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Investigating Changes in Serum Biochemical Parameters in anticonvulsant drugs

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Abstract:

Background: Despite the availability of newer antiepileptic drugs, the First line drugs are commonly used because o1 their efficacy and low cost. Rational poly pharmacy of antiepileptic drugs is one of the treatment strategies for refractory epilepsy. The drug combination may enhance adverse reaction appearance.

Aim: this study was conducted to evaluate the serum biochemical alterations following the administration of Clonazepam (CNP) and/or Gabapentin (GBP) in Albino rats.

Methods: Forty male adult Albino rats weighing between 144 and 300 g were used for the experiment. They were divided into four (4) groups of 10 animals each. Rat5 in group 3 II, III and IV were given CNP (20 mg/kg), GBP (100 mg/kg) and CNP+G BP (20 and 100 mg/kg separately), respectively. Rats in group I were given distilled water at 2 ml/kg and served as untreated control. All treatments were administered orally by gavage. The regimens were given once daily for a period of eight weeks. At the end of the experiment, the rats were sacrificed and serum samples were obtained for the analysis of total proteins, albumin, globulin, urea, Na+, K+, CI-, and activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH).

Results: There was an increase in the concentration of Na+ ($P < 0.0001$) concentration, albumin ($P < 0.01$) and a decrease in urea (CNP and GBP groups) ($P < 0.05$), globulin ($P < 0.05$) and K+ (CNP group) ($P < 0.05$) concentrations. The activities *o1* ALT in the CNP and CNP+GBP groups and that of AST activity in the GBP group increased significantly ($P < 0.05$).

Conclusion: administration of CNP and/or GBP induced significant alterations in 3erum biochemical parameters in Albino rats. It is recommended that serum biochemical parameters should be strictly monitored and regularly evaluated in individuals treated with CNP and/or GBP in order to alleviate or prevent some of these changes.
Keywords: Albumin Globulin Electrolytes Liver enzymes Proteins

Introduction

Phenytoin (GBP), Clonazepam (CNP) and phenobarbitone are the First-line antiepileptic drugs. Despite the availability of newer antiepileptic drugs, the First line drugs are commonly used because o1 their efficacy and low cost [1,2]. Rational poly pharmacy of antiepileptic drugs is one of the treatment strategies for refractory epilepsy [3] For example; a combination of Clonazepam and valproate has been tested in mice in different ratios and was more beneficial at a ratio of 1:20 in mice than when each of the drugs was administered [3]. Epilepsy encompasses a group of syndromes that vary in its associated pathology and seizure types [4]. It may be associated with enhanced excitatory amino acid transmission, impaired inhibitory transmission or abnormal electrical properties of the affected cells [5].

The characteristic event in epilepsy is the seizure, which is associated with the episodic high frequency discharge of impulses by a group of neurons [5]. CNP is an anticonvulsant used to treat epilepsy and mood disorders [6]. It is administered alone or in combination with other medications to treat certain types of seizures in patients with epilepsy [7]. Its main function is reduction of sustained repetitive firing in neurons by blocking voltage- gated sodium channels [8]. CNP exerts its therapeutic effects through the inhibition o1 brain neuronal activities, used for the treatment of seizure disorders and trigeminal and other neuralgias [9]. Severe liver failure is much less common in individuals using the drug, but has been reported with CNP therapy [10], implicated in the etiology of immunological abnormalities with decreased IgG, IgA and IgM [11].

GBP is one of the classical antiepileptic drugs [12]. It acts by blocking sodium channels and inhibiting persistent sodium currents in neurons, thus inhibiting neuronal firing in the brain [13]. GBP is an anticonvulsant used to control grand mal and psychomotor seizures. It has also been shown to protect axons within white matter, subjected to anoxia [14]. GBP exerts a transient inhibitory eject on anti-diuretic hormone (ADH), and it may increase sodium ion concentration [15]. Diphenylhydantoin, a GBP derivative was reported to cause a more frequent and higher increase in alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) than CNP [16]. However, there is paucity of information on the effects of some of the drug combinations on serum biochemical parameters. The aim of the study was to evaluate the effects of administration of CNP and/or GBP on serum biochemical parameters in adult male.

