

The Hypoglycaemic and Hypolipideamic Potentials of Raw and Boiled *Vernonia amygdalina* Leaf Extract on Normal, Diabetic Induced and High Fat Fed Male Albino Rats

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ABSTRACTS

Vernonia amygdalina (VA) though used as folk medicine in managing diabetes yet is high in anti-nutrients. The effect of boiling (to reduce anti-nutrients) on hypoglycaemic and hypolipidemic potentials is not well known. This work, therefore evaluated the hypoglycaemic and hypolipidemic potentials of aqueous extracts of raw and boiled *Vernonia amygdalina* (VA) leaves at a dose of 300mg/kg bodyweight. Rats were divided into three groups (A,B,C) and two treatments (1,2). Each treatment and control has five rats. Group A rats were fed normal diet (RND), group B were diabetic induced rats (DIR) fed normal diet and group C were fed high-fat diet (RHD). Treatments 1 and 2 were given raw VA extract (VAR) and boiled VA extract (VAB) respectively for 14days while the control received saline (with no extract). Blood glucose was monitored every three days and on the 15th day the rats were sacrificed and the blood serum was used to assay for ALP, ALT and AST activities. The result shows that the extracts were able to reduce ($p<0.05$) the blood glucose level of DIR (from 504.20 ± 85.66 mg/ml to 204.20 ± 136.13 mg/ml and 558.80 ± 66.96 to 162.20 ± 56.69 mg/ml within 14days for VAR and VAB extracts treated rats respectively) while diabetic control blood glucose at the end of the study remained significantly ($p<0.05$) high (400.20 ± 64.02 mg/ml) compared with the treated. Liver enzymes (ALP, ALT and AST) activities of untreated DIR (control) were raised significantly ($p<0.05$) when compared with the treated groups. While the control groups of RND and RHD liver enzymes activities were not significantly ($p>0.05$) higher than the treated. From the whole results analysed VAR and VAB reduced the blood glucose level of DIR (treated) and will bring it to normal with longer period of treatment. Also the extracts decreased the ALP, ALT and AST activities of DIR (treated) and so alleviate the liver function impairment associated with diabetic condition.

Keywords, hypoglycaemic, hypolipidemic, *Vernonia amygdalina*, Alkaline Phosphatase (ALP) Alanine Aminotransferase (ALT) and Aspartate Aminotranferase (AST).

1.Introduction

Despite many scientific breakthroughs in the areas of medicinal research in the developed and developing countries, yet diabetes is still a prevalent killer disease. Whiting *et al.* 2011 estimated that 366 million people worldwide are with the disease and it is expected to affect 552 million people by 2030. Although there is paucity of data on the prevalence of diabetes in Nigeria and other African countries, available data suggest that diabetes is now a major health problem in Africa, including Nigeria (IDF 2013; IHRG 2013). This problem of diabetes in developing countries follow the trend of urbanization and lifestyle changes as a result of "Western-style" diet and lack of exercise (ADA 2010). Diabetes is associated with a lot of physical and biochemical alterations that occur in the liver which will affect glucose metabolism and leads to metabolic defects in other organs and tissues including the muscle and adipose (fat) (Buschman 2011). Glycogen accumulation in the liver is seen in 80% of diabetic patients (Stone & Vanthiel 1985). This excess glycogen result in fatty liver which is a useful confirmatory evidence and a secondary cause of diabetes even if the glucose tolerance test is normal. This fatty liver may be as a result of increased transport of fat to the liver from the intestine or to decreased removal of fat from the liver. This shows that there is a pathway that links high fat diets to a sequence of molecular events responsible for the onset and severity of diabetes. Type 2 diabetes has about 70% correlation with hepatic accumulation regardless of blood glucose control (Levinthal & Travil 1999). The liver function abnormalities as a result of Type 2 diabetes leads to elevated transaminases, due to insulin resistance. These amino-transferases, - Alanine Aminotransferase (ALT) and Aspartate Aminotranferase (AST) measure the concentration of intracellular hepatic enzymes that have leaked into the circulation and served as a biomarker of hepatocyte injury. ALP also acts as markers of bilinary function and cholesterasis. The management of diabetic conditions is difficult, costly and the synthetic drugs have a lot of side effects this is why alternative or folk medicine is a necessary option especially among the rural populace of Nigeria where low cost treatment enhances patronage (Iwueke & Nwodo 2008). In this work *Vernonia amygdalina* leaf with hypoglycaemic and hypolipideamic potentials was used for managing/treating diabetes.

