J.Food Pharm.Sci. 5 (2017) 20 – 24

Journal of Food and Pharmaceutical Sciences
jfoodpharmsci
Available online at jfoodphamsci.com

Research Article

Piptadenia Africana: Enhances Weight Gain, Oxidative Stress, Hyperlipidemia in Normal and Hypercholesterolemic Male Wistar Rats

Nwozo Sarah Onyenibe[1*] and Oyinloye Babatunji Emmanuel[1, 2]

[1] *Nutrition and Industrial Biochemistry Unit, Biochemistry Department, FBMS, College of Medicine, University of Ibadan, Ibadan, Nigeria*
[2] Department of Biochemsitry, College of Science, Afe Babalola University, Ado Ekiti, Nigeria

ARTICLE INFO

Received 20/09/2017
Received in revised form 30/09/2017
Accepted 28/10/2017
Available online 10/11/2017

*Corresponding author
Email: sonwozo@yahoo.com

ABSTRACT

Poyphenol rich stem bark of *Piptadenia africana* (PA) is used to treat gastric pain, fever, cough and we evaluated the effect of daily, continual intake of PA for eight weeks in hypercholesterolemic rats. Thirty six rats were divided into six equal groups, A (positive-control); B (cholesterol) (negative-control); C (100 mg/kg bwt PA); D (200 mg/kg bwt PA); E (cholesterol+100 mg/kg bwt PA) and F (cholesterol+200 mg/kg bwt PA). Corn oil served as vehicle for both cholesterol (40 mg/kg/0.3ml) and PA. PA caused increased weight-gain, no enlarged organs but decreased their protein concentrations relative to control. Serum triglyceride, total cholesterol and LDL-cholesterol were elevated in PA only and PA co-treated cholesterol rats relative to both control groups, culminating in high atherogenic index. Lipidperoxidation increased dose dependently while glutathione-peroxidase, glutathione-S-transferase, reduced glutathione, superoxide dismutase and catalase decreased in PA treated groups relative to controls. Histological examination revealed necrosis of hepatocytes in groups D and moderate coagulation of necrosis of tubules of renal medulla in C. Continuous usage of PA may not be totally safe as it enhances weight gain, unhealthy lipid profile, increase chances of cardiovascular disease, elicits oxidative stress and induces organ toxicity.

Key words: atherogenic-index, lipidperoxidation, lipid profile, organ toxicity, *Piptadenia africana*.

1. Introduction

Tremendous rise in the use of phytomedicines could be due to probable efficacy, mild side effects, generally assumed safety and low cost (Leonardo *et al*., 2000). The health-promoting effects of herbal remedies have been attributed to plant secondary metabolites. Phytomedicines could cleanse the body, activate detoxifying enzymes, p clean the gastrointestinal column, scavenge of free radicals, lower plasma cholesterol or are flavoring agent in confectionaries (Clarke, 2000). Isoprenoid derivatives (terpenes, terpenoids, tocotrienols, tocopherol, carotenoids and saponins), phenolics (coumarins, flavones, flavonoids, isoflavones, antrocyanins, lignins and tanins) and others are sugar derivatives such as vitamin C (Sharma, 2009). Adverse plant drug effects include abortifient, stomach ache, dizziness, diarrhea, palpitations, nausea and vomiting (Oyedemi *et al*., 2009). These unwholesome effects could be attributed to some toxic secondary metabolite present in the plant extract, improper dosage for the actual treatment of the various diseases, poor handling of plant drug or total lack of knowledge concerning safety effects of the plant. There is therefore the need to determine safe doses, possible side effects to adverse drug reaction of these medicinal plants used for treating ailments.

P. africana is native to Cameroon (Letouzey, 1969) and aphrodisiac, blood tonic, enema, urethritis and

abortificient effects of the leaves and fruits have been reported (Iwu, 1993). The stem bark is used for gastric pain and fever locally in Noun division, Cameroon (Iwu, 1993). Betulinic acid, cholesterol, 24 (S) – stigmat - 5,22 – dien - 3β – O - glucopyranoside, 5,6 – dimethoxy – 7 – hydroxyflavone, antiquol B, β - amyrine and new lactone derivatives piptadenol A, B and C have been isolated from the stem bark (Mbouuangouere *et al*., 2007). The crude plant extract and 24 (S) – stigmat - 5,22 – dien - 3β – O – glucopyranoside have shown antibacterial and α-glucosidase inhibitory activity (Mbouuangouere *et al*., 2008).