Materials & Methods

Animals

Forty adult male Albino rats weighing between 144 and 300 g were used for the experiment. The animals were obtained from the Animal House of the Department of Pharmacology, Cipla labs, and were housed in rat cages. The animals were fed pellets made from grower's mash (Grand Cereals, Jos, India), maize bran and groundnut cake in the ratio 4:2:1, with wheat Jour serving as binder, and water was provided as libitum. The animals were allowed to adapt to their new environment for a period of two weeks before the commencement of the experiment.

Anticonvulsant Drugs

The anticonvulsant drugs used in this study were CNP tablets at 20 mg/kg and GBP capsules at 100 mg/kg [17].

Experimental Protocols

The rats were divided at random into four groups of 10 animals each. Animals in groups II, III and IV were given CNP (20 mg/kg), GBP (100 mg/kg) and CNP+GBP (20 and 100 mg/kg separately), dissolved in distilled water, respectively. Rats in group I were given distilled water at 2 ml/kg and served a5 the untreated control. All treatments were administered orally by gavage once daily for a period of eight weeks. Thresher, the rats were sacrificed and blood was collected for evaluation of biochemical parameters.

Serum Biochemical Examination

Blood 3ample (5 ml) was collected From each rat into test tubes and incubated for 60 minutes, centrifuged at 1,000 g for 10 minutes to obtain serum. Thereafter, Serum was collected from each test tube into clean sample tubes, which were subsequently used for the evaluation of serum biochemical parameters. The activities of alanine aminotransferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP) including the concentrations o1 electrolytes (Na+, K+ and CI-) and urea also total serum proteins, serum albumin and Serum globulin concentrations were all determined using auto analyzer (Bayer Clinical Chemistry Analyzer, Germany). Lactate dehydrogenase (LDH) activity was estimated based on the principle that LDH catalyzed the conversion of pyruvate to lactate; nicotinamide adenine dehydrogenase (NADH) was oxidized to nicotinamide adenine dinucleotide in the process. The rate of decrease in NAD H was directly proportional to the LDH activity. The LD H activity was estimated using a kit (Optimized Standard Kit; Roche/Hitachi), and the absorbance was read using a spectrophotometer [Shimadzu Double-beam Digital Atomic Absorption/Flame Spectrophotometer Model AA-650 (202-37200), Shimadzu Corporation, Tokyo, Japan].

The research was carried out following the Animal Research Committee of the Ahmadu Bello University, Zaria, Nigeria and National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication number 85-23), revised 1985.

Statistical Analysis

Values obtained were expressed as mean + S EM and subjected to statistical analysis using one-way analysis of variance (ANOVA), followed by Tukey‘s post-hoc test. The Programme used Jorthe analysis was Graph Pad Prism, Version 4.0 for Windows from Graph Pad Software, San Diego, California, USA (www.graphpad.com). Values of $P < 0.05$ were considered significant.