2. *Vernonia amygdalina* food vegetable used in folk medicine

Vernonia amygdalina (VA) named after English botanist William Vernon is a shrub, a member of Asteraceae family with a height of 2-5m and elliptical leaves of about 6mm long which grows mainly in tropical Africa (Aregheore *et al.*, 1998; Adaramoye *et al.* 2008; Ijeh & Ejike 2011). Common names for *Vernonia amygdalina* in Nigeria are bitter-leaf (English) Onugbu (Igbo) Ewuro (Yoruba) Chuar-doke (Hausa) Etidot (Ibibio) Oriwo (Edo) and so on (Igile *et al.* 1995; Ucheck 2004; Oboh & Masogje 2009). Every part of the plant is very important and useful for nutrition and health. *Vernonia amygdalina* is second to *Cassia alata* as the most frequently used vegetable in traditional folk medicine (Abo & Adediwora (2000); Opara *et al.* 2011). Many herbalists and naturopathic doctors recommend aqueous extract of VA to patients as tonic for blood cleansing and for healing various illnesses (Amira & Okobade 2007; Ugwu *et al.* 2011; Eseyin *et al.* 2012). According to Adaramoye *et al.* 2008 *Vernonia amygdalina* has lipid-lowering effects and, may probably serve as a new potential natural product for the treatment of hyperlipidemia. The hepato-protection and hypolipidaemic activities of the aqueous extract of unprocessed VA on diabetic and high fat fed rats can be clearly seen in the activities of their liver function enzymes (ALT, ALP and AST). It has been established that VA vegetable has high anti-nutrients content and so requires processing such as boiling, blanching, soaking, squeeze-washing etc to ensure reduction in anti-nutrients content which makes it safe for consumption (Oboh *et al.* 2005). This is why this work looked at the effects of boiling on the hypoglycaemic and hypolipidaemic potentials of VA extract. The rats serum transaminases were monitored to check the effects of the leaf extracts on the liver after an oral extract treatment of the rats. This work is aimed at evaluating the effects of heat treated aqueous extract of VA on the liver function enzymes (ALP, ALT and AST) of normal, diabetic and high fat fed albino male rats.

3. Materials and Methods

Sourcing of materials

Fresh leaves of *Vernonia amygdalina* (1000g) were bought from Nsukka main market and was authenticated by the Botanist at the Department of Botany, University of Nigeria, Nsukka. Forty five (45) male adult albino rats of Wistar strain weighing between 220 - 240g were procured from Department of Veterinary Medicine, University of Nigeria, Nsukka. Chemicals used were of analytical grade.

3.1 Sample preparation.

Vernonia amygdalina leaves were destalked, washed and drained. The washed leaves were divided into two (2) portions and subjected to different treatments. Fresh leaves (400g) of VA were homogenized with de-ionized water (800ml) using a kitchen blender (Philips, England / China HR 1727,) for 15mins in each batch and extracted for 6 hours. Each homogenized sample was first filtered with cheesecloth and the filtrate was re-filtered under pressure (50°C) through Whatman No.1 filter paper. Each filtrate was centrifuged (Falcon 6/300R, England, CEK- 243-010J) at 10,000×g for 10mins at 4°C -10°C and concentrated to half the volume using rotary evaporator (Diagonal coil, China, RE 300 P) at 80°C. The sample concentrate was freeze dried (Edward freeze dryer, England, Modulyo) to a yield of 13.0g and designated as raw aqueous extract.