PA is used for gastric pain and febrile conditions and we examined the effect of its continual ingestion for eight weeks in hyperlipideamic rats on lipid profile, antioxidant status, markers of liver tissue toxicity and histopathological examination.

2. Materials and Methods

2.1. Plant extract

Dr Ramsey Kandem of Chemistry Department of University of Doula, Cameroon donated PA stem bark extract.

2.2. Experimental animals

Male Wistar rats (36) with body-weight (bwt) (95 - 125g) were purchased from Physiology Department Central Animal House and housed in the Animal House of Biochemistry Department, both in University of Ibadan at room conditions of temperature and humidity. All procedures were carried out in compliance with the protocols approved by the Animal Ethical Committee of Afe Babalola University and care was conducted in accordance with the National Institute for Health guidelines on the care and use of laboratory animals. Rats were acclimatized for two weeks on standard chow and were allowed free access to food and water *ad libitum.* Rats were randomly placed into six equal groups. Group A (control received 0.3ml/kg corn oil); Group B (cholesterol only); Group C (100 mg/kg bwt PA); Group D (200 mg/kg bwt PA); Group E (cholesterol+100 mg/kg bwt PA) and Group F (cholesterol+200 mg/kg bwt PA). Corn oil served as vehicle for PA and cholesterol (40 mg/kg/0.3ml) were administered daily by intubation for 8 weeks. The animals were observed daily sleep, feed psychomotor pattern and mortality.

2.3. Sample collection and assays

After last dose, animals were fasted for 24 h sacrificed by cervical dislocation. Blood samples and visceral organs were harvested and all biochemical assays were as described earlier (Nwozo *et al*., 2011).

2.4. Statistical analysis

All values were expressed as the mean ± S.D of six animals. Data were analyzed using one-way analysis of variance (Anova) followed by students-t test to compare the values between groups, ρ values < 0.05 were considered statistically significant.

3. Results

PA extract either alone or cholesterol did not cause any mortality, sleep pattern and psychomotor activity but feed intake decreased after 5 weeks. Table 1, shows data obtained on body/organ weight changes. All animals gained weight but were not significantly different (ρ<0.05) compared to control. Control rats had only 58.33% increase in bwt, cholesterol-feeding caused 78.33% gain in weight whereas animals on either PA alone or PA plus cholesterol had almost 100% increase in bwt. It is worthy to note that cholesterol alone fed rats (B) had the highest weight of both kidney and liver but it was not statistically significant.

Table 1: Effects of PA extract and cholesterol on the body /organ weights(g) of rats.

Group	Initial wt.(g)	Final wt.(g)	% wt. Gain	Liver wt.(g)	Kidney wt.(g)
A	120.00±0.00	207.50±7.91	58.33±5.27	4.98±0.23	1.33±0.10
B	125.00±0.00	222.92±6.78	78.33±5.43	5.21±0.22	1.43±0.20
C	99.17±0.83	202.08±6.78	103.84±6.90	5.05±0.15	1.27±0.08
D	104.17±2.64	210.42±3.84	102.78±7.09	4.77±0.33	1.28±0.12
E	100.00±0.00	197.92±16.59	97.92±16.59	5.00±0.44	1.34±0.07
F	106.25±2.80	216.67±15.37	104.17±14.12	5.12±0.14	1.36±0.02

Values are expressed as mean ± SD (n=6) for each group [a] and [b] Significant (ρ<0.05) when compared to group A and B respectively

Llipid profile and atherogenic index data are on Table 2. There was a significant increase in triglyceride, total cholesterol and LDL-c in animals fed cholesterol or PA plus cholesterol and this increased dose dependently compared to control. Rats in groups E and F which had both PA extract and cholesterol had higher values of serum triglyceride, total cholesterol and LDL-c which was higher than cholesterol only group B animals and were significant compared to control rats. Thus rats on PA only and those on PA plus cholesterol had raised atherogenic index compared to normal control and negative control groups respectively. HDL-c decreased significantly (ρ<0.05)in rats on PA extract relative to control and groups C and D animals had higher values of HDL-c than cholesterol only exposed group B.