Results

Effect of Treatments on Serum Electrolyte Concentrations

Effect of Treatments on Sodium ion Concentration

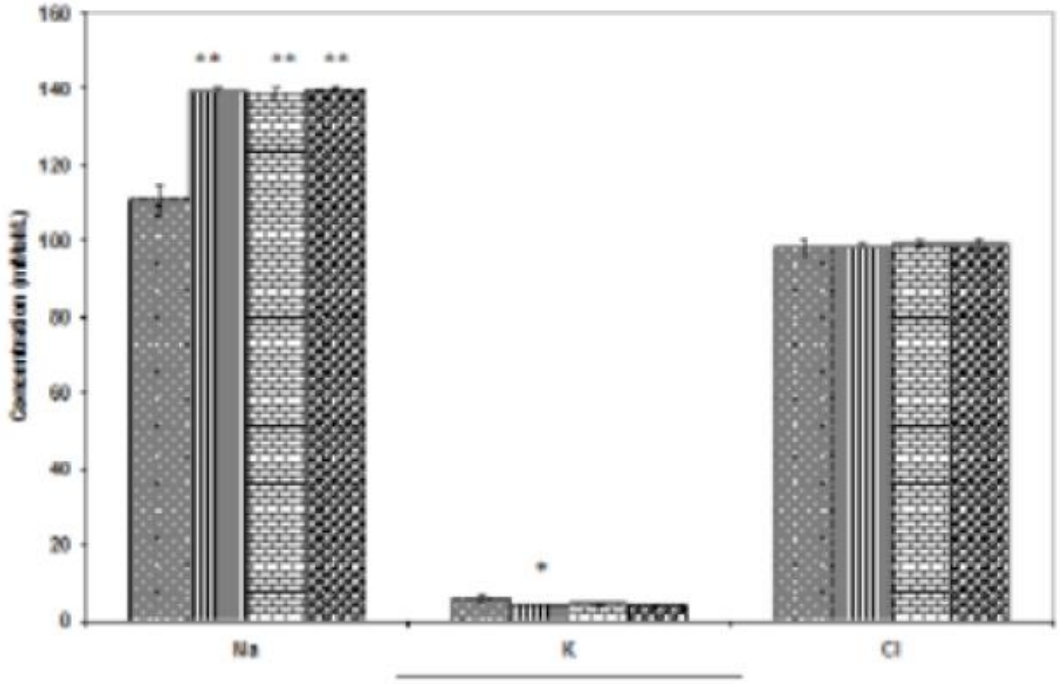
There were significant ($P < 0.001$) increases in Na+ concentrations in the CNP, GBP and CNP +GBP groups, when respectively compared to that of the control group. Change in Na+ concentration in the CNP group was not significantly ($P > 0.05$) different from that of GBP or CNP+GBP group. The difference in Na+ concentration in the GBP group compared to that of the CNP+GBP group was not significant ($P > 0.05$) (Figure 1.0).

Effect of Treatments on Potassium ion Concentration

The changes in K+ concentration in the GBP group when compared to those of the control and the CNP+GBP groups were not significantly different. There was a significant ($P< 0.05$) decrease in K + concentration in the CNP and CNP+G BP group5, when respectively compared to the concentration recorded in the control group. Potassium ion concentrations obtained in between the treatment groups did not differ ($P > 0.05$) (Figure 1.0).

Effect of Treatment on Chloride ion Concentration

There was no significant ($P > 0.05$) change in Cl- concentration in between the group3. The changes in the mean Cl- concentration in the GBP and CNP+GBP groups were not different, when compared to that of the CNP group (Figure 1.0)



#CONTROL-CN P-GBP-CNP+GBP

Figure 1. Effect of administration of Clonazepam (CNP), and/or Gabapentin (GBP) on serum electrolyte concentrations in Albino rats (n = 10)

Na = Sodium ion, K = Potassium ion, CI = Chloride ion

*= P ‹ 0.05 (CNP+GBP Vs Control)

**= P < 0.0001 (CNP, GBP, CNP+GBP Vs Control)

Effect of Treatments on Serum Proteins in Albino Rats

Effect of Treatments on Serum Total Proteins

There were no significant (P > 0.05) changes in the concentrations of total proteins, obtained in between the drug-treated groups, and when the concentration in each of the treatment groups was compared to that of the control group, Figure 2.

Effect of Treatments on Serum Albumin Concentration

Albumin concentrations in the CNP, GBP and CNP+G BP groups were higher (P < 0.01), when respectively compared to that of the control group. There were no significant (P > 0.05) changes in albumin concentrations recorded between the drug-treated groups, Figure 2.

