TOXICOLOGICAL ASSESSMENT OF *PARQUETINA NIGRESCENS* EXTRACTS IN RATS

K.Nsiah[1], E.O.Terlabi[1], E. Woode[2], D.D. Obiri[2], C. Ansah[2], and M.Duwiejua[2]
[1]*Department of Biochemistry and Biotechnology, College of Science,*
Kwame Nkrumah University of Science and Technology, Kumasi, Ghana
[2]*Department of Pharmacology, College of Health Sciences,*
Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

ABSTRACT
The toxicological potential of Parquetina nigrescens was investigated using Sprague-Dawley rats. Liver enzymes like Aspartate and Alanine aminotranferases (AST/ALT), Alkaline phoshatase (ALP), γ-glutamyl transferase (GGT), as well as some haematological indices (red and white blood cells), and urea levels were assayed as evidence of toxicity. The extract, administered (400-1600 mg kg^{-1}, p.o.) for six weeks did not cause any significant ($p < 0.05$) changes in RBC counts in comparison with the mean of the vehicle-treated control. The total WBC count was not affected after 4 weeks of extract administration (400–1600 mg kg^{-1} p.o), but in the sixth week the mean total WBC count in rats treated with 1600 mg kg^{-1} p.o of the extract reduced by 49.0 %.The dose range 400–1600 mg kg^{-1}, p.o did not cause any significant ($p < 0.05$) changes in both the blood AST and ALT levels. The extract, at all dose levels caused an increase of GGT levels within the first two weeks of treatment, implicating the extract in enzyme induction. Mean serum urea levels in all treatment groups remained statistically unchanged over the six-week period. At the dosage range used, the extract did not cause major changes in the biochemical parameters assessed, indicating the liver and kidney had not undergone any toxic assault. However, the authors suggest the WBC count should be monitored when high doses are administered for long period as the highest dose caused a decrease in WBC count.

Keywords: *Toxicity, Liver enzymes, haematology, aminotransferases, haemoglobin*

INTRODUCTION
Several ethno-medical uses have been reported for *Parquetina nigrescens* Fam. Periplocaceae, (Bouquet and Debray, 1974; Irvine, 1961). The plant is widely distributed in the forest and savannah regions of West Africa (Abbiw, 1990). In Cote d'Ivoire, the entire plant, freshly crushed, is used as an abortificient and the leaf juice, added to capsicum extract is for the treatment of menstrual disorders (Adjanohoun *et al.*, 1979). Infusions of pounded and macerated fresh leaves are used to bathe people as a remedy for general fatigue. Erah *et al.* (2003) showed that Jubi Formula® a herbal preparation containing extracts from *P. nigrescens*, *Sorghum bicolour*

and *Harungana madagascarienssis* reversed haematological parameters in *T. brucei brucei*-induced anaemia to normal levels in rabbits.

Datté *et al.* (1996) reported uterotonic, inotropic and spasmogenic effects in extracts from *P. nigrescens.* The spasmogenic effect has also been demonstrated in isolated guinea-pig tracheal tissues in our laboratory (Terlabi, 2000). Additionally, Datté and co-workers (1999) showed sympathomimetic actions for extracts from the plant in isolated portal vein of Wistar rats.

P. nigrescens is the major component of an anti-asthmatic medicine (Tina-A) used at the Centre for Scientific Research into Plant Medicine (CSRPM), Akuapem, Mampong, Ghana. Here, we report the effects of *P. nigrescens* on some biochemical and haematological markers of toxicity in rats as part of our continuing efforts to evaluate the safety of locally used herbal medicines.

MATERIALS AND METHODS

Plant material

The powdered whole plant was collected from the production unit of CSRPM. The powdered material was extracted under reflux condensation for one hour using water as the solvent. The extract was filtered, freeze-dried, weighed and kept in a dessicator. Suitable quantities were reconstituted in normal saline immediately before use.

Animals

Sprague-Dawley rats (180-250 g) of either sex, bred in the animal unit of the Department of Pharmacology, KNUST were used. They were kept in cages, six to a cage and fed *ad libitum* on commercial poultry feed. The animals were also exposed to a 12-hour light-dark cycle at room temperature of 28±2°C.

Experimental Treatment

The animals were randomly grouped (n = 6) and treated with 400, 800 or 1600 mg kg^{-1} p.o. of extract daily for six weeks. Control group received 0.2 ml normal saline.