Fresh leaves of VA (400g) were boiled in 800ml of de-ionized water for 5mins. After boiling and draining the water the sample was homogenised, extracted as described in sample1 and freeze dried (Edward freeze dryer, England, Modulyo). The sample gave a yield of 11.2g and designated as boiled aqueous extract. The extracts were stored in glass bottles and with plastic screw cap and kept in the refrigerator between 4°C - 10°C. Samples 1 and 2 were used for the animal study.

3.2 Classification of Rats

The rats were acclimatized for 7 days and divided into 3 groups (A,B,C) as shown in Table 2 below. Group **A**:- Rats fed normal diet (RND). Group **B**:- Diabetes induced rats (DIR) (normal feeding) Group **C**:- Rats fed high fat diet (RHD). The initial blood glucose level (One touch ultra lifescan glucometer) of all the rats were taken. Group was subjected to 14 hours fast, diabetes was then induced with freshly prepared alloxan Monophosphate (sigma St Louis) at a dose of 140mg per kg body weight intra-peritorially. Diabetic condition was confirmed after 5 days through a blood glucose test. High fat diet (as in Table 1) was used in feeding RHD group for one week after which each group was divided into two treatments and a control each had five rats. Treatment 1 and 2 received 300mg/kg bodyweight of VAR and VAB extracts respectively while the control received 1ml of saline daily (Table 2). Animals in all the groups had free access to water and feed throughout the 14 days of study.

3.3 Blood Glucose Monitoring:

Blood glucose of the serum was monitored using blood glucose meter (One touch ultra lifescan glucometer, California USA, EMC, 89/336EEC) on day 0, 1, 4, 7 10 and 13 each after an overnight fasting.

3.4 LIVER FUNCTION TESTS

Alkaline Phosphatase (ALP):- The quantitative *in-vitro* determination of Alkaline Phosphatase (ALP) in the blood serum was measured by colorimetric method using Randox Alkaline phosphatase assay kit (Randox laboratories Ltd UK). The P-nitrophenylphosphatate was hydrolysed in the presence of ALP to form phosphate and P-nitrophenol. Concentration of ALP was estimated calorimetrically at 405nm after 1, 2 and 3min. The concentration was calculated with the expression:

$$\text{ALP} = (2760 \times \text{Abs @ } 405\text{nm}/\text{min}) \text{ U/L.}$$

Alanine Aminotransferase (ALT):- The quantitative *in vitro* determination of Alanine Aminotransferase (ALT) in the serum was measured by monitoring the concentration of pyruvate-hydrazone formed with 2,4-dinitrophenylhydrazine using Alanine aminotransferase assay kit (Randox laboratories Ltd UK). The absorbance was read at 546nm and ALT activity calculated from standard Table.

Aspartate aminotransferase (AST):- The quantitative *in vitro* determination of Aspartate aminotransferase (AST) in rats serum was measured using Randox Aspartate Aminotransferase assay kit (Randox laboratories Ltd. UK). A 0.1ml volume of blood serum was mixed with 1ml of substrate and incubated at 37°C for 60min. The mixture was added 1ml of 1, 4 - Dinitrophenol hydrazine (chromogen) and allowed to stand for 20min after which 5ml of 0.4N NaOH was added and the solution was allowed to stand for 5min before absorbance was taken at 540nm against the standard blank. The AST activity was calculated from standard Table.

3.5 Statistical Analysis.

The results were analysed statistically using Randomized Complete Block Design (RCBD) and mean separation was by least significant difference (LSD) using the statistical package for social sciences (SPSS) version 20.0. Differences between means were accepted at $p < 0.05$.