Effect of Treatments on Globulin Concentration

Globulin concentration decreased significantly (P < 0.05) in the CNP and GBP groups, when respectively compared to that of the control group. There was no significant (P > 0.05) change in globulin concentration in the CNP+GBP group, when compared to

that o1 the control group. Globulin concentrations in between the drug treatment groups were not different (P > 0.05)

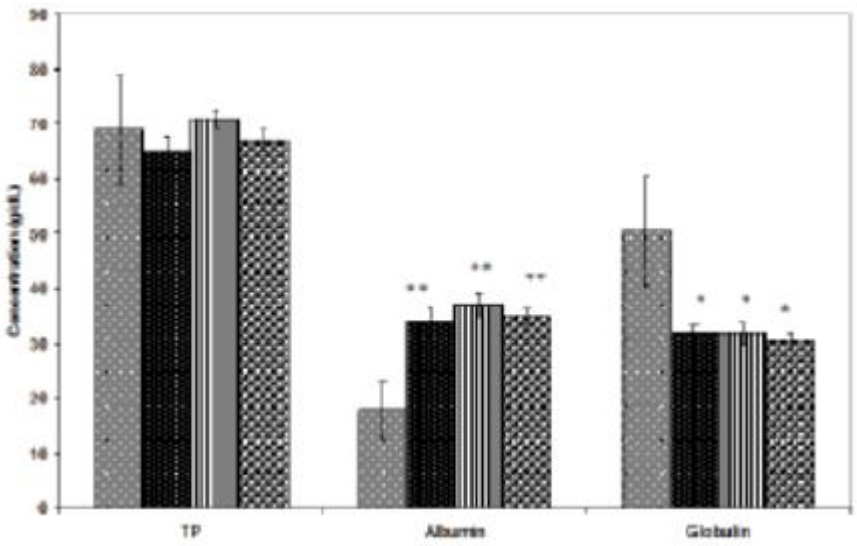


Figure 2. Effect of administration of Clonazepam (CNP) and/or Gabapentin (GBP) on serum proteins in Albino rats (n = 10)

TP = Serum Total proteins; P*= < 0.05 (CNP, GBP, CNP+GBP Vs Control)

** P = < 0.01 (CNP, GBP, CNP +GBP Vs Control)

Effect of Treatments on Serum Urea Concentration

Urea concentration in CNP and GBP groups was lower (P < 0.05) when compared to the value recorded in the control group. There was no change in urea concentrations obtained between the antiepileptic drug-treatment groups, Figure 3.

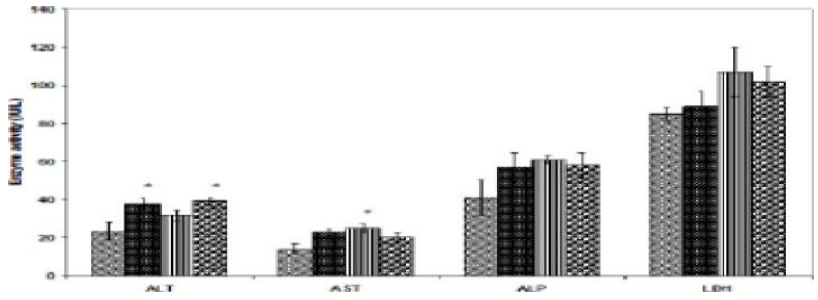


Figure 3. Effect of administration of Clonazepam (CNP) and/or Gabapentin (GBP) on urea concentration in Albino rats (n = 10)

P= ‹ 0.05 (CNP, GBP Vs Control)

Effect of Treatments on Liver Enzyme Activities

Effect of Treatments on Alanine Aminotransferase

The ALT activity in the CNP and CNP +GBP groups rose significantly ($P < 0.05$), when compared to that o1 the control group. The ALT activities in the GBP group and that of the control were not significant. There were also no significant ($P > 0.05$) changes in ALT activities between the drug- treated groups, Figure 4.

Effects of Treatments on Aspartate Aminotransferase

A significant ($P < 0.05$) increase in AST activities was recorded in the GBP group, when compared to that of the control group. Increases in AST activity in the CNP and CNP +GBP groups were not significantly ($P > 0.05$) different, also, no significant ($P > 0.05$) change in AST activity, when the treatment groups were compared, Figure 4.

