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Assessment Of Concentrations Of Micro And Macro Elements In Rats Serum After Oral Administration Of Aqueous Leaf Extract Of Sarcocephalus Latifolius (RUBIACEAE)

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***Abstract*—This study was designed to assess the concentrations of micro and macro elements in rat serum after oral administration of aqueous leaf extract of Sarcocephalus latifolia. The serum and medicinal plant samples were analyzed for Calcium (Ca), Cobalt (Co), Iron (Fe), Potassium (K), Magnesium (Mg), Sodium (Na) and Zinc (Zn) using Atomic absorption spectrophotometer and Neutron activation analysis . The concentrations of the elements in the leaf of the medicinal plants were 8493.0±323.0 (mg/kg) for Ca, 0.1±0.0 (mg/kg) for Co, 145.0±32.0 (mg/kg) for Fe, 9935.0±60.0 (mg/kg) for K, 2403.0±147.0 (mg/kg) for Mg, 34.5±0.3 (mg/kg) for Na and 8.2±1.9 (mg/kg) for Zn. Also the results showed the levels of the elements in the serum in the range of 2.970 ±0.733 to 3.387 ± 0.846 (mg/L) for Ca, 0.013 ±0.003 to 0.016 ±0.003 (mg/L) for Co, 5.954 ±2.091 to 7.210 ±2.974 (mg/L) for Fe, 301.100 ±12.560 to 319.850 ±15.501 (mg/L) for K, 2.776 ±0.343 to 3.000 ±0.289 (mg/L) for Mg, 0.373 ±0.229 to 0.411 ±0.253(mg/L) for Na and 0.573 ±0.126- 0.635 ±0.162 (mg/L) for Zn.The results indicate an increase in the levels of micro and macro elements after oral administration of calculated doses of the aqueous leaf extract of the medicinal plants. The concentrations of these elements were significant (P<.05) against the control group. The levels of these elements in the serum were determined weekly. The pattern of change in the micro and macro elements levels in the the serum was dose dependent. These results suggest that the presence of these elements in the medicinal plants may contribute to the availability of these nutrients in the human body as well.**

Keywords—Neutron activation analysis, Atomic absorption spectrophotometer, micro and macro elements, Sarcocephalus latifolia, rat serum.

I. INTRODUCTION:

Sarcocephalus latifolius (Synonym - *Nauclea latifolia*) is a tree member of the Rubiaceae family, growing commonly in some regions of Africa and used for medicinal purposes in folk medicine [1]. It is commonly found in Senegal, Cameroon, and Nigeria and as far as Sudan, tropical and Southern Africa. The leaves and roots of this plant or its combination in equal proportions have several local uses in the treatment of diseases, like fever, malaria, diabetes, gonorrhea, wounds, coughs, odontalgic problems, stomach aches, disorders of gastrointestinal tract and autonomic system, especially hypertension [2] [3].Studies have shown that medicinal plant contains both organic and inorganic constituents and many of the elements are present in our human body in the wide concentration ranges [4] [5]. Many of them play important roles in blood and organs structuring, for example, various enzymes containing Zn play essential roles in physiological functions and biological synthesis [6]. Fe is responsible for the transport of molecular oxygen in the blood (6). Medicinal plants are being used as valuable sources of food, fiber, fuel, shelter, mineral elements and other items in everyday use by humans and medicine for the prevention of illness and maintenance of human health [7].These medicinal plants can act by supplying necessary micro and macro elements such as Mg, Ca, Zn, V, Cr, Mn, Ni,Se and K which are well known in the treatment of many ailments [8] [9]. Numerous studies have demonstrated that micro and macro elements exist in various forms and kept a dynamic balance status in the human body [10]. Scores of metabolic reactions in the normal physiological processes are known to be related to metal ions. Deficiency or excess of these elements can induce body metabolic disorder and cellular growth disturbance [10]. Every element has its specific importance in the life system though it may be present in the human organism in very minute quantities. Excessive intake of some minerals can upset homeostatic balance and cause toxic side effects. For example, excess sodium intake is associated with high blood pressure and excess iron can cause liver damage. Minerals are inorganic substances, present in all body tissues and fluids and their presence is necessary for the maintenance of certain physicochemical processes which are essential to life [11]. Minerals are chemical constituents used by the body in many ways. Also, severe shortages or self-prescribed minerals can alter the delicate balance in body functions that promotes health. They excess or deficiency is responsible for upsetting the equilibrium and normal functioning of the human system. [12]. In view of the importance of these micro and macro mineral elements in the nutrition of humans, animals and plants and their metabolic inter-relationships which influences other vital factors needed for the survival of living organisms like enzymes, anti-oxidants, vitamins etc, it is important to regularly obtain up-to-date information on the minerals content of commonly consumed plant [13]. It is against this background that this study was designed in order to assess the bioaccumulation of these elements in serum after oral administration of the graded doses of the aqueous leaf extract of *Sacrocephalus latifolius.*

II. Materials and methods

A. Experimental animal

Wister albino rats of either sex weighing about (200g-250g) aged 5-6 months was used for the experiment. They were housed in clean iron cages and fed with standard animal diet. Blood samples were collected through the tail and centrifuged at 4000 rpm for five minutes using centrifuge, (model 800 electric centrifuges B – Bram Scientific and Instrument Co. England) for serum separation. The serum separated was analyzed for elements of interest by AAS (Bulk Scientific Model: VGP 210) using suitable hollow cathode lamps.

