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Full Length Research Article

Biochemical changes in the Liver, Kidney and Serum of rat following chronic administration of cimetidine

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ABSTRACT

The effect of repeated administration of cimetidine, an antiulcer agent, twice daily for 7days on the phosphatase (acid and alkaline) and some function indices of rat liver and kidney was investigated. Sixty-four white albino rats were randomly grouped into two, A and B. Group A which consisted of 32 rats served as the control and were administered twice daily with 1ml of distilled water (the vehicle) for 7days while group B which served as the test group were also administered twice daily with 1ml of cimetidine (2.8mg.kg $^{-1}$ body wt) for 7days. Cimetidine administration (2.8mg.kg $^{-1}$ body wt) resulted in significant increases ($P<0.05$) in the activities of liver and kidney alkaline phosphatase while there was no significant change ($P>0.05$) in the serum alkaline phosphatase activities. Acid phosphatase showed a decreased activity ($P<0.05$) in the liver but an increase ($P<0.05$) in activity in the kidney following the administration of cimetidine. Serum albumin content exhibited significant reduction ($P<0.05$) throughout the experimental period. There was a decrease ($P<0.05$) in the concentration of serum bilirubin from after the third day which was accompanied by a significant increase ($P<0.05$) in creatinine concentration and these pattern of changes were sustained as long as the administration of the agent lasted. The results indicated alterations in the biochemical parameters investigated with a more pronounced effect on the liver function than the renal function tests. It can be inferred that chronic administration of cimetidine repeatedly apart from altering the activities of the phosphatase enzymes, will also adversely affect the functional capabilities of the liver and kidneys. ***(Afr. J. Biomed. Res. 9: 213 – 218)***

Keywords: *Cimetidine, Liver, Kidney, Phosphatases, Albumin, Bilirubin, Urea*

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INTRODUCTION

Cimetidine, one of the most important H_2-receptor antagonists (Bertram, 1989), is frequently used to relief pain from oesophagitis, gastric and duodenal ulcers as well as to provide effective treatment for dyspepsia and symptomatic reflux (Neal, 1992; Rang *et al*., 1995). It functions by blocking the acid secretion stimulated by histamine and gastrin (Rang *et al*., 1995; Altman, 1998). The side effects reported for the chemical compound include constipation, vomiting and nervous system dysfunction (Altman, 1998).

Since cimetidine is a commonly used drug for the management of incidence of ulcer, this study attempts to investigate the effect of its repeated administration on some biochemical parameters of the liver and kidney due to their roles in the detoxification and eventual elimination of chemical agent from the host system. The assay of some key "marker" enzymes in the tissues has previously been shown to be important indicators of monitoring damage to such tissues (Malomo, 2000; Shahjahan *et al*., 2004). The selection and use of certain indices like electrolytes, urea, creatinine and albumin among others also serve to indicate the state of functionality of the two organs (Zilva *et al*., 1991; Whelton *et al*., 1994; Yakubu *et al*., 2003a).

MATERIALS AND METHODS

Sixty-four male white albino rats (*Rattus novergicus*) weighing between 180 - 200g were obtained from the Small Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. They were housed in clean metabolic cages which were cleaned of waste twice daily at 12hr intervals. They were exposed to 12hr each of light and darkness daily at room temperature. Cimetidine was supplied by Unibios Laboratories Ltd., Mumbai, India. P- Nitrophenyl orthophosphate (disodium salt) was a product of Sigma Chemical Co. St Louis, U.S.A. Urea and creatinine assay kits were obtained from Quinica Clinica Aplicada, S.A., Spain while albumin and bilirubin assay kits were obtained from Randox Laboratories, Ltd., United Kingdom. Other reagents used were of analytical grade and were prepared in all glass-distilled water.

