

5¹-Nucleotidase Activity In Albino Rats Treated With Talen® Tablets (Sedative)

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Abstract: Sedative drugs have been used in the management and treatment of Insomnia, each produces some undesirable effects by various mechanisms. Talen®, containing the active ingredient; bromazepam has been used to treat insomnia effectively. This research work examined the hepatobiliary effect of the drug (Bromazepam) in albino rats. Twenty adult male albino rats, distributed into five (5) groups (A, B, C, D and E), with four (4) rats in each group were used in the research. Groups A, B, C and D were given oral treatment of 0.02, 0.04, 0.08 and 0.12mg/kg body weight of drug solution respectively, for seven consecutive days, while group E was kept as the control. Treatment of animals with the drug solution resulted to a decrease in physical activity, body weights, feed and water intake relative to the control. Measurement of the total protein concentration in the serum of the animals did not reveal any significant difference ($P>0.05$) between the test and the control groups. In contrast, the activity of 5¹-nucleotidase recorded in the treated groups were significantly higher ($p<0.05$) than the control. These effects varied with the doses. These findings suggested that Talen® may be toxic to the hepatobiliary system as revealed by increase in 5¹-Nucleotidase levels.

Keywords: Talen, sedative drugs, 5¹-Nucleotidase levels and hepatobiliary system

I. Introduction

Insomnia or sleeplessness is a disorder in which there is an inability to fall asleep or to stay asleep as long as desired (Golub, 2012), while anxiety is an unpleasant state of inner turmoil which is indicated to be one of the major cause of insomnia (sleep disorder). Anxiety and sleep disorders are the most common problems of the recent years and people used to take anxiolytic drugs to over-come these problems (Dolovich *et al.*, 2005). Most of these drugs affect neurohumoral transmission by interfering with the neurotransmitters, blocking the receptors, and hence inhibiting their action (Armett and Ritche 2006). Sedatives which are mostly a member of the benzodiazepines groups are widely used as an anxiolytic, sedative and hypnotic drug. The drug is absorbed completely through the gastrointestinal tract and its peak plasma concentration is reached within 1-4hours after oral administration. The plasma elimination half-life ranges from 7.9 to 19.3 hours. Bromazepam is basic in nature and about 70% of it bound to plasma proteins (Neumeyer and Booth 2005). It is metabolized in the liver into active metabolites which are excreted entirely as glucoside conjugates of 3-hydroxy bromazepam (27%), 3-hydroxybenzoylpyridine derivatives (40%), intact bromazepam (2.3%) and intact benzoylpyridines derivatives (0.66%).

Remy *et al.*, (2003) described a case of cross hepatotoxicity and liver dysfunction for patients treated with tricyclic anti-depressants including bromazepam; the levels of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) returned to normal when the drug was withdrawn. Although sedatives do not relieve pain in themselves, they can be a useful adjunct to analgesics in preparing patients for surgery and are commonly given to patients before they are anaesthetized, or before other highly uncomfortable and invasive procedures like cardiac catheterization and colonoscopy (Bandelow, 2008).

Some sedative including Talen (bromazepam) can cause physiological and psychological dependence when taken regularly over a period of time, even at therapeutic doses (Ebert *et al.*, 2006). Dependent users may get withdrawal symptoms ranging from restlessness and insomnia to convulsion and death. When users become psychologically dependent, they feel as if they need the drug to function, although physical dependence does not necessarily occur, particularly with a short course of use. In both type of dependences, finding and using the sedative becomes the focus in life.

Bromazepam causes similar side effects to other benzodiazepines. The most common side effects reported are drowsiness, sedative, ataxia, memory impairment and dizziness. Impairments to memory functions are common with bromazepam and include a reduced working memory and reduced ability to process environmental information (Cunhar *et al.*, 2008). An experiment on healthy male college students was carried out to explore the effect of four different drugs on learning capacity. It was observed that taking Bromazepam alone at 6mg, 3times daily for 2weeks impaired learning capacities significantly. In combination with alcohol,

impairments in learning capacity became even more pronounced (Iiljequist *et al.*, 2003). Impaired memory, visual information processing and sensory data and impaired psychomotor performance. Ambulatory patients should be warned that bromazepam may impair the ability to drive vehicles and to operate machinery. The impairment is worsened by consumption of alcohol, because both act as central nervous system depressants.

