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Studies on the Consequences of the Administration of Aqueous Extracts of Azadiracta Indica and Morinda Lucida on the Heamatological Parameter and the Activities of Phosphatases in Rats Cellular Tissues

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Abstract

Studies on the effects of the combination of aqueous extracts of Azadiracta indica and Morinda lucida on packed cell volume(PCV) and the activities of phosphatases of rat cellular tissues was investigated. Twenty eight (28) albino rats of six month old of an average weight 182.4g were grouped in to four groups; A, B, C and D. All the rats in the four groups were fed with fowl formulation feeds obtained from poultry store in VOM Jos, Plateau State, Nigeria and water was provided ad libitum for 18 days. Rats in group A, B, C were administered 0.5ml/kg body wt each of A.indica extract, only M.lucida extract, and combination of A.indica and M.lucida extracts orally respectively for 18 days. Group D contained seven rats designated "control". One of the rats from the control was sacrificed at day one, its cellular tissues (liver, kidney, small intestine, and large intestine) were collected and analyzed for PCV, Alkaline Phosphatase (ALP) and Acid Phosphatase (ACP) activities. One of the rats from each of the groups A, B, C and D were sacrificed at every three days interval and these tissues were also collected and analyzed for PCV, ALP, and ACP activities. The result showed a significant decrease in the activities of these enzymes as compared to the control values. The trends of the activities of these enzymes were similar following repeated administration of the extracts, and thereby caused alterations in the activities of both enzymes. This trend when subjected to student t-test analysis, showed significant difference at $P > 0.05$.

Key words: Azadiracta indica, Morinda lucida, PCV, ALP, ACP, Kidney, Liver, Small intestine, and Large intestine

Introduction

Most human diseases results from defect in metabolic routes of which hyperactivity, hypo activity and inhibition of metabolic enzymes are largely involved. These metabolic enzymes are compartmentalized in different tissues. Their elevations or decreases in amount serve as a biomarker for clinical diagnosis of some illness.

Medicinal plant extracts are believed to provide curative measure for several ailments [2]. Morinda lucida is commonly called 'Ugbaikolo' by the Igala tribe of Kogi State, Nigeria. It is abundant in the Northern and

Southern Nigeria [9]. It is used as a good medicament of various illnesses in West Africa for the treatment of ulcer, leprosy, and gonorrhoea. Extracts of the leaves and stem are also recommended in the treatments of various forms of fever, hypertension and cerebral complications [3].

Azadiracta indica is another medicinal plant popularly known as Neem or Nimba. It's common name among the Northern people of Nigeria is "Dongoyaro", Asian tree. In India, the tree is variously known as "Divine tree", "Heal All", "Nature's Drug Store", "Village Pharmacy" and "Panacea for all disease" [7].

Neem contains active ingredient called Azadirachtin [C₃₅H₄₄O₁₆], Mr; 720.71g/mol. Neem proves antimalarial effect[4], anti-inflammatory effect[10], anti-helminthes, antifungal, antipyretic effect[11]. It also showed remedies in obesity, rheumatism, thirst, tumors, vomiting, skin disease, jaundice, diuretic and many others [6]. Investigations on the above named species of plants was carried out to check their effects on the activities of phosphatases, in membrane and tissues and to examine the hematological indices of the selected tissues of experimental animal (albino rats), as a way of providing information regarding proper and better use of these plants for medications.

Materials and Methods

Fresh healthy leaves of *M. lucida* and *A. indica* used were obtained from Bruno and Aladi’s farm respectively both in Okura –Olfafia, Dekina Local Government Area, Kogi State, Nigeria. The leaves were collected, boiled in a mud pot for 30 minutes at 90-105 degree centigrade and the liquid extract was collected into a clean bottle with cover and kept in a refrigerator. A six month old Albino rats (*Rattus Novergicus*) average weights of 182.4 grammes were obtained from animal house of the Nigerian Institute for Trypanosomes and Research (NITR), VOM, Jos, Plateau State, Nigeria.