Administration of cimetidine

The rats which had been maintained on normal rat pellet and water *ad libitum* were allowed to acclimatize for two weeks after which they were randomly grouped into two. Rats in group A (32 rats) which served as the control were given 1ml of distilled water (the vehicle) twice daily at 12hrs intervals for seven days. Rats in group B (32 rats) served as the test group and were administered orally with cimetidine (2.8mg.kg $^{-1}$ body weight) twice daily at 12hrs interval for seven days. The drug and the distilled water were administered at the same time daily (0800-0900hr for morning administration and 1800-1900 for evening administration) throughout the duration of experiment. The animals in the two groups were sacrificed 24hrs after 1, 3, 5 and 7 daily doses.

Serum and tissue preparation

The methods of Akanji and Ngaha (1989), Ngaha *et al* (1989) and Akanji and Yakubu (2000) were used. Briefly, under diethyl ether anesthesia, the neck areas were quickly cleared of fur and skin to expose the jugular veins. The slightly displaced veins were then cut with a sharp sterile blade. The blood was allowed to clot for about 1hr at room temperature and then placed in a refrigerator at 10^{o}C for another 1hr. The sera were collected using dry Pasteur pipette and stored frozen for 12hr. The liver and kidney were homogenized in ice-cold 0.25M sucrose solution (1:5w/v), kept frozen overnight before being used for the various biochemical assays.

Determination of biochemical parameters

Alkaline phosphatase (EC 3.1.3.1) and acid phosphatase (EC 3.1.3.2) activities were determined using the method as described by King and King (1954). Serum albumin concentration was determined using the method of Doumas *et al* (1971). Total protein content of the samples was assayed by the Biuret method (Plummer, 1978). Sodium and Potassium ions content in the serum were measured using the flame photometer (Bassir, 1971). The procedure of Tietz *et al* (1994) was used to determine serum creatinine concentration while the serum urea concentration was determined by the

method of Kaplan (1965). The method of Evelyn and Malloy (1938) was employed to determine the serum bilirubin concentration of the samples. Data obtained were analyzed using Analysis of Variance (ANOVA) and complemented with the Student's t-test (Mahajan, 1997).

RESULTS

Table 1 illustrates the changes in the alkaline phosphatase activities in some rat tissues following the repeated administration of cimetidine. Liver alkaline phosphatase activities increased significantly (P<0.05) by 24hrs after cimetidine administration and the increase was sustained throughout the experimental period (P<0.05). There was an initial significant decrease in alkaline phosphatase activity of the kidney (P<0.05) (Table 1), which was followed three days later by a significant increase in enzyme activity (P<0.05) resulting to about 36% increase by the end of the experimental period (Table 1). These increases in the tissues were not reflected in the serum as there was no significant change in serum alkaline phosphatase activity throughout the duration of experiment (P>0.05) (Table 1).

Table 2 depicts the activities of acid phosphatase during the same period. There was significant reduction (P<0.05) in liver acid phosphatase activities throughout the period of administration. There was however a significant increase (P<0.05) in kidney acid phosphatase activities during the same experimental period. On the contrary, serum acid phosphatase activities did not manifest any significant change (P>0.05) when compared with the control values.

Table 1: Effect of repeated administration of cimetidine (2.8mg.kg $^{-1}$ body weight) on alkaline phosphatase activities of some rat tissues.

Days after Administration	Enzyme activities[a]					
	Liver		Kidney		Serum	
	Control	Test	Control	Test	Control	Test
1	0.35±0.03^{a}	0.91±0.03^{b}	12.21±1.57^{a}	8.80±0.52^{b}	0.79±0.07^{a}	0.84±0.02^{a}
3	0.34±0.04^{a}	0.76±0.04^{c}	11.76±1.66^{a}	16.42±0.67^{c}	0.81±0.06^{a}	0.84±0.04^{a}
5	0.37±0.02^{a}	1.39±0.13^{d}	12.00±1.49^{a}	24.22±0.63^{d}	0.80±0.07^{a}	0.86±0.02^{a}
7	0.36±0.03^{a}	0.82±0.06^{b}	12.36±1.09^{a}	34.60±1.71^{e}	0.83±0.03^{a}	0.79±0.06^{a}

[a]=Enzyme activities are expressed as specific activities in nM.min $^{-1}$mg prot $^{-1}$; Statistical significance were tested with ANOVA and complemented with Student's t-test; Values carrying different superscripts are significantly different (P<0.05); n=8 replicates ±SD.

