**2.580**	**2.209**	**0.941**	**1.490**	**0.941**	**1.153**

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

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

Groups	DNA	RNA	Protein
	mg / whole liver		
Control	1.06 ± 0.13	2.56 ± 0.16	137.82 ± 9.24
0.2	1.24 ± 0.15	1.89 ± 0.37	119.03 ± 6.16
0.4	0.45 ± 0.82	1.34 ± 0.61	114.35 ± 5.29

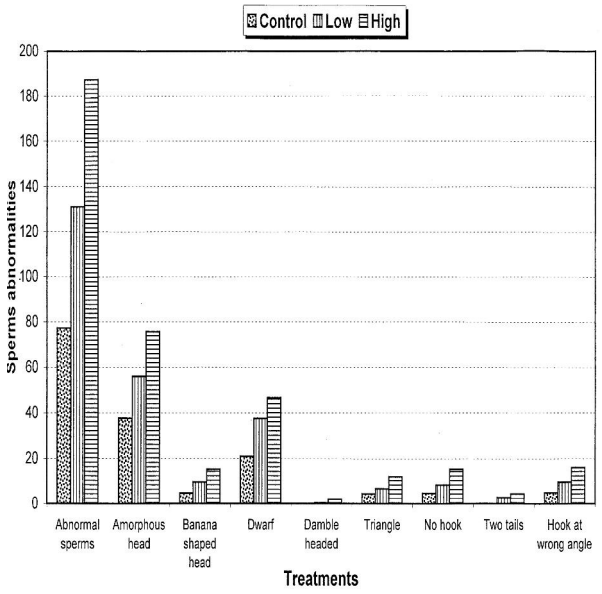


Fig. (1). Incidence of sperm head abnormality in

4. Discussion

Wellbutrin (bupropion hydrochloride), is a new effective antidepressant drug of the amino ketone class. It is a relatively weak inhibitor of the neuronal uptake of norepinephrine, serotonin, and dopamine. The mechanism of action of wellbutrin is unknown. It is presumed that its action is mediated by noradrenergic and dopaminergic mechanisms.

The present study was carried to evaluate the cytogenetic and biochemical effects of wellbutrin on albino male mice. The present study showed that administration of a single dose of (0.2 mg/kg) wellbutrin to male mice for 14 consecutive days caused a slight significant increase in the frequencies of sperm abnormalities and chromosomal aberrations in bone marrow cells and spermatocyte cells.

However, the administration of a single dose of (0.4 mg/kg) to male mice for 14 consecutive days caused a highly significant increase in the frequencies of sperm abnormalities and chromosomal aberrations in germ and somatic cells.

On the other hand, the administration of a single dose of (0.2 mg/kg) of well butrin caused no effect on DNA content but caused a slight decrease in the RNA and total protein content of brain mice. While the administration of a single dose of (0.4 mg/kg) caused a highly significant decrease in DNA, RNA and protein content. This may be as a result of chromosomal aberrations such deletions and breaks which decrease the DNA, RNA and total protein content in the cell brain.

The results are in agreement with El Don and Preskorn (2000) who found that the oral treatment of mice and rats with wellbutrin at doses up to 300 and 150mg/kg/day respectively cause an increase in nodular proliferative lesions of rat liver at a dose of 100 to 300 mg/kg/day but negative results were obtained with mice i.e. no liver lesions .Found no increase in malignant tumors of the liver and other organs seen in either study.

In addition, Jefferson et al., (2006) observed that bupropion produced positive response (2 to 3 times control mutation rate) in 20 of 5 strains in the Ames bacterial mutagenicity test.

Klimek et al., (1985) found an increase in the frequencies of chromosomal aberrations in vivo rat bone marrow cytogentic studies.

Other positive results were obtained by (Tucker, 1983) who studied the effect of oral administration of wellbutrin in rabbits during pregnancy and found an increase in skeletal anomalies at delayed ossification and decrease in the DNA, RNA content in the maternal brain. The same results were obtained by Chan et al., (2005) who found that when women administered bupropion throughout pregnancy a decrease in the fetal body weight and in the DNA and total protein content in the maternal brain.

Similar positive results obtained by Saito et al., (1984) who found that in Chinese hamster 79 cells (bupropion hydrochloride) caused DNA single strand breaks and decrease in the DNA and RNA content.

Also, similar positive results observed by Brambilla et al.,(2009) who studied the genotoxic and carcinogenic effect of antipsychotics and antidepressants drugs They found that wellbutrin has a mutagenic effects when administrated orally to the male rat and mice at a dose equal to the recommended and above the recommended dose for human.

However, negative results were obtained by Way (2007) in testing a fertility study in rats at doses up to 300 mg/kg ,no evidence of impaired fertility were observed.

The present study showed that somatic cells (bone marrow cells) were more sensitive than germ cells (spermatocyte cells) in demonstrating the clastogenic effect of wellbutrin finding were agreement with that of Tates et al., (1977) who

observed that somatic cells were more sensitive to the chemicals than germ cells.

In conclusion

With the contrast to its benefit effect in the treatment of depression. Wellbutrin induces a mutagenic and cytotoxic effect in the male albino mice (germ and somatic cells) and also cause a decrease in the DNA, RNA and total protein content of mice brain. Furthermore, the somatic calls were shown to be more sensitive than the germ cells in demonstrating the effect of wellbutrin. Therefore, wellbutrin should be used with extreme medical care after careful consideration of the risk /benefit ratio.

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