Animal Grouping and Extract Administration

Albino rats were randomly grouped into four in different cages, three cages contained seven rats each and the fourth contained seven rats as control. The extract was administered orally using syringe at 0.5ml (0.5ml/kg body weight) at every 24 hours. All the rats in the four groups were fed with fowl formulation feed obtained from poultry store, Jos, Plateau State, Nigeria and water was provided ad libitum for 18 days. The animal in group A was administered with only *A. indica* extract, group B was administered with *M. lucida* only, group C was administered with the combination of *A. indica* and *M. lucida*. Group “D”, the control group was administered with equivalent of water used in the plant extraction to make them pass through the same stress. A rat in the control group and a rat each of groups; A, B, and C were sacrificed after the 3rd day of feeding, with subsequent sacrifice of one rat each from the four groups at every three day intervals i.e. (3,6,9,12,15,) after the 18th day, the remaining rats in the control group (D), and the experimental groups were sacrificed and their selected tissues were collected as applicable to other days of sacrifice in an ice-cold 0.25M Sucrose solution, homogenized and kept in the freezer over night for total protein and enzyme activity determination.

Tissue Dilution

Tissue homogenate were appropriately diluted using 0.25M sucrose solution as diluents. This was used for both protein and enzyme assay. The dilution factor utilized is given in table 1 below.

Table-1: Dilution Factors

TISSUES	ACP	ALP	PROTEINS
Liver	600	300	30
Kidney	600	300	30
Small intestine	300	150	30
Large intestine	300	150	30

ACP: Acid phosphatase ,ALP: Alkaline phosphatase

Enzyme and Protein Determination

Both the enzymes and proteins assays were carried out at room temperature and the optical densities for the activities of the enzymes and proteins were measured with each having its separate blank sample. Measurements were

done using 1cm light path cuvette in a spectrophotometer [15].

Proteins Determination

To determine protein concentration in each tissue homogenate, Biuret reagent was used. The sample (1.0ml) appropriately diluted was added to 4.0ml of Biuret reagent without distil

water against the blank which contain 4.0ml of Biuret reagent and 1ml of distil water without sample homogenate. These were thoroughly mixed by swirling and were allowed to stand at room temperature for 30 minutes. The corresponding protein concentration was obtained from the standard curve which was plotted using egg serum albumin solution (1g/100ml) as standard protein. The absorbance was then taken at 540nm^[12].

Determination of Acid Phosphatase

The method of^[15] was used for the determination process of acid phosphatase. This method involves the hydrolysis of disodium phenylphosphate (DPP) and its spectrophotometric determination at 540nm followed with observable color in acid buffer as the reaction continues. The reaction was then halted on the addition of 1N solution of sodium hydroxide, after initial incubation at 37°C for 10 minutes. The activities of acid phosphatase were then calculated using;

$$\text{Activity } (\mu\text{molemin}^{-1}\text{ml}^{-1}) = \frac{\text{DE/min} \times 18.8 \times v \times L}{1000 \times V \times F}$$

Where, DE/min = change in optical density per minute, V= total reaction volume, v = volume of enzyme source, L = light path = 1.0cm, F = Dilution factor.

Determination of Alkaline Phosphatase

This determination employed the method of^[14]. Here ALP substrate was dispensed into labeled test-tubes and equilibrated to 37°C for 5 minutes, at an interval of 1 minute, 0.05mL of each of standard, control and sample were added to the respective test-tubes and gently mixed. It was then incubated for 10 minutes and ALP color developer was dispensed into test-tubes and thoroughly mixed. The absorbance was read from spectrophotometric machine at 590nm.

Protein Concentration Determination

The protein concentration of the enzyme samples were calculated comparing their values with standard curve of serum egg albumin which was obtained by the preparation of varying concentrations of serum egg albumin

(1-8mg/ml), and to 1.0ml each of the concentrations, 4.0ml of Biuret reagent was added. These was thoroughly mixed and left for 30 minutes at room temperature. The absorbance was then read at 540nm against the blank. The value obtained was used to plot a graph of optical density against serum egg albumin concentration. The total amount of proteins was then obtained by multiplying with the appropriate dilution factor.