Table 2: Effect of repeated administration of cimetidine (2.8mg.kg $^{-1}$ body weight) on acid phosphatase activities of some rat tissues

Days after Administration	Enzyme activities[a]					
	Liver		Kidney		Serum	
	Control	Test	Control	Test	Control	Test
1	18.02±0.37^{a}	9.44±0.75^{b}	7.73±1.16^{a}	19.80±0.93^{b}	0.47±0.03^{a}	0.49±0.02^{a}
3	17.93±0.54^{a}	12.00±1.95^{c}	7.80±1.10^{a}	26.68±1.11^{c}	0.46±0.04^{a}	0.50±0.01^{a}
5	17.30±1.14^{a}	10.57±1.12^{b}	7.86±1.08^{a}	22.69±0.79^{d}	0.48±0.02^{a}	0.51±0.01^{a}
7	17.80±0.62^{a}	8.16±0.30^{d}	7.77±1.14^{a}	34.20±2.03^{e}	0.49±0.02^{a}	0.53±0.00^{a}

[a]=Enzyme activities are expressed as specific activities in nM.min $^{-1}$mg prot $^{-1}$; Statistical significance were tested with ANOVA and complemented with Student's t-test; Values carrying different superscripts are significantly different (P<0.05); n=8 replicates ±SD.

Table 3:
Effect of repeated administration of cimetidine (2.8mg.kg $^{-1}$ body weight) on some rat liver function parameters

Days after Administration	Albumin (g.L^{-1})		Total Bilirubin (umol.L^{-1})	
	Control	Test	Control	Test
1	34.40±1.65^{a}	36.00±0.71^{a}	10.10±0.08^{a}	11.00±0.11^{a}
3	33.60±2.75^{a}	25.40±1.52^{b}	9.95±0.95^{a}	6.00±0.70^{b}
5	34.80±1.40^{a}	26.00±0.62^{b}	9.90±1.01^{a}	4.80±0.84^{c}
7	35.20±0.90^{a}	28.00±1.00^{c}	10.00±0.94^{a}	5.40±1.52^{c}

Statistical significance were tested with ANOVA and complemented with Student's t-test; Values carrying different superscripts are significantly different (P<0.05); n=8 replicates ±SD.

Table 4:
Effect of repeated administration of cimetidine (2.8mg.kg^{-1} body weight) on some rat kidney function parameters

Days after Administration	Na^+ (mmol.L^{-1})		K^+ (mmol.L^{-1})		Creatinine (mmol.L^{-1})		Urea (mmol.L^{-1})	
	Control	Test	Control	Test	Control	Test	Control	Test
1	136.40 ±0.55^{a}	129.60 ±0.55^{b}	5.70 ±0.07^{a}	5.76 ±0.04^{a}	102.00 ±5.38a	120.40 ±1.14^{b}	6.86 ±0.21^{a}	6.94 ±0.11^{a}
3	135.80 ±0.95^{a}	127.00 ±1.00^{b}	5.71 ±0.08^{a}	5.68 ±0.18^{a}	104.40 ±3.00a	114.00 ±1.63^{c}	6.90 ±0.09^{a}	6.48 ±0.62^{a}
5	136.10 ±0.70^{a}	128.00 ±1.00^{b}	5.64 ±0.18^{a}	5.68 ±0.17^{a}	103.00 ±3.93a	122.00 ±6.00^{d}	6.89 ±0.07^{a}	6.95 ±0.35^{a}
7	136.30 ±0.40^{a}	136.20 ±0.45^{a}	5.78 ±0.08^{a}	5.62 ±0.18^{a}	104.00 ±3.31a	116.00 ±2.00^{c}	6.78 ±0.32^{a}	6.50 ±0.40^{a}

Statistical significance were tested with ANOVA and complemented with Student's t-test; Values carrying different superscripts are significantly different (P<0.05); n=8 replicates ±SD.