B. Sample preparation.

. Plant sample

The collected samples of the selected medicinal plants were freed from twigs and extraneous matter. Soil, grit, sand and dirt were removed by sifting through a sieve. To remove the remnants of adhering foreign matter, the samples were rapidly and thoroughly washed under tap water and with distilled water and then immediately dried at room temperature. The dried plant sample was grinded using a pestle and mortar and sieved. About 250 mg- 300 mg plant samples was weighed onto different polythene films wrapped and sealed for the analysis of the elements by neutron activation analysis (NAA).

C. Analysis of plant samples

The plant sample was analyzed by neutron activation analysis technique. This involves irradiating the sample using Nigeria Research Reactor-1 [NIRR-1] at Centre for Energy Research and Training, ABU Zaria, at a neutron flux of 2.5 x 10^{11} n/cm^2 s, for short half live nuclides and was irradiated for either 1 minute or 5 minutes depending on the nature and activity of the sample. The Long-live nuclides was irradiated for six hours at a neutron flux of 5.0 x 10 ^{11} n/cm^2 s and first counting exercise began four days after irradiation, each sample were counted for 30 minutes to analyze those nuclides with half-lives mainly in the order of hours or few days. [14],[15],[16]. The same batch of samples were recounted for one hour each after nine to ten days decay in order to analyze those nuclides with half-lives in the order of days and years. Finally the identification of gamma ray of product radio-nuclides through their energies and quantitative analysis of their concentration were obtained by using the gamma ray spectrum analysis software, Microsoft windows based software MAESTRO was used for spectrum analysis that is qualitative and quantitative analyses.WINSPAN 2004, [17].

D. Extraction of plants sample

100 g of the powdered leave sample of *Sarcocephalus latifolius* was dissolved in 1 L of distilled water and boiled for 30 minutes. The solution was filtered through a filter paper and evaporated in a water bath. The extract was placed in water bath to concentrate the extracts. The extracts were prepared based on the LD_{50} of 583 mg/kg (body weight) at progressive doses of 250 mg/kg, 450 mg/kg and 583 mg/kg body weight which was administered orally to the treatment groups for 28 consecutive days [18],[19].

E. Collection and handling of blood samples

Blood samples (about 2.0ml) were obtained weekly through the tail of each rat in both the control and the treated group with the aqueous leave extracts of *Sacrocephalus latifolius* medicinal plants. The blood samples were collected into sterile plain tubes and stored in the refrigerator at 21 ^{0} C for analysis of the concentration of micro and macro elements.

F. Analysis of blood Sample

The elements Ca, Mg, Co, Fe and Zn was analysed by atomic absorption spectrophotometer (AAS ,Bulk Scientific Model: VGP 210), while Na and K was analyzed by Flame Photometer (Model Jenway, PFP7).This involves digesting the sample with a mixture of (H_2SO_4 +HNO_3) [20]. After setting the AAS instrument to the right conditions for each element, the respective standard and sample solutions were aspirated into the flame in turn to determine their respective absorbance. Distilled water was always flushed into the flame to re-establish the zero absorbance. For each sample and element, the above procedure was repeated three times. The mean absorbance for each sample solution and standard solutions were calculated and recorded. To prepare a calibration curve for each element, a graph of mean absorbance against corresponding concentrations of the standard solutions was plotted.

III. STATISTICAL ANALYSIS

The data generated were analyzed by Analyze-it version 2.3 statistical software for Microsoft Excel and the significance was set at $P<0.05$.

IV. RESULTS AND DISCUSSION

The concentrations of the macro and micro elements such as Ca, K, Mg, ,Na, Co, Fe and Zn in the serum and the leaf of the selected medicinal plants are given in Table 1 and Table 2 and graphically presented in Fig. 1-7**.**In the *Sarcocephalus latifolius* plant, serum Ca ranged between 1.734 mg/L and 5.023 mg/L, but no significant difference was observed between control group and the group treated with different doses of the extract. The medicinal plants showed a rise in the serum Ca level which was high at the dose (583 mg/kg body weight) of the extract. The distribution level of this element Ca from the different doses of this extracts showed a regular absorption behavioral pattern. This shows that different doses have varied level of this element in the extracts compared with the control group. The concentration of the elements in the serum is dose dependent Fig 1.There were no significant variations of Ca in the serum samples between any of the various doses against control. This suggests that a potentially bioavailable form of Ca cannot be found in leave extract of *Sarcocephalus latifolius* despite the concentration of Ca in the leaves of *Sarcocephalus latifolius* (8493.0±323.0 mg/kg) Table 1. According to [21], when evaluating the food sources of Ca, the Ca content is generally of greater importance than bioavailability. Ca absorption efficiency is fairly similar from most foods sources. Bioavailability of Ca from plant source however can be affected by Ca chelators such as oxalate and phytate. Oxalic acid, like phytic acid, has the ability to bind some divalent metals such as calcium and magnesium thereby interfering with their metabolism [22]. Chelates bind many elements making them nutritionally unavailable, thereby inducing dietary deficiencies [23].