Packed Cell Volume

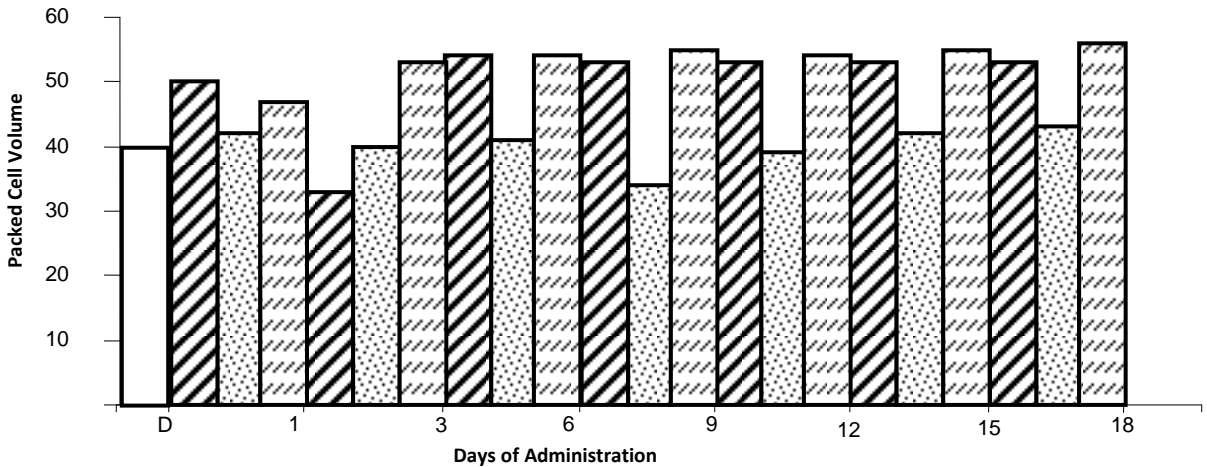
Blood of each of the albino rat in all the groups was collected into a Na-Heparinized microhaematocrit tube, with one ends of the tube sealed with plasticine. These was then placed on a microhaematocrit centrifuge rotor and whirled at 400rev/min for 300 seconds (5 minutes) and their corresponding values were read from the microhaematocrit reader in percentages.

Result

The results of this work were carefully scrutinized in term of the activities of the various enzymes and the hematological indices (PCV level) with regards to the administrations of these extracts. For the enzymes activity, only the combination of *M. lucida* and *A. indica* extract was used, while for packed cell volume determination (PCV), the individual extracts as well, the combination of the extracts was used. The control experiment (with administration of only water) having uniform values were represented D on the days of administration-axis of the graph, while the results of the rest days were represented by the number of daily doses of the extracts that was administered.

A significant increase of 35% of the PCV level above the control level in the group administered with the combined extracts of *M. lucida* and *A. indica*, 29% increase of PCV level was also recorded in the group administered with *A. indica* only, while about 5% increase in PCV level was observed in the group administered with only *M. lucida*. Figure 1 show the graphical representation of the changes in PCV level in these groups of rats.

Figure-1: Showing graphical representation of the changes in PCV level with days of administration of *A. indica*, *M. lucida* and the combination of the two above.



The activity of ACP in the liver, kidney, small intestine and large intestine upon and after the administration of the combined extract of *A. indica* and *M. lucida* was also considered. An initial increase in ACP activity was observed between the first day to the sixth day of administration of the extracts, fluctuation of the ACP activity, followed by a significant decrease in ACP activity between day eighteenth and twenty first of administration of the extract. Figure, 2 below show the graphical representation of the activities of ACP in various tissues studied.