Both albumin and total bilirubin concentrations were decreased in the serum of cimetidine dosed rats. These lower values were manifested after 3days of drug administration (Table 3). The concentrations of sodium ions, potassium ions, creatinine and urea following the administration of cimetidine twice daily for 7days are shown in Table 4. The concentrations of sodium ions were decreased significantly (P<0.05) for the first five days while there was significant increase (P<0.05) in the serum creatinine concentration throughout the period of cimetidine administration. The concentrations of potassium ions and urea in the serum compared favourably (P>0.05) with the control throughout the period of drug administration.

DISCUSSION

The biochemical indices monitored in the liver and kidney are useful 'markers' for assessment of tissue damage. The measurement of activities of various enzymes in the tissues and body fluids plays a significant role in disease investigation and diagnosis (Malomo, 2000), assault on the organs/tissues and to a reasonable extent the toxicity of the drug (Yakubu *et al*., 2003b). Tissue enzymes can also indicate tissue cellular damage caused by chemical compounds long before structural damage that can be picked by conventional histological techniques (Akanji, 1986). Alkaline phosphatase, a 'marker' enzyme for plasma and endoplasmic reticulum (Wright and Plummer, 1974; Shahjahan *et al*., 2004), is often employed to assess the integrity of plasma membrane (Akanji *et al*., 1993). The increase in alkaline phosphatase activities of the liver and kidney (Table 1) may be attributed to either *de novo* synthesis of enzyme molecules or loss of other proteins from the tissues (Wright and Plummer, 1974; Umezawa and Hooper, 1982). The increased enzyme activity might have resulted from increased functional activity of the tissues caused by the drug. Such increase in alkaline phosphatase activities can constitute threat to the life of cells that are dependent on a variety of phosphate esters for their vital process since there may be indiscriminate

hydrolysis of phosphate ester of the tissues. However, the non-significant change in the serum alkaline phosphatase activities (Table 1) is an indication that there was no leakage of the enzyme into the serum. This suggests that cimetidine may be inducing the synthesis of the alkaline phosphatase in the tissues and may not be a plasma membrane labilizer.

Acid phosphatase in its own case is a 'marker' enzyme for the lysosomal membrane (Collins and Lewis, 1971). The significant loss in acid phosphatase activities of the liver (Table 2) may be as a result of damage to lysosomal membrane resulting from drug administration and consequently leakage of the enzymes from the lysosome into the extracellular fluid (Malbica and Hart, 1971) or inactivation of the enzyme molecule by the drug (Akanji *et al*., 1993). We considered it more of enzyme inactivation since there was no corresponding increase in the acid phosphatase activity in the serum.

The observed reduction in albumin and total bilirubin concentrations in the liver (Table 3) indicated liver damage (Naganna, 1989), arising from the administration of the chemical compound. This may be an indication of diminished synthetic function of the liver which may consequently lead to enhanced retention of fluid in the tissues spaces (Naganna, 1989).

The significant decrease in the serum sodium ion concentration following the administration of cimetidine suggests a possible effect on the pump that maintains the constancy of its extracellular concentration even though the serum potassium ion concentration is unaffected. The significant increase in creatinine content of the serum following the administration of cimetidine (Table 4) may be attributed to compromise of the renal functional capacity. The drug might have either interfered with creatinine metabolism leading to increased synthesis or the tissue might have compromised all or part of its functional capacity of tubular excretion (Mitchell *et al*., 1972; Zilva *et al*., 1991). The non-significant effect on the serum urea concentration at the dose investigated may be that the drug has no effect on the urea cycle.

The result of the present investigation has shown that cimetidine is capable of producing alterations in the biochemical parameters investigated. The alterations appears to be more pronounced with the liver probably because it is the organ that is mainly responsible for detoxification of foreign compounds in the body since it has adversely affected the liver functional parameters investigated. The significant loss of liver alkaline phosphatase activity, the inhibitory/inactivation of the acid phosphatase activity, alterations in some of the synthetic functions of the liver and those of the serum sodium ions and creatinine concentrations observed here may explain some of the side effects like nervous system dysfunction experienced with the use of cimetidine.

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