Table 1.Concentration of macro and micro elements (mg/kg) in the leaf of *Sarcocephalus latifolius*

Element	*Sarcocephalus latifolius* (leaf)
Ca	8493.0±323.0
K	9935.0±60.0
Mg	2403.0±147.0
Na	34.5±0.3
Co	0.1±0.0
Fe	145.0±32.0
Zn	8.2±1.9

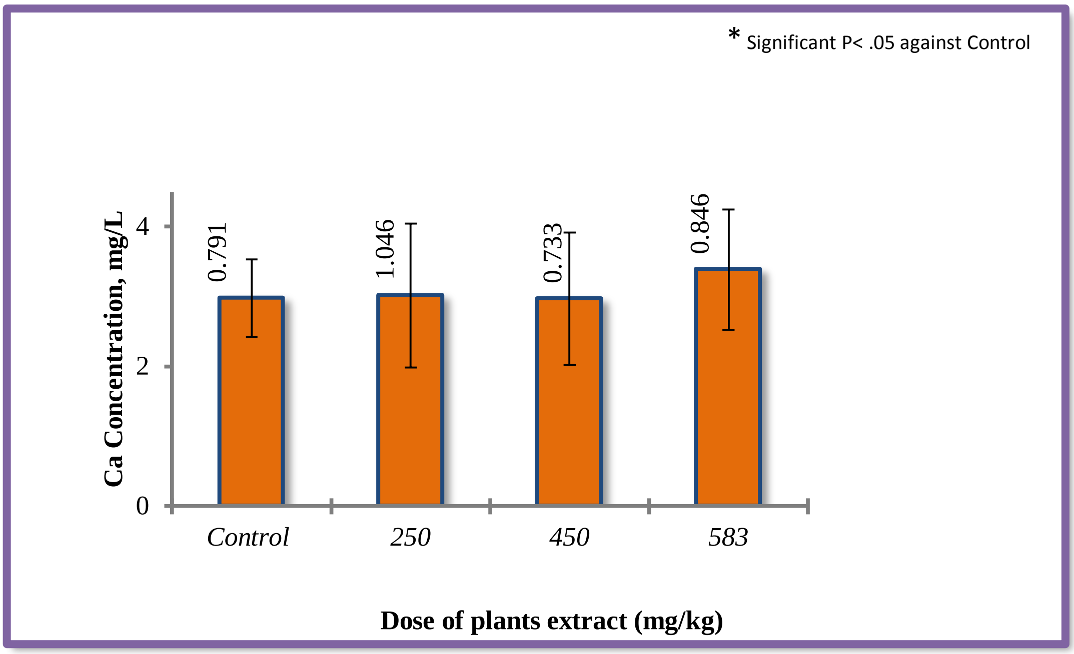

Fig ure1 Calcium concentrations in serum following oral administration of different doses of water extracts of *Sacrocephalus latifolius*

The serum level of potassium after oral administration of *Sacrocephalus Latifolius* plant extract K, serum level ranged between (199.600 – 336.400) mg/L. The concentration of K also increased in a dose dependent manner found to be highest at the dose of 583mg/kg body weight (319.850±15.501) mg/L. There were significant differences between control (280.217 ±35.874) mg/L group and 250 mg/kg (307.700 ±16.235) mg/L and 583 mg/kg [319.850 ±15.501] mg/L treatment groups respectively. K concentrations in serum suggest that the plants species present bioavailable forms of K in their leaves extract that are generally dose dependent. But *Sarcocephalus latifolius* presented the most bioavailable form of K as at the 250 mg/kg body weight dose, as it indicated a significant variation against the control group. *Sarcocephalus latifolius* content is [9935.0±60.0 mg/kg]. Regulation of plasma K is by renal excretion and movement of K from extracellular fluid to intracellular fluid. If these mechanisms are functioning normally, the amount of K ingested has little effect on plasma K. However, if one or more of the regulatory mechanisms is faulty, then the amount of K ingested can exacerbate abnormalities in plasma K. However, K absorption has been strongly linked to Na and Ca in its regulation of hyperkalaemia a physiological condition in which K blood level of between 3.8 mmol/L and 5.2 mmol/L is exceeded [24].