Two rats in each group were anaesthetised with ether every two weeks and blood collected by cardiac puncture for biochemical and haematological analyses.

Sera from the groups were assayed for Aspartate aminotransferase (AST, EC.2.6.1.1)), Alanine aminotransferase (ALT, EC2.6.1.2), Alkaline phosphatase (ALP, EC3.1.3.1) and γ-glutamyl transferase (GGT, EC2.3.2.2) using the Randox test kit according to the manufacturer's instructions.

Urea levels in sera were determined by the Urease-Berthelot colorimetric method (Facet and Scott, 1960).

For haematological studies, blood was collected into tubes containing 4% dipotassium ethylenediamine tetra-acetic acid (EDTA). Red and white blood cells were counted manually under a light microscope in a Neubauer haemocytometer. Haemoglobin concentration was determined by the cyanomethaemoglobin method (Dacie and Lewis, 1991).

STATISTICAL ANALYSIS

Data were expressed as mean ± SEM (n = 6). Means were compared using one-way analysis of variance followed by Bonferroni's multiple comparison test if the overall ANOVA was significant (Wallenstein *et al.*, 1980). Unpaired t-test was used for comparison of two means.

RESULTS

Haematology

The extract, administered (400-1600 mg kg^{-1}, p.o.) for six weeks did not cause any significant ($p < 0.05$) changes in RBC counts in comparison with the mean of the vehicle-treated control (Fig. 1).

The total WBC count was not affected after 4 weeks of extract administration (400 – 1600 mg kg^{-1} p.o). However in the sixth week, the mean

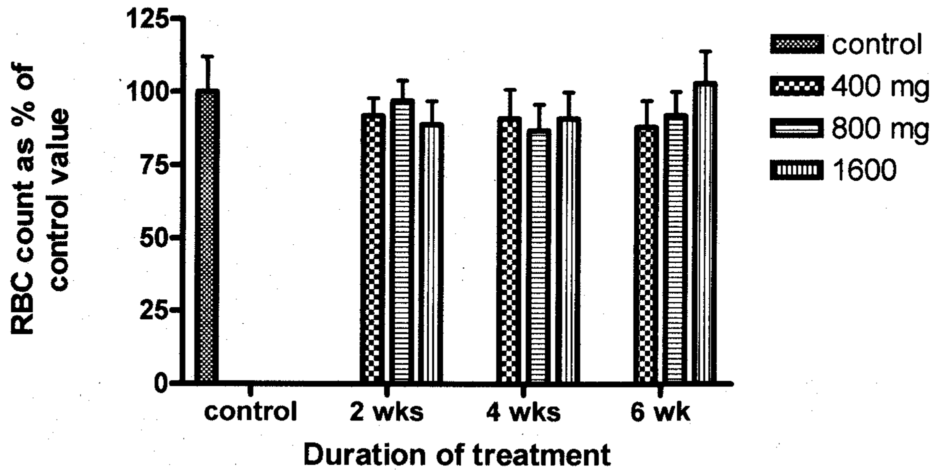


Fig 1: Effect of *P. nigrescens* extract on RBC count of Sprague-Dawley rats. Extract was administered at 400, 800 and 1600 mg kg^{-1}, p.o. Results are presented as mean ± S.E.M (n = 6).

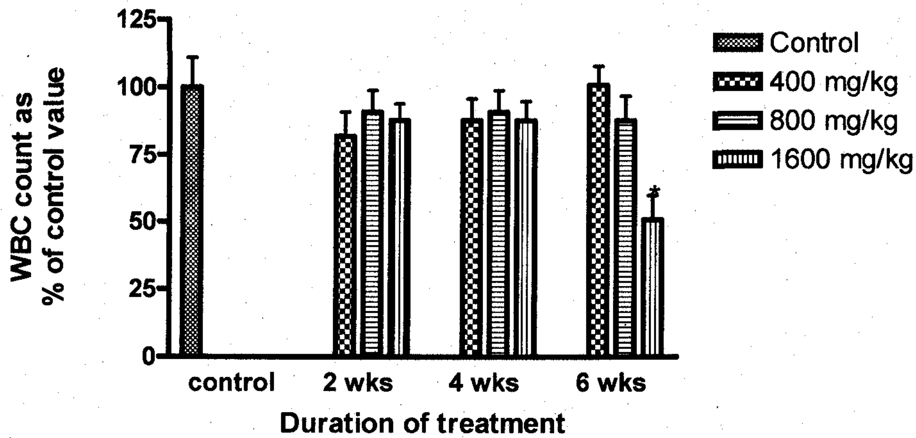


Fig. 2: Effect of *P. nigrescens* extract on total WBC count of Sprague-Dawley rats. Extract was administered at 400, 800 and 1600 mg kg^{-1}, p.o. Results are presented as mean ± S.E.M. (n = 6). *=signifcant at P≤0.05, ANOVA.