Table 2: Effects of Piptadenia africana on lipid profile parameters and atherogenic index of the rats

Group	HDL-C (Mmol/l)	T-Chol (Mmol/l)	TAG (Mmol/l)	LDL-C (Mmol/l)	Atherogenic index
A	86.12±2.32[b]	122.25±17.06[b]	156.64±3.12[b]	48.18±10.24[b]	0.26
B	52.38±2.01[a]	238.07±11.17[a]	228.36±3.62[a]	159.16±12.13[a]	0.64
C	75.90±2.40[b]	161.97±19.96	180.28±6.50[b]	56.17±16.33[b]	0.38
D	61.64±0.81[ab]	178.16±21.29	187.62±3.96[ab]	62.83±11.38[b]	0.48
E	36.40±4.22[ab]	251.71±13.94[a]	231.46±2.99[a]	164.55±8.62[a]	0.80
F	33.24±2.34[ab]	273.75±34.68[a]	256.17±18.80[a]	218.00±24.25[a]	0.89

Values are expressed as mean ± SD (n=6) for each group [a] and [b] Significant (ρ<0.05) when compared to group A and B respectively

Data obtained for total protein, LPO and antioxidants level of the liver and kidney homogenate are on Tables 3 and 4 respectively. Cholesterol feeding caused decrease in GPX, GSH, SOD, GST, CAT while MDA values were elevated in both organs. Rats on only the plant extract had increased activity in oxidative markers compared to cholesterol only group B rats and these were still lower than control, although MDA values were slightly increased compared to control.

Table 3: Effects of PA on liver tissue GPX, GSH, LPO, SOD, GST and CAT of the rats

LIVER						
Group	GPX	GSH	LPO	SOD	GST	CAT
A	21.73±0.61	41.42±2.18[b]	2.89±0.31	2.74±0.22[b]	16.20±0.73	31.28±2.54
B	16.57±3.07	18.21±3.81[a]	4.47±0.38	1.43±0.23[a]	13.89±2.00	24.32±5.17
C	18.78±0.96	21.79±5.85[a]	3.37±0.35	2.58±0.21[b]	15.63±0.78	28.76±1.78
D	19.12±1.85	19.08±6.02[a]	4.29±0.77	1.50±0.19[a]	14.47±2.44	26.42±2.85
E	15.01±1.21	15.21±4.34[a]	4.60±0.20	1.41±0.20[a]	13.31±1.07	22.84±3.51
F	13.79±1.13[a]	14.08±1.98[a]	5.67±0.19[a]	1.20±0.28[a]	11.57±1.16	20.19±3.16

Values are expressed as mean ± SD (n=6) for each group [a] and [b] Significant (ρ<0.05) when compared to group A and B respectively

Table 4: Effects of PA on kidney GPX, GSH, LPO, SOD, GST and CAT in U/mg protein concentration of the rats

KIDNEY						
Group	GPX	GSH	LPO	SOD	GST	CAT
A	51.21±2.94	32.67±2.16[b]	4.97±0.68[b]	2.51±0.34[b]	17.36±2.37	48.70±4.8
B	33.92±4.09	23.58±4.56	8.41±0.55[a]	1.25±0.24[a]	14.47±1.07	30.53±6.58
C	41.86±3.31	29.96±3.33	5.67±0.32	2.23±0.34	15.63±0.78	37.57±6.98
D	35.87±2.15	27.79±2.40	7.75±1.08	1.41±0.20	15.05±1.72	35.78±4.73
E	27.12±0.85[a]	12.21±1.75[a]	10.49±1.01[a]	1.27±0.26[a]	13.31±1.07	28.62±9.67
F	25.84±3.14[ab]	8.67±0.99[a]	13.27±0.58[ab]	0.97±0.14[a]	12.73±0.73	20.41±4.76

Values are expressed as mean ± SD (n=6) for each group [a] and [b] Significant (ρ<0.05) when compared to group A and B respectively

Groups E and F rats on both PA and cholesterol had statistically significant increase (ρ<0.05) in LPO compared to control in both organs and in the kidney it was significant compared to cholesterol only group B.

