Cytogenetic and Biochemical effects of Antidepressant 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 animals 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 animals 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 animals 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 animals 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 2010; 3(6):121-126]. (ISSN 1554 – 0200).

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, (Rush, 2010).

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 (IsHak., 2009).

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 (Malhi et al., 2003).

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 (Paquette et al., 2009).

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 Williams, (2006).

Wellbutrin Sr (bupropion hydrochloride) is a newer antidepressent 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 (Karam-Hage et al., 2010).

Wellbutrin (bupropion hydrochloride) is a selective catecholamine (norepinephrine and dopamine) reuptake inhibitor, it has only a small effect on serotonin reuptake. The antidepresant 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; Hoim and Spencer, 2000).

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 tricyclic 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 C13H18CINOHCL.

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 ad 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 Wyrobeck 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 (Agarwal, et al., 1994). 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, 50 metaphase spreads were examined for each animal, scoring the different types of chromosomal abnormalities.

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
Deoxy-ribonucleic acid, ribonucleic acid and the brain of each animal, fresh or frozen

2.2.3. Statistical analysis: The brain of each animal, fresh or frozen

and methods of total protein were determined according to the Sambrook et al. (1989).

Snedecor and Cochran (1980).

to analysis of variance (ANOVA) according to randomized design. The obtained data were subjected

cromosomal aberrations followed complete

according to the methods of Schneider, (1945) and Sambrook et al. (1989).

Deoxy-ribonucleic acid, ribonucleic acid and total protein were determined according to the methods of Burton, (1956), Khafagy et al. (1980) and Lowery et al. (1951), respectively.

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°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 Schenider, (1945) and

Sambrook et al. (1989).

3.1. Cytogenetic Analysis:

3.1. 2. Chromosomal aberrations:
morphous, hooked…etc were recognized in all

controlled groups of wellbutrin. Analysis of these

aberrations showed that overall 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. 2.1. 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 hyperploidy).

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 periploidy (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 (1.24±0.15 mg/whole brain), while animals given the higher dose (0.4 mg/kg/b.w.) showed a highly significant decrease in brain DNA content (0.45±0.82 mg/whole brain) compared with control (2.56±0.16 mg/whole brain).

Also animals given wellbutrin at the levels of 0.2 and
0.4 mg/kg body weight showed a significant decrease in RNA content (1.89±0.37 and 1.34±0.61 mg/whole brain), respectively compared with the control (2.56±0.16 mg/whole brain). The total protein content of the brain decreased significantly at the different dose levels (119.03±6.16 and 114.35±5.29 mg/whole brain) compared with control (137.82±9.24 mg/whole brain) and this decreased was dose-dependent.

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

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Abnormal Sperms</th>
<th>Amorphous head</th>
<th>Banana Shaped head</th>
<th>Dwarf</th>
<th>Double Headed</th>
<th>Triangle</th>
<th>No hook</th>
<th>Two tails</th>
<th>Hook at wrong angle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>77.66±0.58</td>
<td>37.67±0.58</td>
<td>4.67±1.15</td>
<td>21.00</td>
<td>0.00</td>
<td>4.33±1.15</td>
<td>4.67±0.58</td>
<td>0.00</td>
<td>5.00</td>
</tr>
<tr>
<td>0.2 mg/kg/day</td>
<td>131.00±1.00</td>
<td>56.00±0.58</td>
<td>9.67±0.58</td>
<td>37.67</td>
<td>0.33</td>
<td>6.67±0.58</td>
<td>8.33±0.58</td>
<td>2.67</td>
<td>9.67</td>
</tr>
<tr>
<td>0.4 mg/kg/day</td>
<td>187.30±2.08</td>
<td>75.67±0.58</td>
<td>15.33±1.53</td>
<td>46.67</td>
<td>2.00</td>
<td>12.00±1.00</td>
<td>15.33±1.53</td>
<td>4.33</td>
<td>16.00</td>
</tr>
<tr>
<td>LSD at α 0.05 level</td>
<td>2.746</td>
<td>6.067±0.00</td>
<td>2.307±0.58</td>
<td>3.586</td>
<td>1.331</td>
<td>2.746±1.00</td>
<td>1.998±0.58</td>
<td>0.941</td>
<td>1.762</td>
</tr>
</tbody>
</table>

Means of different letters (a, b, c and d) in the same column are significantly different. The column without letters is not significant.

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

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Structural Aberration</th>
<th>Numerical Aberration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x-y Univalent</td>
<td>Autosomal Univalent</td>
</tr>
<tr>
<td>0</td>
<td>1.00±1.00</td>
<td>1.33±1.00</td>
</tr>
<tr>
<td>0.2 Mg/kg/day</td>
<td>2.67±0.58</td>
<td>4.33±0.58</td>
</tr>
<tr>
<td>0.4 Mg/kg/day</td>
<td>4.33±0.58</td>
<td>6.67±0.58</td>
</tr>
<tr>
<td>LSD at α 0.05 level</td>
<td>1.490</td>
<td>1.632</td>
</tr>
</tbody>
</table>

Means of different letters (a, b, c and d) in the same column are significantly different. The column without letters is not significant.

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

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Structural Aberration</th>
<th>Numerical Aberration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chromosomal gaps</td>
<td>Chromosomal Break</td>
</tr>
<tr>
<td>Control</td>
<td>2.33±1.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>0.2 Mg/kg/day</td>
<td>6.00±0.00</td>
<td>2.67±0.58</td>
</tr>
<tr>
<td>0.4 Mg/kg/day</td>
<td>8.67±0.58</td>
<td>4.33±0.58</td>
</tr>
<tr>
<td>LSD at α 0.05 level</td>
<td>1.490</td>
<td>0.941</td>
</tr>
</tbody>
</table>

Means of different letters (a, b, c, d) in the same column are significantly different. The column without letters is not significant.

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>DNA (mg / whole brain)</th>
<th>RNA (mg / whole brain)</th>
<th>Protein (mg / whole brain)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.06 ± 0.13</td>
<td>2.56 ± 0.16</td>
<td>137.82 ± 9.24</td>
</tr>
<tr>
<td>0.2</td>
<td>1.24 ± 0.15</td>
<td>1.89 ± 0.37</td>
<td>119.03 ± 6.16</td>
</tr>
<tr>
<td>0.4</td>
<td>0.45 ± 0.82</td>
<td>1.34 ± 0.61</td>
<td>114.35 ± 5.29</td>
</tr>
</tbody>
</table>

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 wellbutrin 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 of brain mice. 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 (Brustolim, et al., 2006) 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 cytogenetic 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 cells were shown to be more

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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.

5-References


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