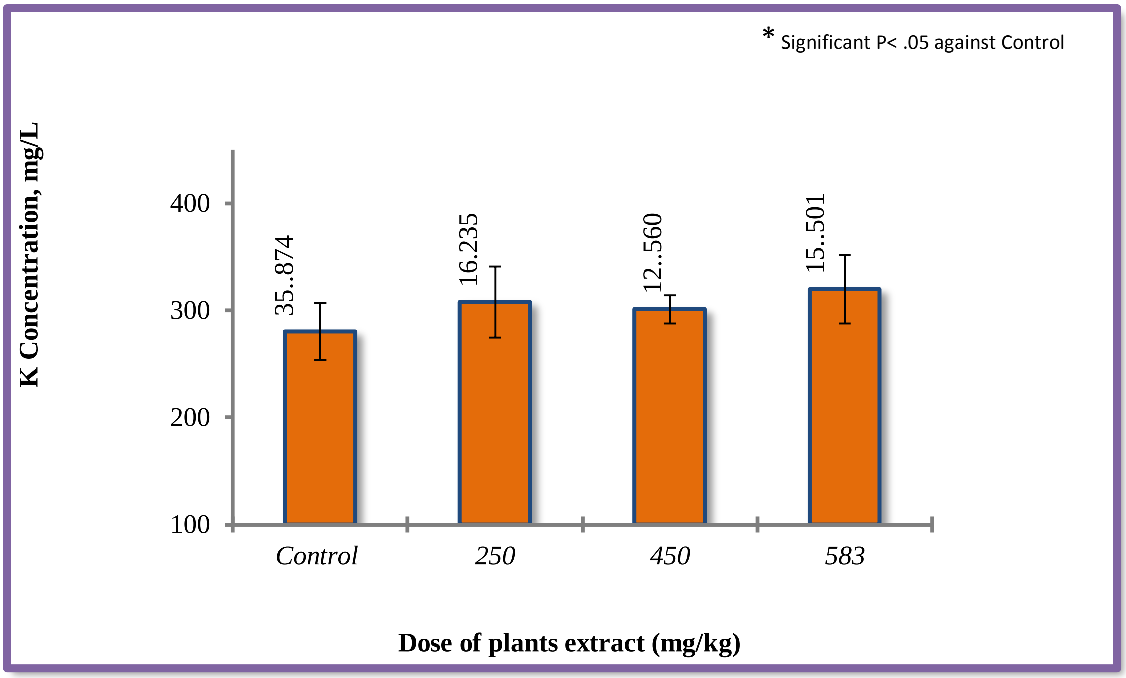

Figure 2 Potassium concentrations in serum following oral administration of different doses of water extracts of *S. latifolius*

In the groups treated with *Sacrocephalus latifolius* plant extract, Mg in serum ranged between 1.899 mg/L and 3.517 mg/L. There were significant differences between control (2.385 ±0.397) and 450 mg/kg (2.911 ±0.308) and the 583 mg/kg [3.000 ±0.289] mg/L treatment groups respectively Fig 3. The concentration of Mg in rat serum after oral administration of Sacrocephalius *latifolius* plant extracts Mg ranged from (1.899 - 3.517) mg/L. The result shows that there was significance difference [P<.05] between control groups (2.385±0.397)mg/L at 450 mg/kg [2.911±0.308] mg/L and at 583 mg/kg body weight, (3.000±0289) mg/L treatment groups respectively as shown in Fig. 5 and Table 1. This indicate that continuous used of these plants could add to the initial concentration of this element in the body which may result in toxic effect due to build up of Mg in the blood stream [25].Mg concentration in serum imply that *Sarcocephalus latifolius* presents bioavailability potentials that are dose dependent. Mg is very important element because it is actively connected with the transport of Ca and K [26]. Another factor that was found to be responsible for the bioavailability of Mg in serum is pH [27]. This result suggests that medicinal plants could be good sources of magnesium supplements. This is suggestive that the presence of these elements in the medicinal plants may contribute effectively to the macro and micronutrients requirement in humans as well.

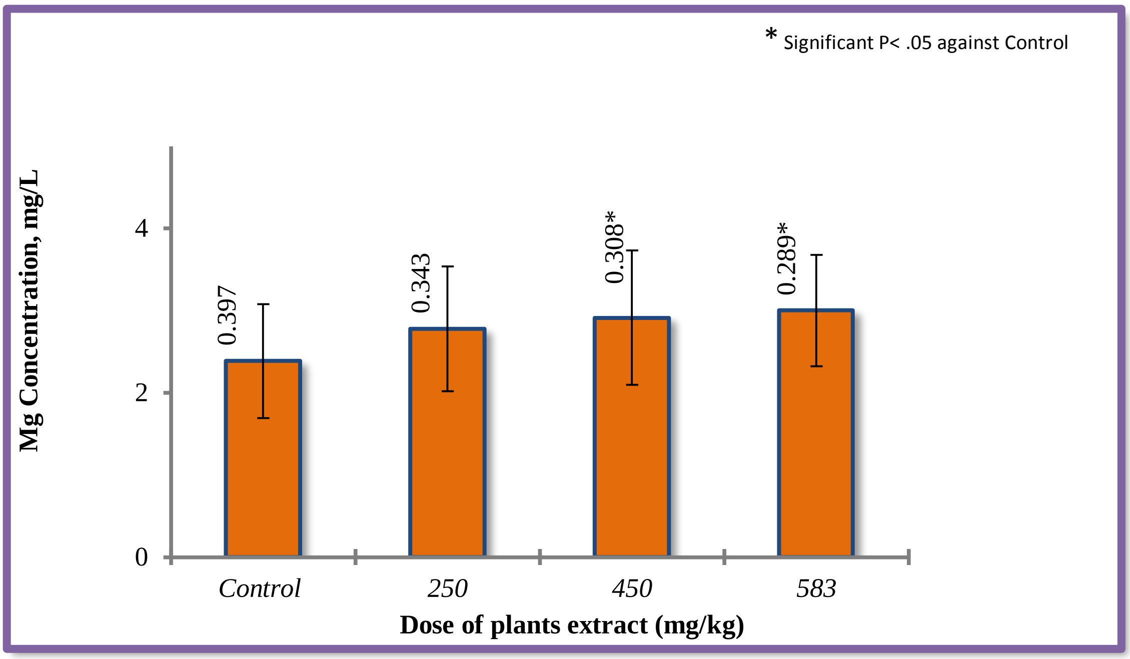

Figure 3 Magnesium concentration in serum following oral administration of different doses of water extracts of *Sacrocephalus latifolius*