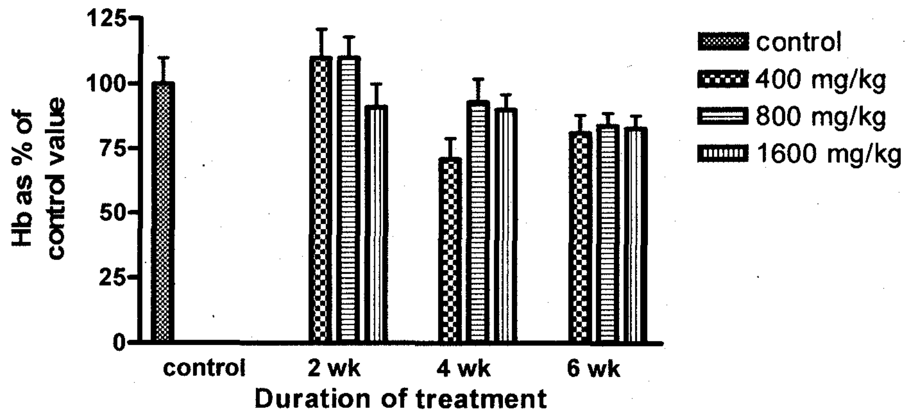


Fig.3 Effect of P. nigrescens extract on haemoglobin concentration of Sprague-Dawley rats. Extract was administered at 400, 800 and 1600 mg kg^{-1}, p.o. Results are presented as mean ± S.E.M (n = 6).

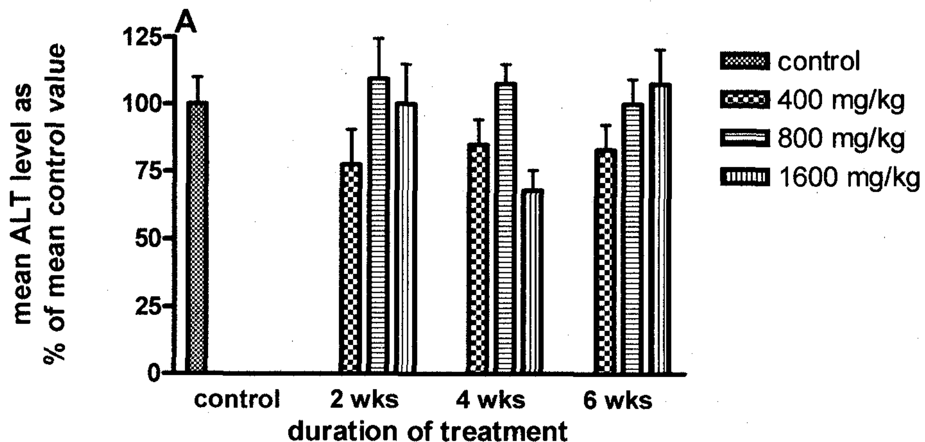
total WBC count in rats treated with 1600 mg kg^{-1} p.o of the extract reduced by 49.0 % (Fig. 2).

In comparison with the mean control value, the extract had no significant effect on the haemoglobin concentration of rats in all treatment groups over the six-week period (Fig. 3), which correlates well with the RBC count.

Blood Biochemistry

Administration of the extract over the dose range 400 – 1600 mg kg^{-1}, p.o did not cause any significant ($p < 0.05$) changes in both the blood AST and ALT levels (Figs. 4A and 4B)

Similarly, administration of the extract (400 – 1600 mg kg^{-1}, p.o.) caused no significant ($p < 0.05$) change in alkaline phosphatase levels in the second and fourth weeks (Fig. 5). However, at the end of the sixth week, while ALP levels in rats receiving 400 mg kg^{-1}, p.o. of the extract