Figure-2a: Graphical Representation of the Effects of Administration of *A. indica*, and *M. lucida* on rat liver and kidney acid phosphatase

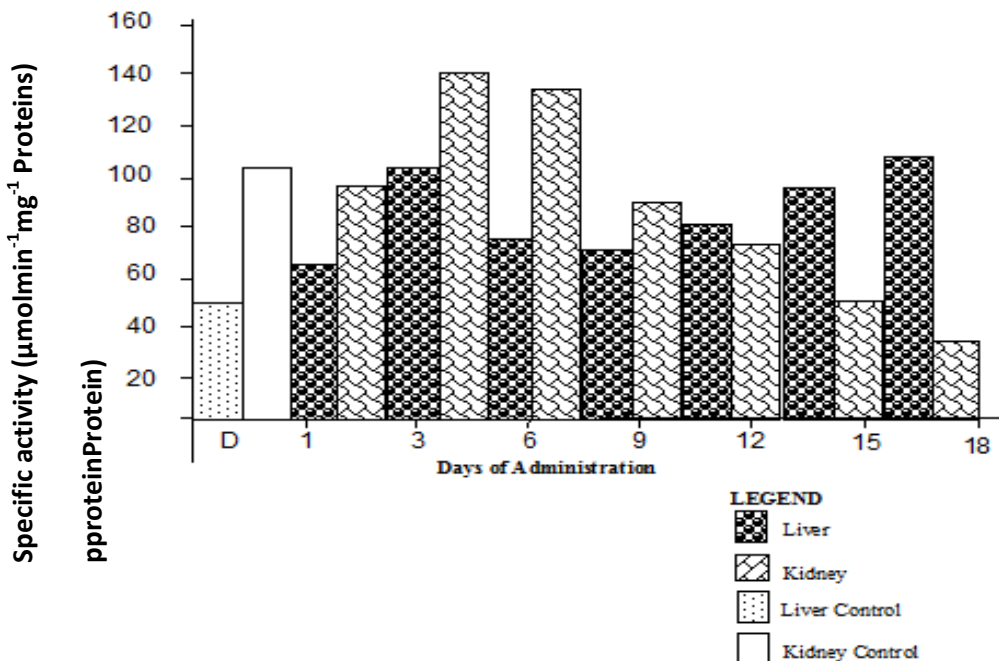


Figure-2(b): Graphical Representation of the Effects of Administration of *A. indica*, and *M. lucida* on rat intestine acid phosphatase

Proper analysis of the activity of alkaline phosphatase (ALP) showed that, the activities of ALP follow similar trends with that of ACP. This is equally represented graphically in figure 3 below.