Effect of Treatments on Alkaline Phosphatase Activity

Changes in ALP activity in the CNP, GBP and CNP+GBP groups, when respectively compared to that of the control group were insignificant ($P > 0.05$). The increase in ALP activity in the GBP group, when compared to that of the CNP or CNP+GBP group wa3 insignificant. Similarly an insignificant increase wa5 recorded in the CNP +GBP group when compared to that of the CNP group, Figure 4.

Effect of Treatments on Lactate Dehydrogenase Activity

There were no significant ($P > 0.05$) changes in LDH activity in the CNP, GBP and CNP+GB groups, when respectively compared to that o1 the control group. There was a relative, but insignificant ($P > 0.05$), decrease in LDH activity in the CNP group, compared to either that of the GBP or CNP +GBP group. Although the LDH activity in the GBP group increased over that recorded in the CNP+GBP group, the difference in the activities was not significant, Figure 4.

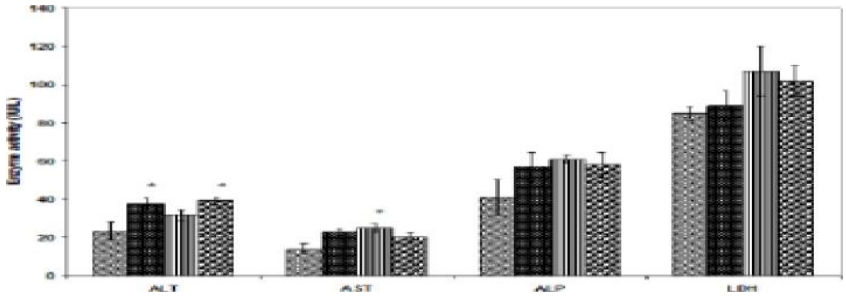


Figure 4. Effect of administration of Clonazepam (CNP) and/or Gabapentin (GBP) on serum liver enzyme activities in Albino rats (n = 10)

ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, ALP = Alkaline phosphatase, LDH = Lactate dehydrogenase.

*P= < 0.05 (ALT = CNP+GBP Vs Control; AST = GBP Vs Control)

Discussion

The increase in Na+ concentration in the antiepileptic drug (AED)-treated groups agrees with the findings of others [18] that demonstrated an increase in Na+; but disagrees with that of others [19], who reported hypernatremia following the administration of CNP and carbamazepine. The result of the present study also disagrees with that of others [9], who obtained hypernatremia following CNP administration. According to Liamis et al [15], GBP exerts a transient inhibitory effect on ADH release by the posterior pituitary gland, and it increases water reabsorption in the collecting duct of the kidneys, GBP has also been shown to reverse the hypernatremia induced by CNP therapy. The reason for the decrease in K+ concentration in the CNP-treated group is not known. However, hypokalemia may be observed with increased activity of the adrenal cortex, and this may cause the body to reclaim Na+ from the urine in exchange for K+ excretion. Thus, high Na+ concentration was obtained in the present study with relative decrease in serum K+ for the CNP group.

The increase in albumin concentration observed in the present study may be as a result of over- production of cortisol by the adrenal glands [20]. The arene oxide metabolites of GBP and CNP may cause oxidative stress [10]. GBP group had the greatest increase in serum albumin, indicating pronounced increase in cortisol production. The decrease in globulin concentration in the AED-treated groups agree with the results of Ashraji [21] who showed that CNP therapy caused decrease in IgA and IgG levels. GBP was also reported to cause an induction of transient selective IgA deficiency. Rats co-administered with CNP and GBP had the greatest decrease in globulin concentration, indicating higher decrease in immunoglobulin's A and G. This finding may be due to the synergistic effects of the drugs. The decrease in urea concentration in the AED-treated groups may be due to impaired protein metabolism, apparently, due to hepatic dysfunction. This is because the liver converts proteins into urea for excretion. This impairment may be due to persistent assault on the liver by the drugs or their metabolites, since the liver is the site of metabolism and detoxification of the drugs. The metabolism of these AEDs results in the formation of toxic epoxides from the action of cytochrome P450s, resulting in hepatic dysfunction [22].