Benzodiazepines require special precaution if used in elderly, pregnant, child, alcohol or drug dependent individuals with comorbid psychiatric disorders (Authier *et al.*, 2007). The clinical consequence is that the elderly should be treated with lower doses than younger patients and increment should be made gradually, depending on the response of the patient, in order to avoid over sedation or neurological impairment. The drug Talen (bromazepam) is pregnancy category D, a classification that means that it has been shown to cause harm to unborn child. The product information leaflet warns against breast feeding while taking bromazepam. There has been at least one report of sudden infant death syndrome linked to breast feeding while consuming bromazepam (Martens, 2004). Benzodiazepines share a similar mechanism of action with various sedative compounds that act by entrancing the GABA_A receptor. Talen (bromazepam) binds to the GABA (gamma-aminobutyric acid) receptor GABA_A causing a conformational change and increasing the inhibitory effect of GABA.

5¹-Nucleotidase (5¹-ribonucleotide phosphohydrolase 5¹-NT), an intrinsic membrane glycoprotein present as an ectoenzyme in a wide variety of mammalian cells, hydrolyzes 5¹-Nucleotides to their corresponding nucleosides (Sunderman, 2000). Despite its ubiquitous distribution, serum concentration of 5¹-Nucleotidase appear to reflect hepatobiliary disease with considerable specificity. It has been observed that serum 5¹-NT was clinically useful for differential diagnosis of hepatobiliary diseases, the enzyme activity being increased only in hepatobiliary disease.

Assay of 5¹-Nucleotidase activity have value as an addition to measurement of nonspecific total alkaline phosphatase (AP) in patients with suspected hepatobiliary diseases (Novo and Tutor, 2002).

Aim/Objectives

The adverse effects of sedative drugs have been widely reported. The present work investigated the effect of Talen® on the hepatobiliary system of albino rats by measuring the blood nucleotidase levels after treating them with the drug (Talen®).

II. Materials And Methods

Collection of Albino Rats

Twenty male albino rats (80-230g) were obtained from the breeding unit in the Zoology Department of the University of Nigeria Nsukka (UNN) in a steel cage and were transported down to Abakaliki.

Collection of Drug Sample:

Bromazepam (Talen^(R)) tablets (1.5mg concentration) was purchased from Jabera Pharmacy Abakaliki, Ebonyi State, Nigeria.

Preparation of drug sample

A total of 90mg of Talen® (Bromazepam) was suspended in 400ml of distilled water to obtain a concentration of 0.02mg/ml

Animal handling and treatment

Animal grouping

The animals were divided into five groups of four rats per cage to acclimatize for seven days in a well-ventilated room (25±2°C) and 12hours light/dark cycle at the animal house of the Zoology Department, Ebonyi State University, Nigeria.

Measurement of the Weight of the Animals

The weight of the animals was taken daily using an Electronic weighing balance. The results obtained were used to monitor weight changes and determine the volume of the sample to be administered to each of the animals.

Administration of Sample

Animals were regularly fed on a standard diet with growers mash and water on daily basis for seven (7) consecutive days. The samples were orally administered to the animals using 1ml syringe. The animals in group A, B, C and D were given 0.02, 0.04, 0.08 and 0.12mg/kg body weight respectively while the animals in group E were orally treated with distilled water (Vehicle) for seven (7) consecutive days and considered as a control group.

Collection of Samples from the Animals

After seven (7) days of administration with the drug sample, feeds were withdrawn from the animals for 24hours (fasted Overnights) and blood samples were collected into a sterile bottle by means of cardiac puncture under mild anesthesia using chloroform.

Chemicals and reagents

All the chemicals and reagents used in this research were of analytical grade.

Determination of 5¹-Nucleotidase level

Assay principle

The 5¹-Nucleotidase (5¹NT) assay is based on the enzymatic hydrolysis of 5¹-monophosphate (5¹IMP) to form inosine which is converted to hypoxanthine by Purine Nucleoside Phosphorylase (PNP). Hypoxanthine is then converted to uric acid and hydrogen peroxide (H₂O₂) by xanthine oxidase (XOD). The formation of uric acid is measured spectrophotometrically.

Statistical Analysis

The results of this analysis was expressed as mean ± standard deviation, the Data obtained were subjected to one way analysis of ANOVA.

III. Results

Physical Observation

On the first day, the animals were very active before the drug was administered. After administration, a remarkable decrease in physical activity was observed. Subsequent days gave rise to further decrease in physical activity especially in groups D, C, B and A, decrease in feed and water intake as the days progressed, while the animals in the control group (group E) increased in physical activity, increase in feed, water intake also noticed among the control group.