4 RESULTS AND DISCUSSION

4.1 Effect of extract on blood glucose level

The results of the effect of 300g/kg bodyweight treatment with VAB and VAR showed a non significant gradual but steady reduction in blood glucose of RND (from $94.8 \pm 5.26\text{mg/ml}$ to $80.40 \pm 1.5\text{mg/ml}$ and from $95.8 \pm 12.32\text{mg/ml}$ to $71.80 \pm 7.29\text{mg/ml}$ for VAR and VAB extracts respectively).

The blood glucose level of RND treated with VAB was significantly ($p < 0.05$) lower than the corresponding value for the control group from day four till the end of the treatment (see Fig 1).

All the rats before the induction of diabetes had normal blood glucose level between 63mg/ml and 123mg/ml. Five days after the induction and prior to treatment, the fasting blood glucose levels of the DIR (453.67mg/ml - 576.67mg/ml) were significantly ($p < 0.05$) higher than the initial fasting blood glucose level of DIR, RND and RHD (see Fig 1). Four days into the extract treatment the blood glucose level was reduced by 34.68% and 36.71% respectively for VAR and VAB extracts treated DIR while the control reduced by 6%. Continuous daily administration of the extracts for 14 days caused a significant reduction ($p < 0.05$) in the blood glucose level of DIR (from 453.67mg/ml to 175.67mg/ml and 576.67mg/ml to 142mg/ml for VAR and VAB respectively) when compared with the diabetic control group (from 551.67mg/ml to 368.0mg/ml). Physical examination of the liver of rats also showed that diabetic control had a darkened red colour indicating liver toxicity/damage.

There was a significant ($p < 0.05$) rise in blood glucose level (from 92.33mg/ml to 106.33mg/ml) of RHD between the initial blood glucose level (i.e. before the high fat diet feeding) and the value in day zero (the day extract administration began). Both the treated and control of RHD did not show any significant rise in blood glucose level within the first one week of extract administration but between the 10th and 13th day the blood glucose level (106.67mg/ml) of CTRL became significantly ($p < 0.05$) higher than that of the treated groups (see Fig 1).

4.2. Effect of extract on the liver function enzymes (ALP,ALT and AST).

The ALP activity for rats treated with VAR and VAB were significantly higher than the value for the control in RND, but all were within the normal range (20U/L – 140U/L). The control group of DIR showed higher ($p < 0.05$) ALP activity (238mg/ml) than the ALP values for DIR treated (146mg/ml - 176mg/ml) and all were above the normal range. The VAR treated rats of RHD showed higher ALP activity (43.00U/L) than those treated with boiled VA (39.0U/L). However both the treated and control groups of RHD had ALP activity within the normal range. The DIR treated groups showed high ALP activity (136.67U/L-170U/l) above the normal range of ALP and higher than the value reported by Ojiako and Nwanjo (2006) on VA treated diabetic rats (88.4U/l - 104 U/l). The DIR control group ALP activity (253.33U/l) did differ ($p < 0.05$) from the ALP value in rats treated with VAR and VAB extracts.

Figure 2 shows the effect of VAR and VAB extracts on the enzyme Alanine Aminotransferase (ALT) in rats. Rats on normal diet (RND) treated with VAR and VAB had ALT activity of 43.57U/L and 40.04U/L

respectively while the control had a higher ($p>0.05$) activity of 52.91U/L. The control diabetic rats showed higher ($p<0.05$) ALT activity, than the diabetic treated with VA extracts. Ojiako and Nwanjo (2006) reported a lower value with VA (raw and boiled) extracts (18.60 U/L and 22.4 U/L) for treated diabetic group than the value observed in this study (40.04U/l and 43.57U/l). RHD group had slightly elevated ALT activity compared to the value in RND. The control group of RHD showed a significantly higher ALT activity (103.8U/L than the value (74.43U/L - 77.34U/L) for treated group.