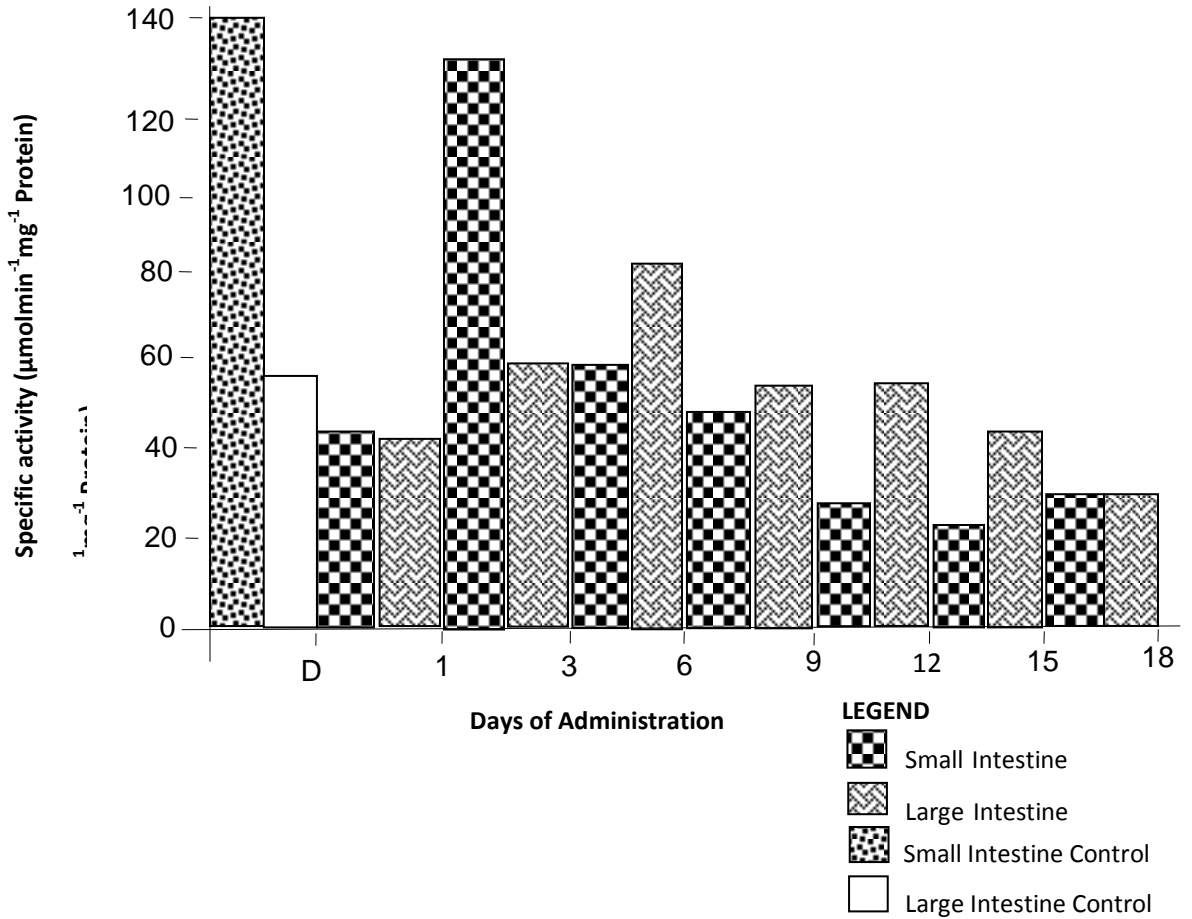


Figure-3a: Graphical Representation of the Effects of Administration of *A. indica*, and *M. lucida* on rat liver and kidney acid phosphatase