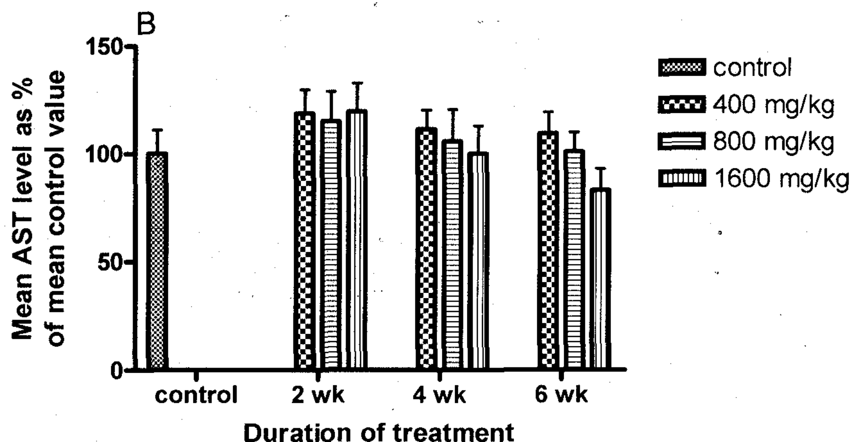


Fig.4: Effect of *P. nigrescens* extract on blood Aspartate aminotransferase (**A**) and Alanine aminotransferase (**B**) of Sprague-Dawley rats. Extract was administered at 400, 800 and 1600 mg kg^{-1}, p.o. Results are presented as mean ± S.E.M (n = 6).

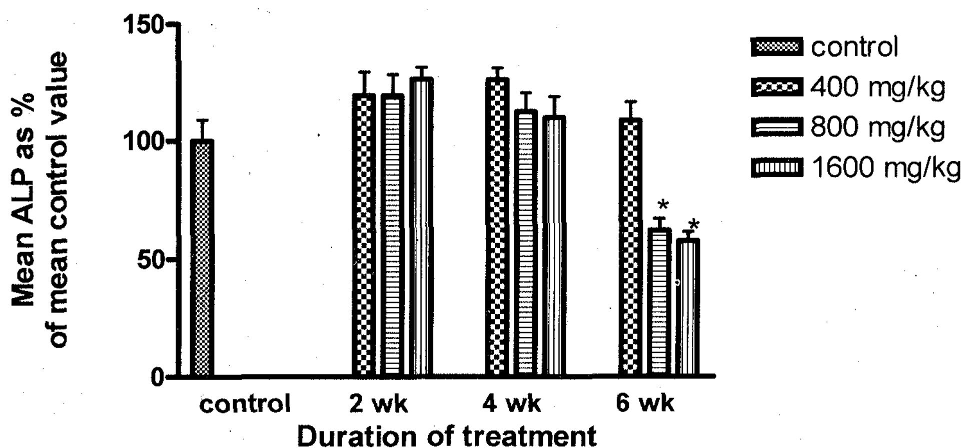


Fig.5: Effect of *P. nigrescens* extract on blood Alkaline phosphatase (ALP) of Sprague-Dawley rats. Extract was administered at 400, 800 and 1600 mg kg^{-1}, p.o. Results are presented as mean ± S.E.M (n = 6). *=signifcant at $P \leq 0.05$, ANOVA

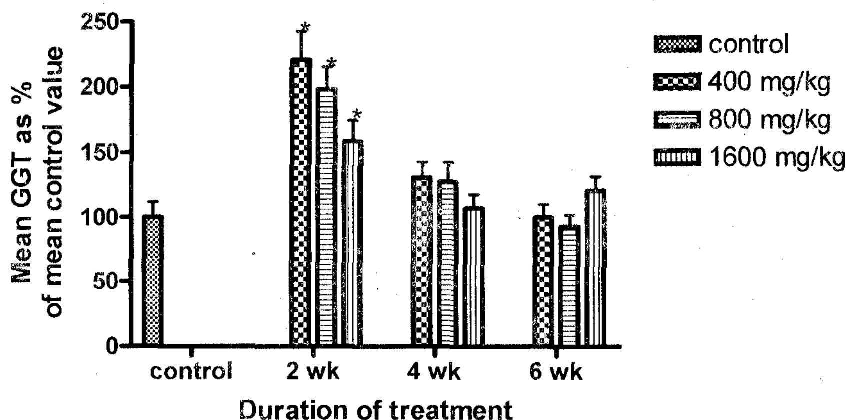


Fig.6: Effect of *P. nigrescens* extract on blood γ-glutamyl transferase (GGT) of Sprague-Dawley rats. Extract was administered at 400, 800 and 1600 mg kg^{-1}, p.o. Results are presented as mean ± S.E.M (n = 6). *=signifcant at P≤0.05, ANOVA.