In RND, there was no remarkable difference in the AST activity between rats treated and the control group. The VAR showed higher ($p>0.5$) AST activity (54.37 U/L) than the VAB (45.43 U/L) and the control (50.33U/L). The diabetic rats both treated and untreated had high AST value but the control (114.22U/L) was significantly ($p<0.05$) higher. Ojiako and Nwanjo (2006) reported a lower AST value (10.6 U/L to 44.4 U/L) for diabetic treated groups than was observed in this study.

4.3 Discussion

This reduction observed in the blood glucose level of RND treated with VAR and VAB seem to suggest that the extracts had hypoglycaemic potential which help to maintain the blood glucose level in normal person. The extracts VAR and VAB significantly reduced the serum glucose level of diabetic rats by 61.28% and 58.03% respectively. Similarly, repeated administration of these extracts on DIR for longer periods is likely to bring the blood glucose to normal.

The hypoglycaemic properties observed in the VA extracts suggest synergistic interaction of phytochemicals and antioxidants. According to Abraham *et al.*, 2011 the phytochemical composition of *Vernonia* extracts show presence of polyphenols which suppress post-prandial hyperglycaemia and glucose transport across the small intestine. Saponin is known to delay glucose transfer from stomach to small intestine there by controlling the rate of absorption (Pandey and Rizvi, 2009). Epicatechin has a restorative effect on the pancreatic beta cells against alloxan damage by producing more insulin to quicken the uptake of glucose into the cells. Also flavonoid as an antioxidant reduces/reverse the effects of diabetes on the rats' pancreatic cells. These observations agrees with the report of Akpaso *et al.*, 2011 that some phytochemicals in VA contribute to the blood glucose lowering by reversing pancreatic damage caused by induction of diabetes and increase insulin production. Yeh *et al.*, 2003 and Uraku *et al.*, 2011 observed that micronutrients such as Zinc, Copper, Manganese and Vanadium found in VA also activate the insulin and antioxidant enzymes strengthening the hypoglycaemic potency of VA extracts. The rise ($p<0.05$) in blood glucose level of RHD (control) may be as a result of complication due to excessive fat intake and the body metabolism of the rats which may have reduced the activities of insulin. This rise in blood glucose may lead to complication that can accelerate the onset of Type 2 diabetes if not checked. The low blood glucose level of the treated RHD may be associated with the lipid lowering effect of VA extracts on rats fed high cholesterol diet as reported by Adaramoye *et al.*, 2008. The results of liver function enzyme activities showed that VAR and VAB extracts were not likely to have damaging effect on the liver of experimental animals. The RND and RHD treated with 300mg/kg bodyweight of VAR and VAB aqueous extracts did not significantly increase the ALP, ALT and AST activities when compared with their controls. Even-though treated DIR groups had high liver enzyme activities when compared with the respective values for RND and RHD but were significantly ($p<0.05$) lower than the activity of enzymes in DIR control. This agrees with report of Ojiako and Nwanjo, 2006 on the slight increase of ALP and AST of treated diabetic rats. This change in the activity of enzyme shows the effect of insult in the body of experimental animals that is, in increasing or decreasing of the activity of enzyme. Also changes in the liver enzymes is clinically used to evaluating toxicity or damage of any extraneous substance to the living system (Uboh, *et al.*, 2012).