There was an increase in ALT activity in the CNP and CNP+GBP-treated groups. This finding disagrees with the report of McNamara [23], who observed moderate elevation of ALT activity with GBP therapy. These changes were transient and may be due in part to induced synthesis of the enzymes. Transient elevation of ALT activity with CNP therapy may be due to hepatocellular damage [24]. The highest ALT activity obtained in the group co-administered with CNP and GBP demonstrated greater hepatocellular injury than in the CNP group. AST activity was found to be the highest in the GBP-treated group control. This finding indicates that GBP may cause more damage to the organs (liver, cardiac and skeletal muscles, kidneys, brain and blood cells), where the enzyme is found. Aldenhövel [25] reported that increases in AST, ALT and ALP activities are more frequent

and higher with diphenylhydantoin than with CNP. Except in the case of ALT activity, which was higher in the CNP group, the AST and ALP (although, insignificant) activities were highest by 40.7 P« and 35 P», respectively in the GBP group in the present study. [24], also reported increased activities o1 ALT, AST and ALP with long-term GBP therapy in rats, attributed to hepatocellular damage. Gabapentin, phenobarbital and Clonazepam have been 3hown to be highly toxic to the skin, liver, brain, kidneys and gastro-intestinal tract [26]. The predisposition to the toxic effects of GBP and CNP is presumed to be a consequence of an inherited deficiency in the detoxifying enzyme(s) epoxides hydrolase [27].

Conclusion

In conclusion, administration of CNP and/or GBP induced significant alterations in serum biochemical parameters in Wistar rats, which, in most cases may be transient, and can reverse when the drugs are discontinued. It is, however, recommended that serum biochemical parameters should be strictly monitored and regularly evaluated in individuals treated with CNP and/or GBP.

ETHICAL APPROVAL: Uruk University College of Pharmacy [UUCOP] Ethical Committee
CONSENT TO PARTICIPATE: Informed consent was taken from each subject before their enrolment in the study.
HUMAN AND ANIMAL RIGHTS: The study conducted in adherence with Helsinki Ethical standards.
CONSENT FOR PUBLICATION: Authors transfer the copyright to International Journal of Medical Sciences.
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CONFLICT OF INTEREST: The authors declare no conflict of interest, financial or otherwise.
DATA AVAILABILITY: The data was available in Uruk University College of Pharmacy [UUCOP] set and available on request.

References

1. Abbondazo SL, Ivey NS and Frizzera G. Dilantin associated lymphadenopathy: Spectrum of histopathologic patterns. Ame J Surg Path, 1995; 19: 675-686.
2. DeVriese AS, Philippe J and Van Reuterghem DM. Clonazepam hypersensitivity syndrome: report of 4 cases and review of the literature. Medicine, (BaItimore),1995; 74: 144-151.
3. Thakur S, Saraswathy GR and Maheswari E. Influence of vitamin C on Gabapentin induced haematotoxicity and oxidative stress in rats. Inter J Pharmacol Res and Inno, 2011; 2: 32-39.
4. http://www.ascp.com/publications/tcp/2000/mar/forum.shtml.

