Hematological Profiles and Alkaline Phosphatase Enzyme of Tissues in Male Mice Treated with Nifedipine Medication

Thikra Abd Aun Hassan[1], Nuha Yaarub Al-Harbi[2], Walla Salih Hassan[3], Hala Mohi Al_Saily[3]

[1]Assistant Prof., University of Karballa, Science College, Biology Dept, [2]Professor, [3]Assistant Prof. University of Babylon, Science College, Biology Dept.

Abstract

It is well recognized that certain types of medications for treatment of antihypertensive , known as Calcium channel blockers (CCBs) and calcium ion antagonists are two types of calcium ion antagonists., only work by blocking the free passage of Ca+. Nifedipine, amlodipine, felodipine, diltiazem, and other medications are among them..Fifteen Adult male mice weighing 180-250 gm were randomly divided into three groups and housed at the College of Science's animal house, where they were fed a regular mice diet of water and pellets ad libitum.. Tthe control group (n=5) were treated with 1ml of distilled water, the 2nd group (n=5) were treated orally with 20 mg/kg body weight of Nifedipine while the 3rd group (n=5) were treated orally with 60mg/kg by oral gavage for 35 days body weight of Nifedipine. The treatment was discontinued for 24 hours before the animals were sacrificed..

The data indicated biochemical parameters alkaline phosphatase (ALP) were found nonsignificant increased in kidney and testicular tissues,but hematological parameters no any variance has been shown in both Hb,PCV,RBC, HCT, MCV, RDW and platelets in all test groups, whereas MCHC did show major increases in both dosage groups of nifedipine, but WBCs count data suggested a decrease in 20mg/Kg dosage only.We showed that nifedipine could reduce lymphocyte counts Substantial increases in monocyte and neutrophil counts were achieved at doses of 60 mg/kg BW when compared to the control group, and significant increases in monocyte and neutrophil counts were achieved at doses of 20 BW when compared to the control group.,Above results indicate that the variations caused by nifedipine drug can have slight negative effects on the parameters of kidney, testis enzymes and hematological parameters.

Key words: *Hematological profiles, Alkaline phosphatase enzyme, nifedipine medication, male mice*

Introduction

Nifedipine is a calcium channel blocker and is divided according to the chemical structure into three categories [(1)]. It is used a vasodilation of the blood vessels, that the drug has a chemical composition, has the chemical formula C17H18N2O6 and a molecular weight of 346.3. [(2)].The drugs that block calcium channels are used as the essential choice for the treatment of hypertension include calcium channel blockers,angiotensin-converting enzyme(ACE)inhibitors, angiotensin II receptor type1 (AT1) antagonists, diuretics, b -blockers, and a -blockers the mechanism by which each drug lowers blood[(3).] Calcium channel blockers block calcium to flow out of through the L-type voltage-dependent calcium channel at the standard of vascular smooth muscle, thereby damage the excitation-contraction process[(4&5)]. that fundamentally extend vessels and nondihydropyridine derivatives that, lower cardiac activity with a decrease of its muscle contraction[(6)]. Intestinal ALP, Placental ALP, Germ cell ALP, and tissue nonspecific alkaline phosphatase (L/B/K) ALP are the four parts of this enzyme, which are separated into four divisions based

Corresponding author:
Nuha Yaarub Al-Harbi
sc.nuha.yaarub@uobabylon.edu.iq

on the place of tissue expression. [7].In normal serum, more than 90% of total ALP activity is attached to bone and liver isoenzymes. The relative ratio of the individual enzyme in serum is related to the intensity of lesion of the tissue from which it is produced. Thus, one cannot foresee the relative rate of the different forms of enzyme in the patient's body.An estimate of ALP activity from blood can detect abnormalities in certain health conditions, including liver disease and, bone disease[8,9].