Figure 2 showed significant ($p<0.05$) rise in ALP, ALT and AST activities of all the DIR especially with uncontrolled diabetic condition. This may suggest that the bile ducts have been blocked by fat which agrees with the report of Nwangwu *et al.*, 2011. Obstruction increases the level of the enzyme (ALP) in the plasma through leaching of intestinal ALP (Iweala and Obidoa, 2009) The obstruction of the bile ducts reflected as deficiencies in bilirubin metabolism (eg reduced hepatocyte uptake, impaired conjugation of bilirubin and reduced hepatocyte secretion of bilirubin). Treated DIR ALP was significantly reduced by the extracts though it did not bring it down to normal level but longer use of the extracts may normalize the ALP activity. According to Uraku *et al.*, 2011 the reduction in ALP of diabetic rats by plant extracts may be due to certain compound in plant extracts that can exchange reaction with the -SH groups of the enzyme and the proteins in the body and so inhibit the enzyme activity. Rats fed VAB extract had a significantly lower ALP activity than those fed VAR extract indicating that five minutes boiling may have reduced the phytochemical content of VA which may act as hepatotoxicity. Though it is not likely that consumption of (300mg/kg bodyweight) unprocessed VA will lead to hepatotoxicity because according to Akinola *et al.*, 2009 oral aqueous administration of VA does not produce toxicity. The higher activity of AST enzyme in the VAR fed rats may suggest higher active ingredients in VAR or it may mean that the presence of anti-nutrients in the unprocessed leaves (VAR) could raise the AST activity unlike the boiled (VAB) with reduced anti-nutrient content. The ALT and AST of RND and RHD were normal

suggesting that the extracts had effect on the fat accumulated in the liver of rats fed with high-fat.

The high AST activity and decrease in AST/ALT quotient (in the control group of DIR) recorded in this study may be indicative of liver mal-function as a result of the high blood glucose level. The ratio of AST to ALT is used to differentiate between causes of liver damage. Elevated AST activity is not specifically caused by liver damage as it is also used as a cardiac marker. This damage according to Ode et al., 2011 leads to leaking of enzyme into the blood where they can be measured as indicators of cell damage. In addition high level of insulin resistance that accompanied diabetes and fatty liver condition, observed in the diabetic control group showed that the vegetable extracts were effective in maintaining the liver even at diseased condition, this agrees with the report of Nwangwu *et al.*, (2011). From the results analysed, consumption of *Vernonia amygdalina* vegetable should be encouraged as a normal meal for non sick persons to help prevent accumulation of fat in the liver which is responsible for increased ALP, ALT and AST activities.

5. Conclusion

From the results, blood glucose monitoring and liver enzyme function tests showed that VAB extract did not differ ($p > 0.05$) significantly from VAR in hypoglycaemic and hypolipidaemic activities. The control groups of RND, DIR and RHD had higher blood glucose level and liver function enzyme activities than the respective treated groups. The DIR treated had increased liver enzyme activities which were above normal range but the control had abnormally higher activities than the DIR treated. In conclusion, boiling of VA leaves for five minutes before consumption should be encouraged because it reduced the anti-nutrients and toxicity but had no adverse effect on the hypoglycaemic and hypolipidaemic properties of VA extracts. There is need for further research on the long term effect of VA on the liver. Also the absorption of minerals and vitamins while receiving VA treatment to avoid in-balance in the body nutrients should be studied.

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Feed Formulation

Table 1: Formulation of feed for animal study:

S/N	Items	Quantity of items {for normal feed(Kg)}	Quantity of items { for high-fat feed (Kg)}
1	Wheat offal	20	18
2	Maize	44	44
3	Soybean meal	8	8
4	Palm kernel cake	16	16
5	Fish meal	2	2
6	Bone meal	4	4
7	Salt	0.25	0.25
8	Methionine	0.25	0.25
9	Lysine	0.25	0.25
10	Vita-min. Premix	0.25	0.25
11	Oil (palm oil)	5	7
Total		100	100

Table 2:Classification of rats according to treatments.

Treatment	CLASS A Rats fed normal diet (RND)		CLASS B Diabetes induced rats (DIR)		CLASS C Rats fed high-fat diet (RHD)	
	No. of rats	Extract given	No. of rats	Extract given	No. of rats	Extract given
1	5	VAR	5	VAR	5	VAR
2	5	VAB	5	VAB	5	VAB
Control	5	No extract (saline)	5	No extract (saline)	5	No extract (saline)
Total	15		15		15	