Data obtained on tissue protein concentration, serum ALT, AST and ALP levels are on Table 5. Cholesterol intake caused protein concentration in the organs to decrease. PA only treated groups had increased organ protein concentration compared to those on cholesterol only and it was lower when compared with the control group. The effect of administration of PA extract at 100 and 200 mg/kg bwt for 56 days significantly (ρ<0.05) increased serum levels of ALT and AST, only ALP was found to decrease in groups treated with extract compared to control.

Table 5: Effects PA on liver toxicity markers in U/l and tissue protein concentration mg/dl of the rats

Group	ALP	ALT	AST	Kidney prot	Liver prot
A	3.11±0.38	60.17±4.76[b]	33.62±4.11[b]	10.15±1.43[b]	18.04±1.41
B	2.30±0.14	85.33±1.92[a]	56.88±2.64[a]	6.73±0.42[a]	13.16±1.61
C	2.67±0.28	66.70±1.73[ab]	39.00±1.47[b]	8.86±0.27	16.54±1.53
D	2.59±0.21	75.60±1.53[a]	56.53±0.62[a]	7.43±0.70	14.09±2.14
E	2.07±0.025	103.87±2.22[a]	66.36±0.59[ab]	5.82±0.47[a]	12.74±0.68
F	1.78±0.11[a]	132.30±6.92[a]	73.41±1.26[ab]	4.68±0.24[a]	10.87±0.29[a]

Values are expressed as mean ± SD (n=6) for each group [a] and [b] Significant (ρ<0.05) when compared to group A and B respectively

Histopathological examinations of kidney and liver sections are on Figs 1 and 2. Cholesterol intake caused moderate widespread thinning of hepatic cord, this was similar to group E on (cholesterol+100 mg/kg bwt PA) but Group F (cholesterol+200 mg/kg bwt PA) which had higher dose of the extract had moderate multifocal single cell necrosis of the hepatocytes and mild Kupper cell hyperplasia. Cholesterol intake caused necrosis in the renal medulla and a few tubules had tubular casts, while cholesterol and extract treated groups had moderate sloughing of the epithelium of the tubules in the renal medulla. Control rats had no visible lesion and animals on PA only had moderate congestion of renal blood vessels.

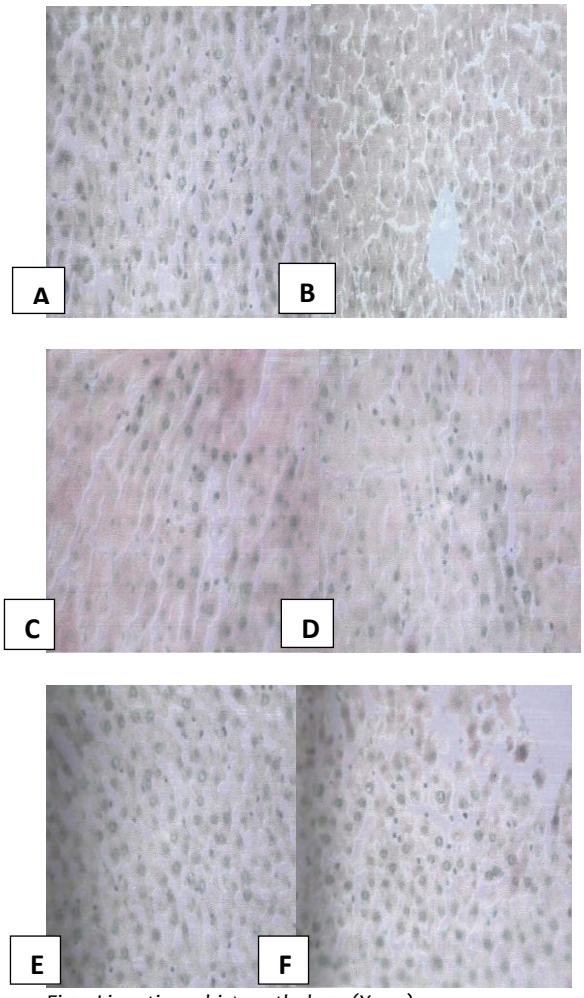

Fig 1: Liver tissue histopathology (X400)

A: Moderate multifocal single cell necrosis of hepatocytes
B: No visible lesions
C: Moderate multifocal single cell necrosis with mild kupffer cell hyperplasia
D: No visible lesions or autolysis
E: Moderate widespread thinning of hepatic cords
F: Moderate widespread thinning of hepatic cords