The result obtained from treatment with extracts of *Sacrocephalus Latifolius* shows that, Na concentrations ranged between (0.007- 0.760) mg/L. The only significant difference [P<.05] was between the control [0.320±0.193] and the dose of 450 mg/kg body weight (0.411±253) treatment groups as shown in Fig 4. Na concentration in serum samples showed the widest variations within all groups studied, suggesting a highly unstable dynamics of Na in serum. The result generally indicated that both plant species do not potentially present reliable bioavailable forms of Na in leaves extracts. Although there seems to be a significant variation against control at 450 mg/kg body weight dose with the extract of *Sarcocephalus latifolius.* The concentration of osmotically active plasma solutes, of which sodium is the most important, governs these controls [28]. This is perhaps the reason why Na variations is so diverse due to its close link with water content, hence dilution the effects of dilution dynamics.

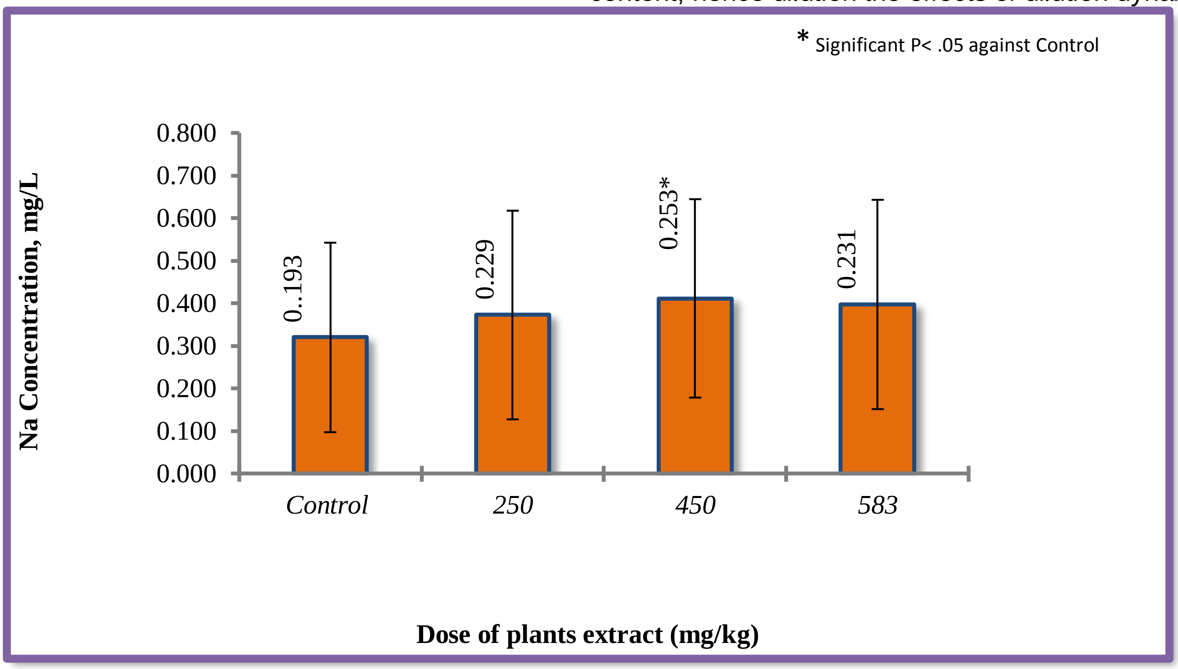

Figure. 4 Sodium concentration in serum following oral administration of different doses of water extracts of *Sacrocephalus latifolius*

The mean values for serum Co levels at different weekly intervals after oral administration of the medicinal plants have been given in [Table 2] and graphically presented in (Fig 5). In *Sacrocephalus latifolius* plant extracts, there were significant differences between the control group

(0.004±0.001mg/L) and all other dose groups of 250 mg/kg (0.014±0.003), 450 mg/kg body weight (0.016±0.003 mg/L and 583 mg/kg body weight, 0.013±0.003 mg/L) respectively. This group of rats showed an increase in the cobalt serum level at different doses compared to the control group. The evident rise in the cobalt serum level of the rats treated with this plant extracts could be due to the high contents of this element in the administered doses and absorption pattern by the rats. The results of Co concentration in serum samples generally suggests a dose dependent effect as there were statistically significant (P<.05) variations at the 583 mg/kg body weight dose for the medicinal plant. Although the result clearly indicated that *Sarcocephalus latifolius* presented bioavailability of Co. The absorption and bioavailability of Co have been reported to be dose dependent, with very low doses being almost completely absorbed, whereas larger doses are less well absorbed. Nutritional factors also influence absorption, for example, absorption is reduced by amino acids, and increased in Fe deficiency [29], [30], [31].