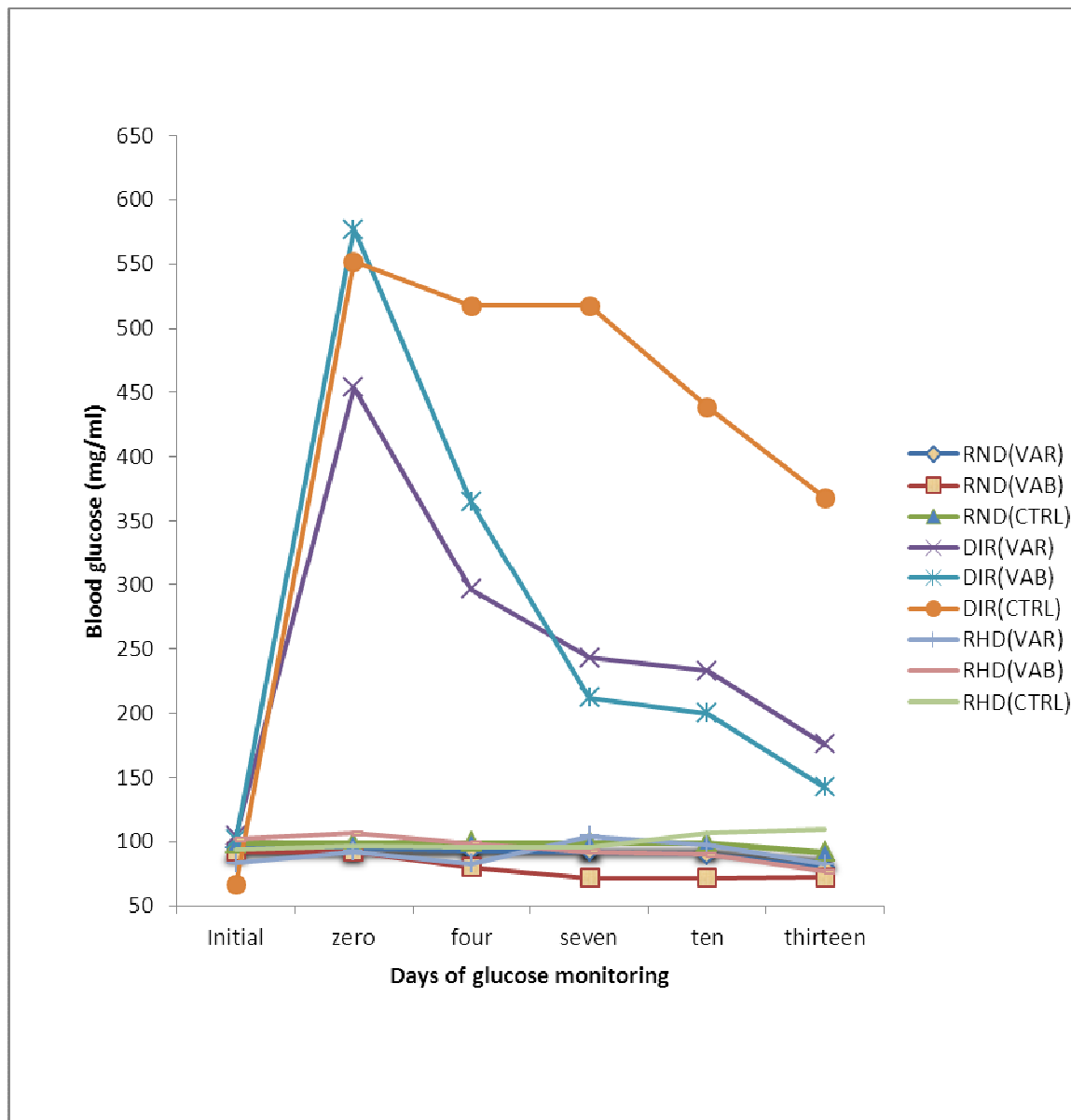


Fig 1: Blood glucose monitoring or RND, DIR and RHD

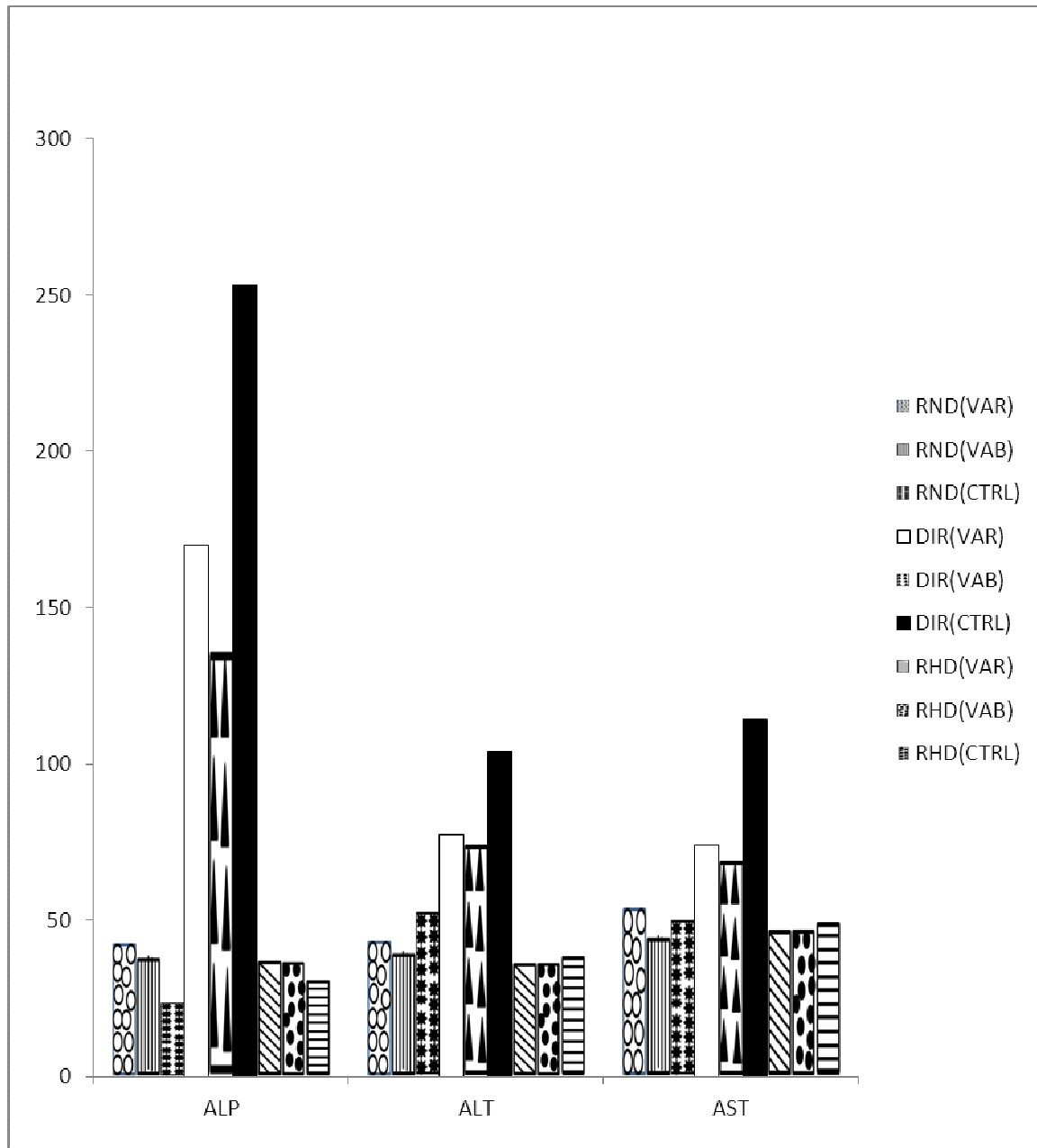


Fig 2: Liver function tests for RND, DIR and RHD

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