Materials and Methods

Animals and drugs:

The animals, weighing 180-250 gm, were housed in the College of Science's animal home and fed a regular mouse diet of water and pellets ad libitum. The control group (n=5) received 1ml distilled water, the second group (n=5) received 20 mg/kg body weight of Nifedipine orally, and the third group (n=5) received 60 mg/kg.for 35 days body weight of Nifedipine. The treatment was discontinued for 24 hours before the sacrifice of the animals The study employed nifedipine, which was bought from a local pharmacy. Based on human dosage comparisons, the concentration of these medications was generated by dissolving known milligrams in known volumes of distilled water. and body weight of animals.[10]

Hematological Assay:

For hematological analysis, blood was taken in a test tube containing 20 mg/mL ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. In one milliliter of each sample, the amount of red blood cells (RBCs) and white blood cells (WBCs), as well as hemoglobin, hematocrit, and other parameters, were counted. MCH, MCHC,MCV ,RDW neutrophil, lymphocyte, and monocyte counts using an automated blood analyzer (Sysmex KX21, Japan).

Tissue homogenate & Enzyme assay :

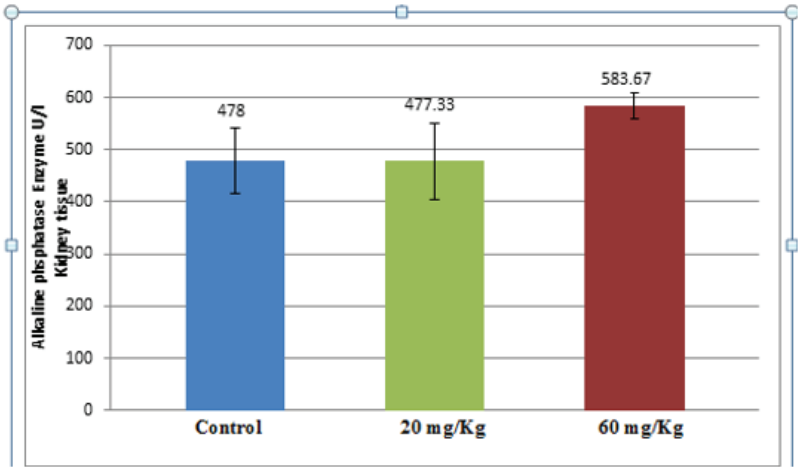
Kidney parts and testis were weighed and homogenized immediately to obtain a 50 percent (w/v) homogenate in ice-cold medium with 50m Tris-Hcl buffer (PH 7.5) and centrifuged at 10.000 for 30 [11] minutes; the supernatant was utilized as an enzyme source for several reflotron tests.