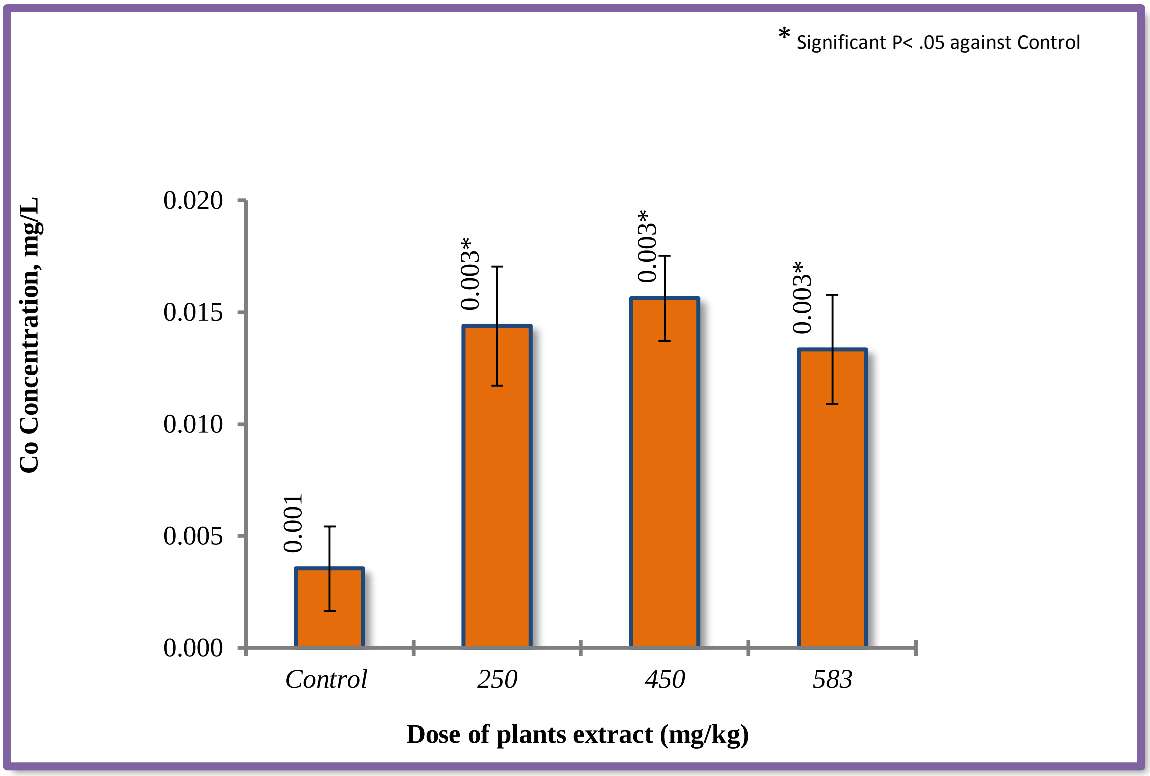

Figure 5 Cobalt concentrations in serum following oral administration of different doses of water extracts of *Sacrocephalus latifolius*

In *Sacrocephalus Latifolius* plant groups, serum Fe level ranged between (2.625 - 14.475) mg/L and a significant difference was observed between control group (4.561±2.072) mg/L at 583mg/kg (7.210±2.072) mg/L.In Comparism with other elements, the concentration of Fe were much higher in the single dose as shown in Table 1 and Fig. 6 compared to the control group. This could be clearly attributed to the much higher concentration of iron in the serum and the selected medicinal plants. This reveals that this plant could serve as good sources of iron for human body. Continuous use of this plant could also contribute very well to the iron content in the body. However, this plant should be used with caution to avoid assimilating excess iron. [32].Fe concentration in serum samples suggests that *Sarcocephalus latifolius* have equivalent bioavailable form of Fe in the leave extracts as their samples showed significant (P<.05) variation at 583 mg/kg body weight dose against control. Thus Fe absorption was found to dependent on the doses. Studies have shown that low levels of dietary Cu and Ca inhibit Fe absorption [33],[34],[35],[36]. But the most frequent existing Fe absorption inhibitor is probably phytic acid (or phytate when in the form of salt) [35], [37]. Another significant regulator of Fe concentration in serum is the mediation of hepcidin, which acts by down-regulating Fe release by enterocytes and macrophages [38], and by internalizing and degrading ferroportin, leading to decreased export into the circulation of absorbed and released Fe [39]. On the whole, the result of this study show concordance to the fact that Fe absorption is also generally influenced by dose levels [39].