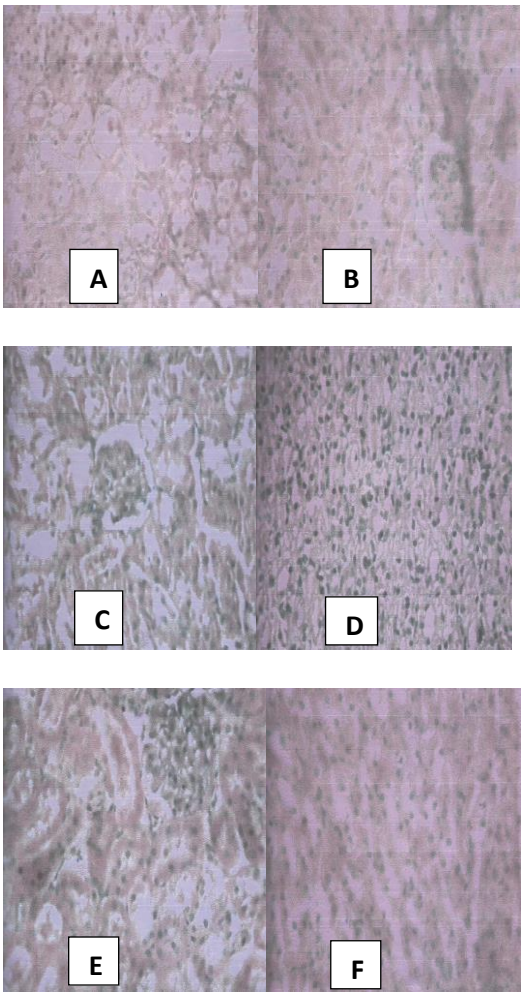

Fig 2: *Kidney tissue histopathology (X400)*

A: No visible lesions
B: Moderate coagulation necrosis of the tubules in the renal medulla and a few tubular cast
C: Moderate congestion of renal blood vessels
D: Moderate coagulation necrosis of some tubules in the renal medulla
E: Moderate sloughing off of the epithelium of the tubules of the renal medulla
F: Moderate sloughing off of the epithelium of the tubules of the renal medulla

4. Discussion

PA extract did not cause mortalities, changes in sleep pattern and restlessness but gradually decreased feed-intake after 5weeks. Decreased food-intake should have caused decrease in body-weight but all rats gained weight. Cholesterol feeding has been shown to enhance fat deposition, increase body-weight and elevate serum lipid levels (Nwozo *et al*., 2011). Thus the observed increase in bodyweight of cholesterol only fed rats is not surprising but the enhanced increment in animals on both cholesterol and PA extract might be a pointer to the deleterious effect of continual intake of this plant for 56 days duration by either obese persons or those with elevated serum lipid or persons with coronary heart disease (CHD).

PA caused total cholesterol, LDL-c and triglyceride increased and a marked decrease in HDL-c (Table 2). HDL-c is important in the transportation of cholesterol from cells and arteries to the liver for catabolism and is

considered good cholesterol (Leudeu *et al*., 2009). Significant decrease in HDL-c by the PA relative to control and increased serum lipids have been identified as one of the risk factors in atherosclerosis and coronary health disease (Lüscher *et al*., 2014; Malika *et al*., 2007). Elevated serum lipids in groups C, D, E&F could be caused by secondary metabolites, especially cholesterol which has been isolated from the stem bark (Letouzey, 1969; Iwu 1993). This calls for caution in continuous administration of this medicinal plant by people predisposed to CHD, hypertension and overweight as evidenced by elevated atherogenic index.

Oxidative stress occurs when reactive nitrogen /oxygen species (RNS/ROS) generated exceeds the antioxidant capacity (Valko *et al*., 2006). Cholesterol feeding caused decrease in GPx, GST, CAT, SOD, GSH while LPO increased in both the kidney and liver. Administration of PA or plus cholesterol resulted in a significant increase in LPO. LPO is a well-established mechanism of cellular injury in both plants and animals, and is used as an indicator of oxidative stress. LPO in biological membrane alters structural architecture and could affect membrane integrity and alter enzyme function. The notetable decrease in the activities of the enzymatic and non-enzymatic antioxidants (SOD, CAT, GPx, GST and GSH) indicates that the administration of PA in dose and study duration elicited oxidative stress. Glutathione acts as the first line of defense against prooxidant stress and we observed 100% decreases in GSH. PA caused considerably oxidative stress as reflected by high MDA and lowered CAT, SOD, GST, GPx and GSH in the study and groups E&F were worse hit, thus supporting the need for caution in the continual usage of the extract by obese and CHD persons.