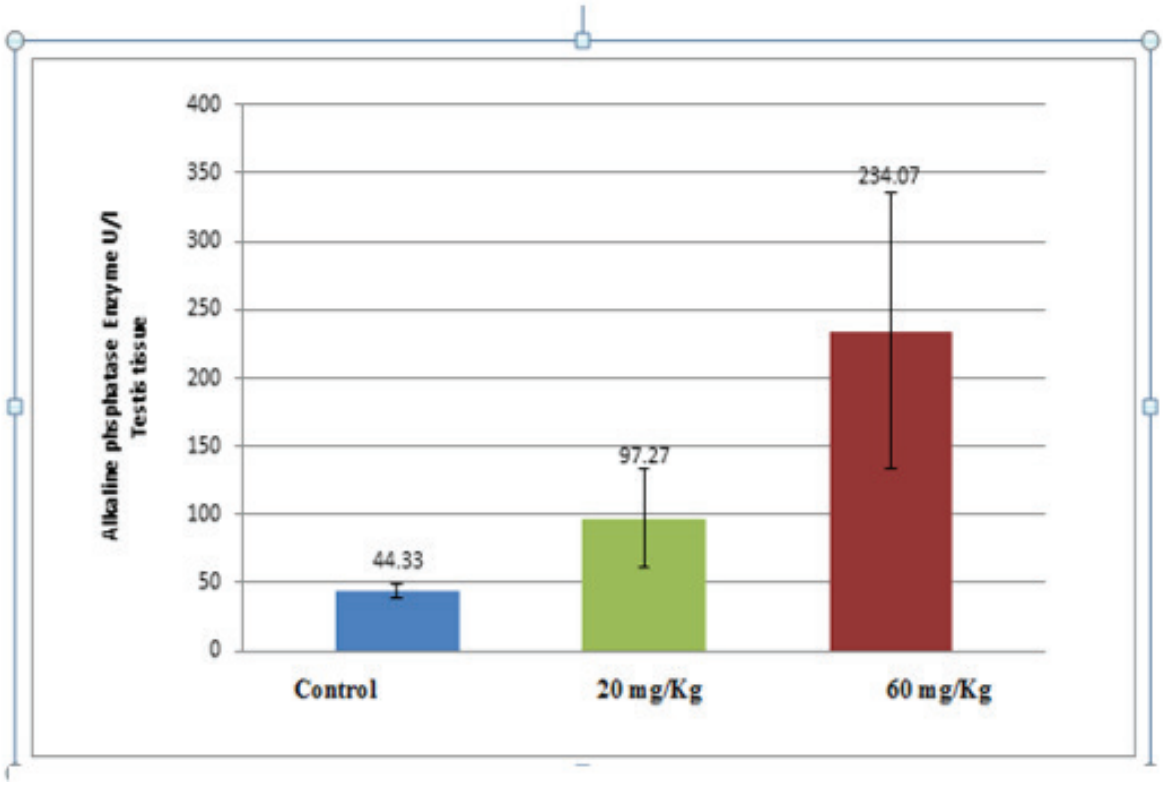
Statistical Analysis

All research used (SPSS) system/version 23 to analyze the data. The data is presented as a mean S.E. The analysis of variance (ANOVA), the Least Significant Difference (LSD) test, and Duncan were used in this study to compare means (percent).

Results and Discussion

Current study showed that in nifedipine treated mice there was non significantly increased in alkaline phosphatase enzyme in kidney tissue but this study revealed a significant increased in ALP testicular tissue when compared with the control group ((fig.1& fig.2).





The finding of the current study showed that in nifedipine treated mice there was non significantly increased in Alkaline phosphatase enzyme in kidney tissue after homogenate in two dosages 20mg/Kg (t=0.007,p=0.995). Also , in 60mg/Kg (t= 1.551,p=0.196) when compared with the control group(Fig.1).There are many studies that confirm high some enzyme due to the use of nifedipine treatment such as alkaline phosphatase, CPK, LDH, SGOT, and SGPT [12]. There is ALP an enzyme in many tissues and is available especially in the liver, bones, and kidneys, As this ALP enzyme in serum is high in patients with conditions that are hyperfiltration, such as diabetes mellitus (DM) and metabolic syndrome (MS)[13&14].

Other studies have shown that the predominant form of ALP enzymes in normal testis found in which accounts for about 95% of alkaline phosphatase is the tissue unspecific (liver-bone-kidney) isozyme with only effect amounts of placental-like alkaline phosphatase present[15]. Elevated ALP levels can be seen with worsening magnitude of damage on the testicular architecture after nefidipine drug treatment[16].

Table 1: Student's t – test: comparison of means between study group.

Treatment (Nifedipine medication)	Enzyme Concentration U/l (ALP)		t values	P Value
	Kidney	Testes		
20mg/Kg	477.33 ± 6.960	62.067±73.035	t=5.660	0.005 Significant
60mg/Kg	583.67±24.54	322.67±6.936	t=10.236	0.001 Significant

In the present study(Table 1) increase in ALP in 20mg/Kg treatment of nifedipine in kidney tissue compare with testicular tissue in the same dose . The Alkaline phosphatase levels of kidney tissue was increased compared to the enzyme itself in testicular tissue in 60mg/Kg with drug treatment. Therefore, changes in blood parameters remain due to the non - significant increase in the enzyme of kidney tissue[17] An enzyme ALP is mainly found in the cells membranes, which this enzyme have a biological role in transport. [14)(19).

Table 2: Complete blood count parameters in control , Nifedipine 20 and 60 mg /Kg for mice.

Hematological Parameters	Control	Nifedipine 20mg/Kg	Nifedipine 60 mg/Kg
RBCs (×10^6/µL)	9.59±0.41 a	10.01±0.79 a	11.37±0.7 a
Hb(g/dL)	45.00±5.77 a	43.33±6.69 a	44.10±0.55 a
HCT(%)	43.87± 4.12 a	38.67±5.38 a	48.60± 2.70 a
MCH(pg)	13.27±0.27 a	14.03±0.69 a	14.13±0.03 a
MCHC(g/dL)	29.23±0.82 a	32.60±0.64 b	33.00±0.30b
MCV(µm³)	45.53±2.28 a	46.10±0.70 a	43.30±0.50 a
WBCs(×10^3/µL)	16.07±1.37 b	5.00±2.05 a	13.37±1.57 b
Lymphocytes(×10^3/µL)	12.07±1.27 b	4.97±1.13 a	7.30±0.00 a
Monocytes(×10^3/µL)	1.73±0.03 ab	1.27±0.47 a	2.70±0.50 b
Neutrophils(×10^3µL)	2.23±0.13 a	0.47±0.12 a	4.93±1.07 b
PLT(×10^3µL)	275.00± 2.17 a	187.00± 55.05a	197.00±6.92a
RDW(%)	18.57±0.39 a	19.23±0.66 a	17.20± 1.10 a