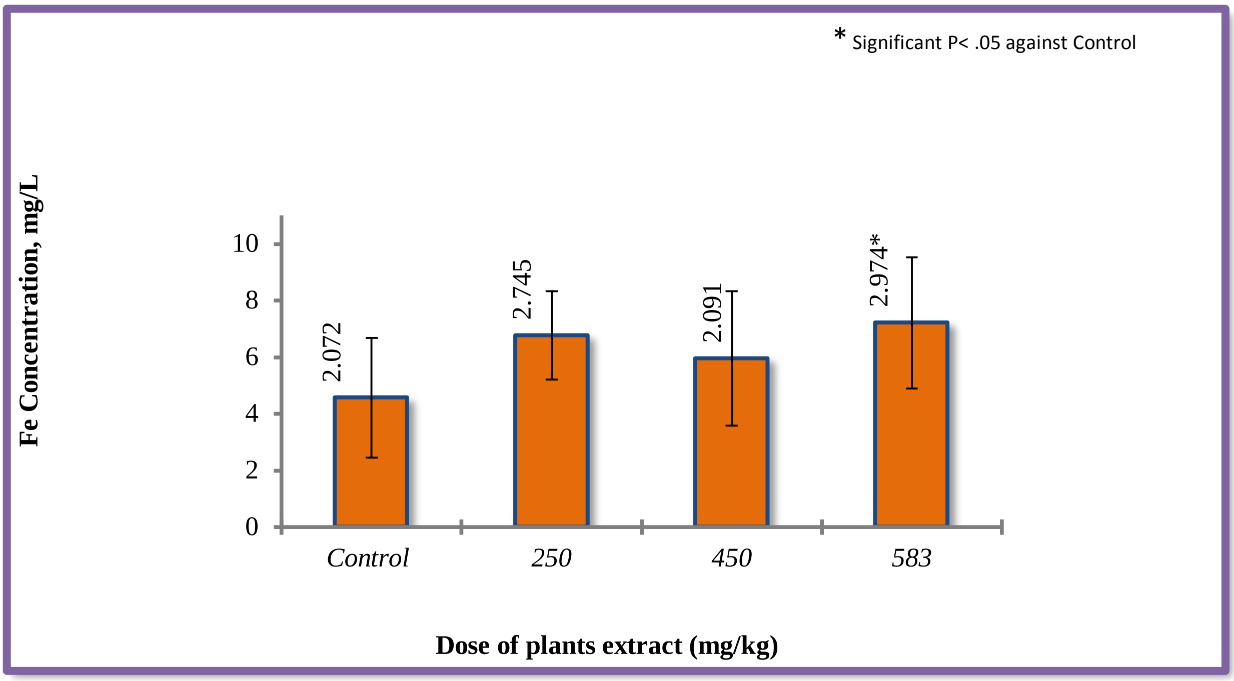

Figure 6 Iron concentrations in serum following oral administration of different doses of water extracts of *Sarcocephalus latifolius*

The effects of Zn concentration in rat serum after oral administration of Sacrocephalus *latifolius are* presented in Table 2 and Fig. 7 respectively. Serum Zn level ranged from (0.297 ± 0.884) mg/L in *Sacrocephalus latifolius* treatment groups.

There was significant difference between the control groups [0.389±0.061] and all other treatment groups. 250 mg/kg body weight (0.573±0.126) 450 mg/kg [0.608±0.121] and 583 mg/kg body weight (2.635±0.1620) mg/kg respectively. The results showed a regular absorption behavioral pattern between the treatment groups. The bioaccumulation of Zn in all the treatment groups was in a dose dependent manner. Zinc is the only elements

that activates 300 enzymes. Zinc concentrations in human body range from 200 – 300 mg which has been shown to play a significant role in the metabolism of amino acids and protein [40]. This study revealed that use of this plant could add to the amount of Zn in the human body as demonstrated in rat serum. The result of this study is also indicative that this medicinal plant could serve as potential source of this element. The value obtained in the serum is lower than those in the normal human body. The use of this plant could serve the purpose of supplying the daily requirement and economically serve this purpose of zinc supplementation.

Zn concentration in serum corresponds with the trend observed with K in serum. The results of Zn suggest that both plants species present bioavailable forms of Zn in their leaves extract that are generally dose dependent. On the other hand, bioavailability of Zn is enhanced by dietary protein, but plant sources of protein are also generally high in phytic acid [37], [41].