Table 1: Effect of *P. nigrescens* extract on serum urea in rats

Dose mg kg^{-1} p.o	Serum urea levels as % of mean control value at:		
	Week2	Week4	Week6
Control	100 ± 11.3		
400	78.3 ± 11.1	106.2 ±14.3	88.4 ±9.8
800	74.3 ± 8.9	76.3 ± 9.9	101.4 ± 15.3
1600	82.1 ± 10.9	100.4 ± 9.7	84.3 ± 9.8

Results are presented as mean ± SEM (n = 6, ANOVA).

remained statistically unchanged, the mean levels in rats treated with 800 and 1600 mg kg^{-1}, p.o were significantly ($p < 0.05$) reduced to 62.1 ± 9 % and 57.5 ± 7 % of the mean control value respectively (Fig. 5).

The extract, at all dose levels caused an increase (400 mg/kg: 120%, 800 mg/kg: 98.3 % and 1600 mg/kg: 58.6 %) of GGT levels within the first two weeks of treatment (Fig 6). In the 4th and 6th weeks the mean GGT concentration in all treatment groups had returned to levels comparable to that in the control group (Fig. 6).

Mean serum urea levels in all treatment groups remained statistically unchanged over the six-week period (Table 1)

DISCUSSION

Blood and its cellular elements, the liver and the kidneys are the major target sites particularly prone to toxicity from drugs and chemicals. Currently, the potential toxicity of herbal medications as with orthodox remedies, especially on long-term usage is not in doubt. We were prompted by the diverse pharmacological actions reported on *P. nigrescens* (Dattè *et al.*, 1996; Terlabi, 2000) and the routine use of Tina-A[a], the major component of which is *P. nigrescens* extract, for the treatment of asthma at the CSRPM, to investigate the possible toxicity of the aqueous extract of *Parquetina nigrescens*.

In the present study, we used haematological and biochemical markers of damage to the liver and kidney in Sprague-Dawley rats as evidence of toxicity. At the dosage range used, the extract did not cause major changes in the biochemical parameters assessed. Our present observations and earlier work by Terlabi (2000) together confirm that the extract is unlikely to be toxic to the organs in question.

Erah *et al.,* (2003) reported the reversal of *T. brucei brucei*-induced anaemia in rabbits by Jubi Formula, a polyherbal mixture containing *P. nigrescens*. However, whether *P. nigrescens* contributed to the anti-anaemic effect of Jubi Formula is unknown. In our study, we found no effect of *P.nigrescens* extract on blood parameters in Sprague-Dawley rats. The haemoglobin level remained unchanged after 6 weeks at all dose levels. This effect was reflected in RBC counts which was also unchanged

Similarly, the extract had no apparent adverse effects on the WBC count. However, the highest dose used (1600 mg/kg) significantly reduced the WBC count by 49.0 % after six weeks. It must be noted that this dose translates to an equivalent of 112 g extract for a 70 kg adult, a very unlikely dose for any condition in man. Nevertheless caution should be exercised in using the extract at high doses for long periods, particularly in the event of associated infections.

Damage to the structural integrity of the liver is reflected in increased level of serum transaminases (Schmidt *et al.*, 1975; Gadgoli and Mishra, 1997). These enzymes are cytoplasmic in location and are released into systemic circulation after cellular damage (Sallie *et al.,* 1991). We found no evidence of liver damage in this study as the enzyme levels remained unchanged.

It is noteworthy that GGT activities in treated animals in the second week were significantly ($p < 0.05$) higher than in control animals. This burst of GGT activity could be attributed to enzyme induction. Cholestatic and obstructive disease do result in increased GGT levels. However, the absence of a concomitant rise in ALP, rules out a disease-induced effect. Liver damage is therefore, unlikely to be the cause of increased GGT activity.

When the liver is exposed to some foreign compounds, some drug metabolising enzymes are induced to enhance biotransformation of the compounds (Rawlins, 1980). It is claimed (Callbreath, 1992) that the synthesis of glutathione and the movement of amino acids into cells are in part regulated by the activity of GGT. According to Wamnamethee et al., (1995) raised GGT is indicative of oxidative stress contingent upon glutathione depletion. Elevated activity of GGT could serve to provide the cell with necessary substrates for the *de novo* synthesis of GSH (Rajpert-De Meyts *et al.*, 1992). It has to be noted that GGT is a plasma membrane-bound enzyme that catalyses the first step in the degradation of extracellular GSH, the components of which are used for the *de novo* synthesis of GSH intra-cellularly. It is therefore plausible that some components of *P. nigrescens* extract are metabolised to intermediates that require conjugation with glutathione. Depending on the demand of GSH stores, it could be exhausted resulting in induction of GGT.