Different letters refers to significant difference between groups

Similar letters refers to non significant difference between groups

According to the results of hematological assays, there were no significant differences in the quantity of RBCs, hemoglobin (Hb), hematocrit (HCT), MCH, MCV, RDW, and PLT in two groups treated with nifedipine.. In both dose and MCHC, there was a considerable rise. groups of 20 and 60 mg/kg when compared with the control group (32.60±0.64 and 33.00±0.30), respectively while WBCs decreased significantly in group of 20 mg/kg in comparison with control group ($p < 0.05$) . However there were insignificant differences observed in lymphocyte , monocyte and neutrophils in group when compared with control group($p>0.05$). In our study ,there is significant decrease in WBCs count in nifedipine treated with 20mg/kg and. As indicated above, There was also an decrease in the lymphocytes in both nifedipine dosage. Polymorphonuclear cells, including monocytes and lymphocytes, make up peripheral blood leukocytes. Advanced glycation end products (20), oxidative stress (21,22), angiotensin II (23), and cytokines may all activate polymorpho- and mononuclear leukocytes (24) Several inflammatory markers have been linked to a variety of diseases, according to mounting evidence., [25&26] Angiotensin II levels were significantly elevated in patients on

Nifedipine manifesting gingival overgrowth compared to the other 2 groups (p)[27]. The pro-inflammatory role of angiotensin is explained by the positive relationship between WBC and this molecule; increasing Angiotensin appears to exert its effects through boosting the production of pro-inflammatory cytokines such as IL-6 and IL-8 (6), which are effective inducers of WBC formation. (4th). Inhibition of angiotensin synthesis has been linked to a decrease in the number of white blood cells. [22]. More intriguingly, earlier investigations [23,24] have found a relationship between higher WBC count and hypertension, which could be due to angiotensin's inflammatory and vasoconstrictor effects. Π.

The effects of nifedipine-induced hepatitis are comparable to the effects of verapamil, another calcium channel blocker. With mild infltration of lymphocytes, neutrophils, and eosinophils and an extension of portal tracts with a mixed infammatory infltration of lymphocytes, histiocytes, neutrophils, and eosinophils, the parenchyma architecture might be conserved [18]&[27]. Towering serum ALP levels are often observed in renal osteodystrophy. The enzyme also rises in patients with conditions that are associated with hyperfiltration, such as diabetes mellitus (DM) and metabolic syndrome (MS) [9,10]. Alkaline phosphatase (ALP) is a membrane homodimeric enzyme that catalyzes the hydrolysis of organic pyrophosphate [8]. ALP is present in a variety of tissues, and is especially abundant.

Angiotensin has been hypothesized as an unique pro-inflammatory mediator generated by adipose tissue that has avital participation in vasoconstriction and the production of inflammatory factors such IL-6, IL-8, and vascular cell adhesion molecule [28] [29]. Inhibition of angiotensin synthesis has been linked to decline in number of white blood cells. [30]. More intriguingly, earlier investigations [23,24] have found a relationship between higher WBC count and hypertension, which could be due to angiotensin's inflammatory and vasoconstrictor effects.. Other study indicated that people who took the following medications, Diagnostic tests revealed that patients with a decreased relative lymphocyte count needed angiotensin converting enzyme inhibitors, -blockers, and mineralocorticoid receptor antagonists.. Relative lymphocyte percentage is a straightforward, low-cost, and widely available immunological measure with prognostic potential. In HHF, current risk classification techniques lack immune statussurrogates [31].

Conflict of Interest: The authors of this paper declare that he has no financial or personal relationships with individuals or organizations that would unacceptably bias the content of this paper and therefore declare that there is no conflict of interests.

Source of Funding: The authors have no sources of funding, so it is self-funding research.

Ethical Approve: We declare that the study does not need ethical approval.

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