Changes In Body Weight

The average body weight of animals in groups A, B, C, D and E after seven (7) days of drug administration is shown below. The animals in Group A, B and C, D showed an insignificant decrease (p<0.05) in weight, while the animals in Group E, which were not treated (control group) showed an insignificant increase (p<0.05) in weight.

Table 1: Show changes in the body weight of animals during 7 days of administration

Day	Group A±SD	Group B±SD	Group C±SD	Group D±SD	Group E±SD
1	82.5±5.00	120.0 ± 5.97	120.0±5.97	137.5±7.58	150.0±3.32
2	82.5±2.58	119.5 ± 5.13	117.5±5.00	129.5±7.07	152.5±2.18
3	80.5±3.58	117.5±5.00	117.0±5.77	124.0±4.14	155.0±5.25
4	79.0±4.17	115.0±5.77	112.5±5.00	119.5±6.57	155.0±5.52
5	76.0±3.90	112.5±5.00	109.0±5.63	115.5±3.00	156.0±6.77
6	75.0±2.06	110.0±4.77	105.5±5.00	112.0±3.14	157.5±5.13
7	73.5±3.57	107.0±4.84	103.5±5.00	109.0±3.07	157.5±6.46

LEGEND

Group A = 0.02mg/kg body weight
 Group B = 0.04mg/kg body weight
 Group C = 0.08mg/kg body weight
 Group D = 0.12mg/kg body weight
 Group E = Control

Table 1: above shows the average weight of albino rats treated with tallen for seven days. The mean body weight of the animals in groups A to D decreased while the control group increased.

Table 2: Average protein concentration, 5¹NT activity and average specific 5¹ NT activity.

Animal group	Average total protein (mg/ml) ±SD	Average activity 5NT (µl) ±SD	Specific 5NT activity (µl/mg/ml)±SD
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A	23.33±2.53	0.33±0.07	43.52±5.37
B	29.77±2.13	0.37±0.57	61.18±2.15
C	41.19±3.97	0.50±0.02	112.29±4.06
D	46.08±3.54	0.54±0.01	141.87±4.22
E	18.39±3.10	0.14±0.03	25.74±3.73

LEGEND

Group A =	0.02mg/kg body weight
Group B =	0.04mg/kg body weight
Group C =	0.08mg/kg body weight
Group D =	0.12mg/kg body weight
Group E=	Control

Table 2 above shows that the average 5¹-NT activity and average specific 5NT activity in serum of groups A to D were found to be significantly higher ($p<0.05$) than the control and the average total protein concentration of the groups were found to be insignificantly different ($p<0.05$).

IV. Discussion

Serum 5¹-Nucleotidase level was investigated with the view of establishing hepatotoxicity in albino rats treated with Talen® (Sedative).

A decrease in physical activity, feed and water intake was observed in the groups that were treated with the drug, while the animals in the control group were physically active. The exact biochemical mechanism responsible for the decrease in the physical activity, feed and water intake is a recommendation for further study. Hence, it may be attributed to the chemical constituents of the drug administered to the rats.

Sedatives have been reported to influence various body processes such as muscle relaxation, sedation, hypnotic (sleep-inducing), anxiolytic (anti-anxiety), anticonvulsant, properties and overall body metabolism of the organism (Bateson, 2002).

The reason behind the decrease in average body weight of the treated rats relative to the control is still not fully understood, this may be partly related to the reported decrease in food and water intake caused by the introduction of the drug solution into the animals.

The ability of the drug to produce a significant difference in total protein levels in the treated groups compared to the control group suggest that the chemical constituent of the drug at the doses administered may influence the rate of protein synthesis and degradation significantly.

The dose dependent nature of the increase in 5¹-NT activity agrees with the general principle of drug effect, the higher the dose, the higher the effect. In a study isolated 5¹-NT elevation, noticeable in an unselected group of patient at veteran affair hospital was observed.

In consideration of the issue that has been discussed in this research, care must be taken and the doses strictly maintained, when this sedatives are administered to prevent overdose and the risk of liver disease.

V. Conclusion

The observations made in this work suggest that Talen® may induce metabolic responses, which may include hepatocellular injury. Thus, care must be taken in the use of this sedatives for the management and treatment of insomnia, since the drug was able to increase the activity of 5¹-NT. However, further research is needed to ascertain the actual mechanism behind the reported observation.

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