Renal damage prevents excretion of urea and other waste products. Serum urea levels remained unchanged, suggesting that the extract probably has no potential of inducing renal damage.

CONCLUSION

We conclude from our results that the aqueous extract of *Parquetina nigrescens* is unlikely to provoke hepatic or renal damage, though WBC count should be monitored when high doses are administered for long periods.

REFERENCES

Abbiw, K.D. (1990). Useful Plants of Ghana. Kew: Intermediate publications and Royal Botanic gardens.

Adjanohoun, E.J., Adjakide, V., Ahyi, M.R.A. and Ake-Assi, L. (1979). Contributions aux études ethnobotanique. Floristique en République du Benin. Medicine traditionalle et pharmacopée, Agence de Cooperation Culturelle et Technique, 420-421

Bouquet, A. and Debray, M. (1974). Plantes medicinales de la Cote d'Ivoire. Paris: ORSTROM Editions, pp. 55-60. In: Dattè JY, Ziegler A, and Tillement J (1999). Sympathomimetic effects of *Parquetina nigrescens* (Periplocaceae) extract in isolated portal vein smooth muscle, *General Pharmacology* 32: 551 – 556

Callbreath, D.F. (1992). Clinical Chemistry: A Fundamental Textbook. USA: WB Saunders Co. pp207-209.

Dacie, J.V., Lewis, S.M. (1991). Practical Haematology. 7th ed. New York: Churchil Livingstone

Datté, J., Ziegler, A. and Tillement, J. (1999). Sympathomimetic effects of *Parquetina nigrescens* (Periplocaceae) extract in isolated portal vein smooth muscle. *General Pharmacology* 32: 551-556

Datté, J., Offoumou, A.M. and Manda, O.M. (1996). Uterotonic effects of hydromethanolic extract of *Parquetina nigrescens* (Periplocacae) on spontaneous contractile activity in the isolated myometrium of pregnant rats. *J. Ethnopharmacol.* 53: 15 – 20.

Erah, P.O., Asonye, C.C. and Okhamafe, A.O. (2003). Response of Trypanosoma brucei brucei-induced anaemia to a commercial herbal preparation, *Afr J Biotechnol* 2: 307-311

Facet, J.K., Scott, J.E. (1960). A rapid and precise method for the determination of urea. *J Clin Pathol* 13: 156-159

Gadgoli, C. and Mishra, S.H. (1997). Antihepatotoxic activity of *Eichorium intybus. J. Ethnopharmacol.* 58: 131-134

Irvine, F.R. (1961). Periplocaceae. In: Woody Plants of Ghana. 1st ed. London: Oxford University Press.

Rajpert-De Meyts, E., Shi, M., Chang, M., Robison, T.W., Groffen, J., Heisterkamp, N., Forman, H.J. (1992). Transfection with gamma-glutamyl transpeptidase enhances recovery from glutathione depletion using extracellular glutathione. *Toxicol Appl Pharmacol.*, 114: 56 - 62

Rawlins, M.N. (1980). Methods of studying drug metabolism in man; *Topics in Therap.* 6: 86 – 94

Sallie, R., Tredger, J.M. and William, R. (1991). Drugs and the liver. *Biopharmaceutical Drug Disposition.* 12: 251-259

Schmidt, E., Schmidt, F.W., Mohr, J., Otto, P., Vido, I. Wrogeman, K. and Herfarth, C. (1975). Liver morphology and enzyme release. Further studies in the isolated perfused rat liver. In: Keppler D(ed). Pathogenesis and Mechanism of Liver Necrosis. Medical and technical Lancaster

Terlabi, E.O. (2000). Anti-asthmatic and other pharmacological properties of *Parquetina nigrescence.* MPhil Thesis, Department of Biochemistry, KNUST, Kumasi, Ghana

Wallenstein, S., Zucker, C.L. and Fleiss, J.L. (1980). Some statistical methods useful in circulation research. *Cir. Res.* 47: 1 – 9

Wamnamethee, G., Ebrahim, S., Shaper, A.G. (1995). Gamma-glutamyl transferase: determinants and association with mortality from ischemic heart disease and all causes. *Am J Epidemiol.* 142: 699 - 708