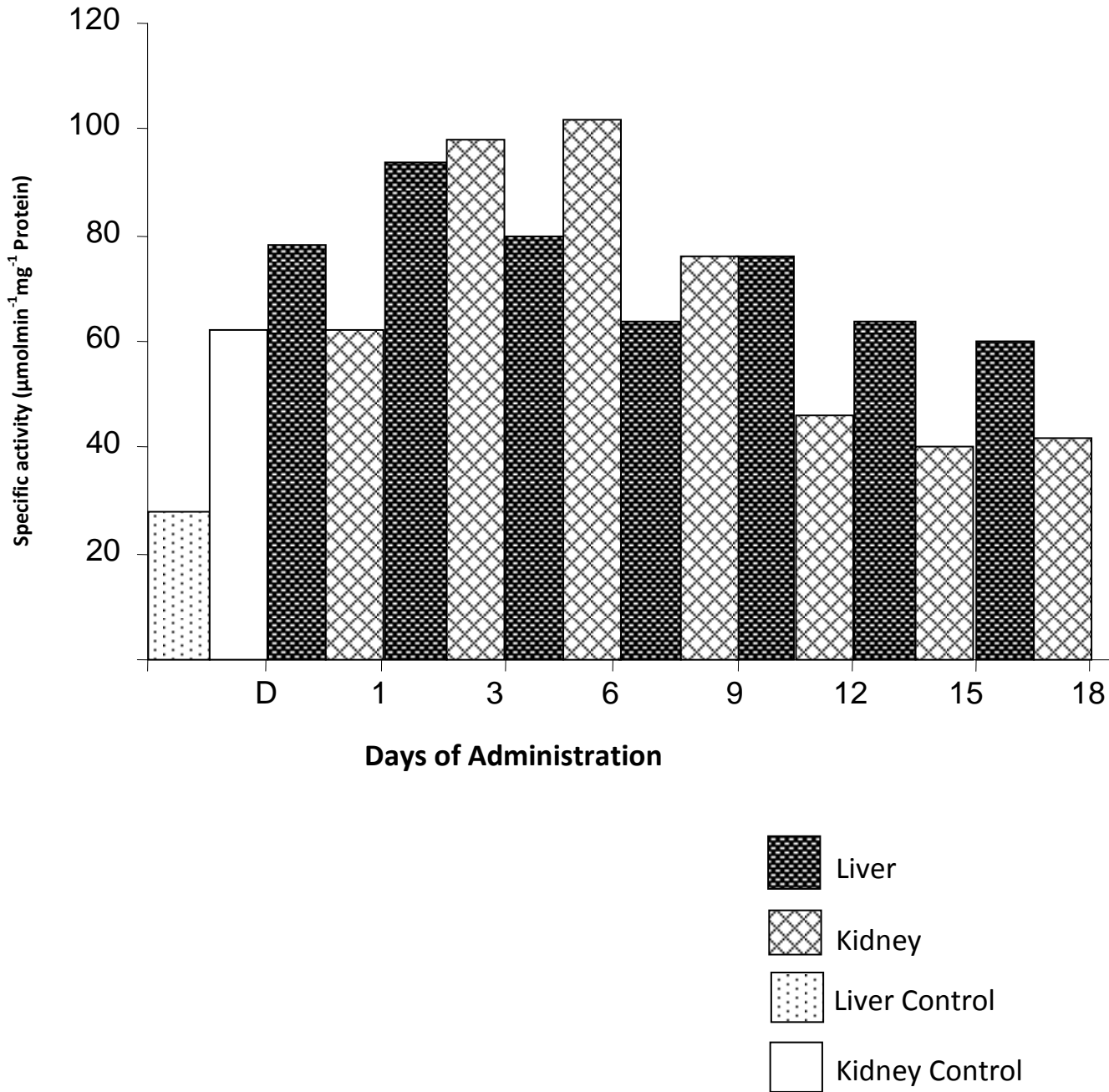
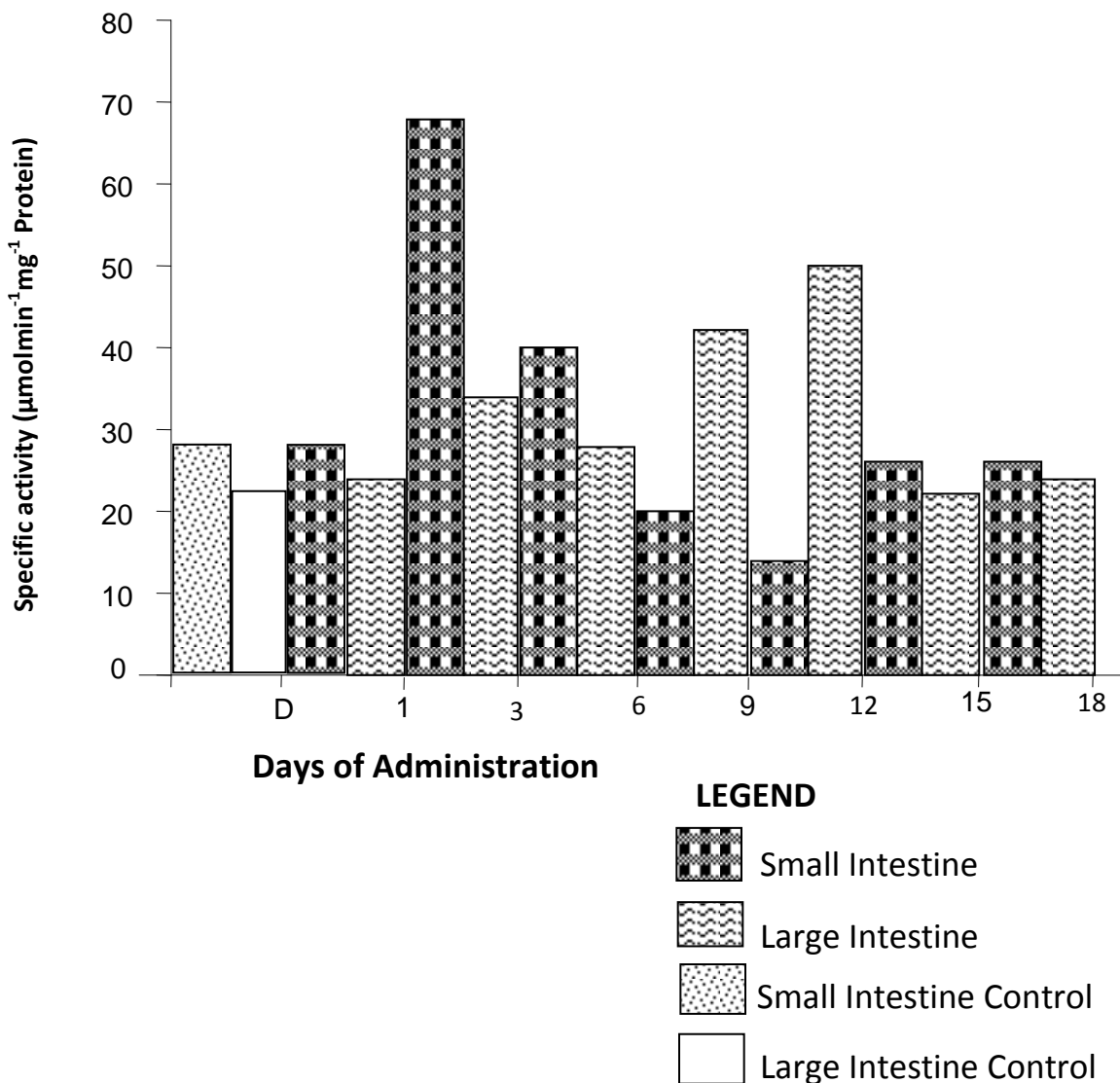