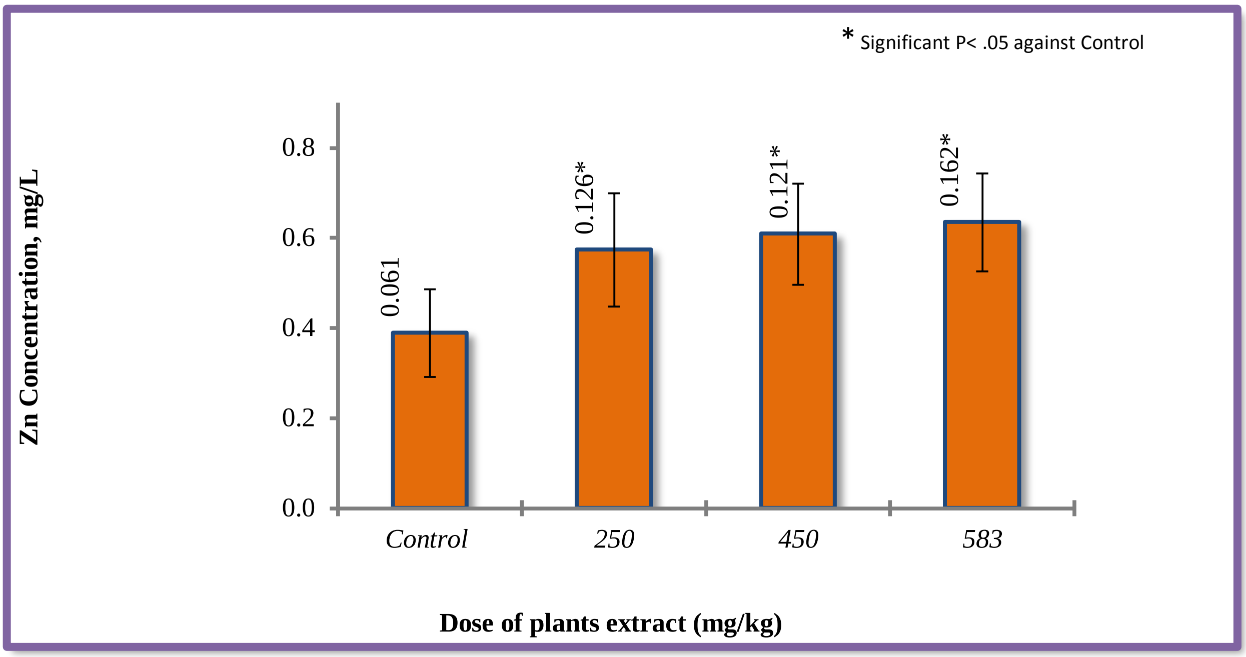

Figure 7 Zinc concentrations in serum following oral administration of different doses of water extracts of *Sarcocephalus latifolius.*

Table 2: Serum Elements Concentrations (mg/L) after oral administration of aqueous leaf extracts of *Sarcocephalus latifolius.*

Element	Plant	Dose (mg/kg)	Mean ±SD	SE	Min	Max	Median
Ca	*Sarcocephalus latifolius*	Control	2.983 ±0.791	0.228	1.961	4.712	2.704
		250	3.019 ±1.046	0.302	1.849	5.023	2.742
		450	2.970 ±0.733	0.212	1.734	4.532	2.756
		583	3.387 ±0.846	0.244	2.667	4.875	2.978
Co	*Sarcocephalus latifolius*	Control	0.004 ±0.001	0.000	0.003	0.005	0.003
		250	0.014 ±0.003	0.008	0.003	0.100	0.008
		450	0.016 ±0.003	0.008	0.003	0.100	0.008
		583	0.013 ±0.003	0.008	0.003	0.100	0.005
Fe	*Sarcocephalus latifolius*	Control	4.561 ±2.072	0.887	2.675	9.875	2.898
		250	6.767 ±2.745	1.081	3.996	13.800	5.068
		450	5.954 ±2.091	1.181	2.997	13.750	3.775
		583	7.210 ±2.974	1.147	4.345	14.475	5.200
K	*Sarcocephalus latifolius*	Control	280.217 ±35.874	10.356	199.600	320.000	288.600
		250	307.700 ±16.235	4.687	272.000	319.600	313.800
		450	301.100 ±12.560	3.626	279.600	318.800	299.700
		583	319.850 ±15.501	4.475	279.600	336.400	317.800
	Sarcocephalus latifolius	Control	2.385 ±0.397	0.115	1.899	2.945	2.509
		250	2.776 ±0.343	0.099	2.274	3.397	2.729
		450	2.911 ±0.308	0.089	2.519	3.517	2.812
		583	3.000 ±0.289	0.083	2.544	3.442	2.988
Na	*Sarcocephalus latifolius*	Control	0.320 ±0.193	0.056	0.007	0.478	0.421
		250	0.373 ±0.229	0.066	0.008	0.607	0.431
		450	0.411 ±0.253	0.073	0.017	0.760	0.490
		583	0.397 ±0.231	0.067	0.022	0.686	0.482
Zn	*Sarcocephalus latifolius*	Control	0.389 ±0.061	0.018	0.297	0.539	0.383
		250	0.573 ±0.126	0.036	0.397	0.884	0.570
		450	0.608 ±0.121	0.035	0.437	0.803	0.578
		583	0.635 ±0.162	0.047	0.391	0.840	0.661

V. CONCLUSION

Accumulation and magnification of micro and macro elements in blood serum through the administration of extract and consumption of herbal remedies can cause hazardous impacts on health. The concentrations of macro and microelements (Ca, Na, K, Cu, Fe, Zn, Co, Mg), in rat serum was determined after oral administration of calculated doses of aqueous leaf extracts of selected medicinal plant, to investigate their absorption behavior. Results generated from these animal studies closely points towards a definite increase in the levels of serum micro and macro elements in a dose dependent manner. This study on rat serum was merely a preliminary investigation to determine the bioaccumulation of micro and macro elements in the serum after oral administration of doses of crude extract of *Sacrocephalus latifolius*. These results suggest that the presence of elements in medicinal plants may contribute to the availability of these elements in the human body as well

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