5. Riley RJ, Kitteringham NR and Park BK. Structural re9uirements for bioactivation of anticonvulsants to cytotoxic metabolites in vitro. B J Clin Pharmacol, 1989; 28: 482-487.
6. Almgrem M, Nyengaard JR, Persson B and Lavebralt C. Clonazepam protects against neuronal hyperplasia and abnormal gene expression in the megencephaly mouse. Neurobiol Dis, 2008; 32: 364-376.
7. Porter RJ and Meldrum BS. Antieizure drugs. In: Basic and Clinical Pharma cology, 10th Ed, Pp. 374394, New York, McGraw- Hill, 2007.
8. Macdonald, RL and Meldrum, BS. Principles of antiepileptic drug action. In: Mattson RH and Meldrum BS. (Eds.) Antiepileptic Drugs, 4th Ed, Pp. 61-78, Raven Press, New York, 1995.
9. Schmidt D. Efficacy o1 new antiepileptic drugs. Epi Cur, 201 1; 11(1): 9-11. Spielberg SP. In vitro analysis of idiosyncratic drug reactions. Clin Bioch, 1986; 19: 142-144.
10. Vijay P, Yeshwanth R and Bairy KL. Effect of Gabapentin sodium on biochemical Parameters of reproductive function in male albino Albino rats. J Physiol and Biomed Sci, 2002; 22(2): 14-18.
11. Hoshino C and Hoshi T. Clonazepam-induced agammaglobulinaemia clinically mimicking diffuse panbrochioloitis. B Med J Reports, 20 11; doi:10.1136/bcr.11.2010.3535
12. Kserk , Haugvicovä P and Mares P. Age-dependent Gabapentin effects oncortical stimulation in rats. Physiol Res, 1998; 47: 143-149.
13. Bryan CH and Waxman SG. Neuroprotection by sodium channel blockade with Gabapentin in an experimental model of glaucoma. Investit Ophthal and Visual Sci, 2005; 46(11): 4164-4169.
14. Fern R, Ransom BR, Stys PK and Waxman SG. Pharmacologic protection of CNS white matter during anoxia: Actions of Gabapentin, Clonazepam and diazepam. J Pharm Expe Ther, 1993; 266: 1549-1555.
15. Liamis G, Milioni3 HJ. and ElisaJ M. A review of drug-induced hypernatraemia. Clin Kidney J, 2009; 2(5): 339-346.
16. Sun, M, Van Rijn CM, Liu Y and Wang M. Combination of Lamotrigine and valproate in diHerent dose proportion in maximal electroshock seizure model in mice. Epi Res, 2002; 51(1-2): 5-11.
17. Rang HP, Dale MM, Ritter JM and Flower RJ. Mechanism of action on antiepileptic drugs. In: Rang and Dale Pharmacology, 6th Ed, Pp 578-584, Churchhill Livingstone, 2007.
18. Rakesh KR, Surendra R and Thangam J. Effect of valproic acid and Clonazepam on learning and memory in rats. I nd J PharmacoI,1991; 23(30: 185-188.
19. Kolb SJ. and Litt B. Management of epilepsy and comorbid disorders in the emergency room and intensive care unit. In: Ettinger, A. B. and Devinsky, 0.

(Eds.). Managing Epilepsy and Co- existing Disorders. Boston. Butterworth-Heinemann, 2002; Pp. 515-535.

20. Kaslow J. Proteins-albumin, globulins. 2011. Cited April, 2012. Available from http://www.drkaslow.com/html/proteins-albumin-globulins-html
21. AshraJi M, Hosseini SA, Abolmaali S, Biglari M, Azizi R, F arghadan M, Samadian A, Shaghafi S, Mombeini H, Saladjegheh N, Rezaei N and Aghamohammadi A. Effect of anti-epileptic drugs on serum immunoglobulin levels in children. Acta Neurol Belgica, 2010; 110: 65-70.
22. Swann AC. Major system toxicities and side-effects of anticonvulsants. J Clin Psychiatry, 2001; 62 s14: 16-2 1.
23. McNamara JO (ed.), Pharmacotherapy of epilepsy. In: Goodman and Gilman's The Pharmacological Basis al Therapeutics, 11th Ed, Pp. 501-525, New York: McGraw-Hill, 2006.
24. Ekaidem IS, Akpanabiatu MI, Uboh FE. and Eka OU. Vitamin B 12 supplementation: effect3 on some biochemical and haematological indices al ratson Gabapentin administration. Biokemistn, 2006; 18(1): 31-37.
25. Aldenhövel HG. The influence of long-term anticonvulsant therapy with diphenylhydantoin and Clonazepam on serum on aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase. E Arch Psych and Neurol Sci, 1988; 237(5): 312-316.
26. Misra U K, Kalita J and Rathore C. Adverse drug reaction: Gabapentin and Clonazepam cross reactivity: report of a case and review of literature. Postgraduate M ed J, 2003; 79: 703-704.
27. Salawu F and Danburam A. Hyponatraemia during low-dose Clonazepam therapy. Ann of Afri Med, 2007; 6(4): 207-208.