Figure-3(b): Graphical Representation of the Effects of Administration of *A. indica*, and *M. lucida* on rat intestinal alkaline phosphatase



Discussion

The packed cell volume or haematocrit is a measure of the relative mass of red cells present in a sample of whole blood. There are several methods of determining PCV. Methods such as centrifugation, calculation methods, and

radioisotope dilution methods can be used^[8]. In this research work, a centrifugation method was adopted for the PCV determinations.

Results of the rats PCV showed that, the group administered with the combination of *A. indica* and *M. lucida* showed an appreciable increase

in PCV value above those groups administered single extracts of *A. indica* and *M. lucida*, but a corresponding increase in PCV value in all the groups as the numbers of days of administration of the extracts increased. There was a 35% increase in PCV level in the group administered combinations of *A. indica* and *M. lucida*, a 29% increase in the group administered single extracts of *A. indica*, and a 5% increase in the group administered only *M. lucida*.

An increase in activity of alkaline phosphatase (ALP) in all the tissues was observed during the administrations of the combined extracts from day 3rd to 6th. After day 6th, there was a decrease in the activity of the enzymes in the various tissues. These sequential reductions in the enzyme activity could be traced to the presence of chemical compounds (physiochemical) present in the extracts^[1]. Also, because the plant extracts was believed to contain toxins (phytotoxins)^[5], this could cause damage to the plasma membrane leading to leakages of the enzyme from the site of locations, thereby leading to lower concentrations of the enzymes.

Specific activity of acid phosphatase (ACP) in the kidney, small intestine and large intestine showed gradual reductions below the control level with increase in days of administrations. These decreases in the activity of ACP in these tissues from day 12th to day 18th of administration could be as a result of the administered extracts causing inhibition to the activity of ACP. Also, fluctuation in the large intestine of the intestinal ACP was observed, but with a normal trend of decrease as with other tissues. The decrease in ACP activities in the various tissues examined could be linked to disruption of the membrane integrity of these tissues by the administered extracts.

Conclusion

Increased activities of ACP and ALP after administration of the extract signified the response of the enzyme to the constituents of the extracts, or as a result of activation or high concentration of the enzyme with lower substrate concentration. Decreased activities of these enzymes resulted from inactivation caused by the extracts, inhibition of the enzyme activities, blockage active site of the enzymes

from the constituents of the extract, substrate inactivation^[13], by the extracted substrate constituents, substrate analogue formation by the extracts which compete for the active site of the enzyme.

Decrease and increase in the activities of the enzymes in its respective tissues, provides information for the diagnosis of various diseases in the liver, kidney and other tissues of the body.

Packed cell volume (PCV) determination showed that the combinations of the extract of *A. indica* and *M. lucida* could increase the level of red cells present in the whole blood, hence, a good option for anemic patients and other related issues.

I hereby recommend the use of *A. indica* and *M. lucida* for various homes due to its positive result.

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