Activities of tissue enzymes and body fluids can be used to evaluate the extent of assault/ toxicity of chemicals on organs/tissues (Malomo, 2000; Yakubu *et al*., 2003). Aminotransferases are usually low in the blood and only increases by leakage from damaged tissue. PA caused a significant increase in serum ALT and AST activities, this further increased in rats co-administered cholesterol compared to either A or B groups. Liver tissue damage is characterized by a rise in serum enzymes like AST, ALT, ALP etc (Brautbar & Williams, 2002). Increased AST and ALT indicates liver toxicity and lipidperoxidation were consistent with histopathological result of liver and kidney sections. Cholesterol-feeding caused moderate widespread thinning of hepatic cord and necrosis in the renal medulla and PA failed to ameliorate this as supported by E and F in Figs 1 and 2 respectively where we had moderate sloughing of the epithelium of the tubules in the renal medulla.

5. Conclusion

Daily continual administration of PA, especially to hypercholesterolemic rats caused increased bodyweight, elicited lipidperoxidation, elevated serum lipids/ transaminases and necrosis in liver and kidney. PA is a medicinal plant used in Cameroon for stomach pain and febrile ailments should be used with caution by patients who are hyperlipideamic, CHD and obese.

Reference

Brautbar N, Williams II J. Industrial solvents and solvent and liver toxicity: risk assessment, risk factor and mechanism: review. International Journal of Hygiene and Environmental Health (IJHEH). 2002; 205: 479-491.

Clarke M. "Saponin for health." Nutritkion J. 2000; 2: 16

Iwu MM. Handbook of African Medicinal plants. CRC press, Boca Raton, Florida. 1993.

Leonardo DCL, Franco A, Gustavo ATL, Luciano MA, Luis FMES, Gabriel PDS, et al.Toxicological evaluation by *in-vitro* and *in-vivo* assays of an aqueous extract prepared from *Echnindorus macrophyllus* leaves Toxicol Lett. 2000; 116:189-196.

Letouzey R. Manuel d Botanique Forestière de l' Afrique Tropicale, II A Imprimerie Jouve, Paris 6e, France; 1969.

Leudeu BCT, Tchiengang C, Barbe F, Nicolas B, Gueani JL. *Ricindendron heutelotti* (Bail) or *Tetracarpidium conophorum* (Mull) oils fed to rats lower blood lipids. Nutri. Res. 2009; 29(7): 503-509.

Lüscher TF, Landmesser U, Eckardstein AV, Fogelman AM. High-density lipoprotein vascular protective effects, dysfunction, and potential as therapeutic target. Circ Res. 2014; 114: 171-182.

Mallika V, Goswami B, Rajappa M. Atherosclerosis pathophysiology and the role of novel risk factors, a clinical biochemistry perspective. Angiology. 2007; 58: 513 - 522.

Malomo SO. Toxicological Implication of Ceftriaxone Administration in Rats. Nig. J. Biochem. & Mol. Biol. 2000; 15(1): 33-38.

Mbouuangouere RN, Tane P, Choudhary MI, Djemgou P, Ngadjui BT, Ngamga D. Piptadenol A-C and α-glucosidase inhibitor from *Piptadenia africana* Res J. Phytochemistry 2008; 2(1): 27-34.

Mbouuangouere RN, Tane P, Ngamga D, Djemgou P, Choudhary MI, Ngadjui BT. Piptaderol from *Piptadenia africana.* Afr J Trad CAM. 2007; 4(3): 294-298.

Nwozo SO, Orojobi F, Adaramoye OA. Hypolipidemic and antioxidant potentials of *Xylopia aethiopica* seed extract in hypercholesterolaemic rats. J. Med. Food. 2011; 14 (1/2): 114-119.

Oyedemi SO, Bradley G, Afolayan AJ. Toxicological effect of aqueous stem bark extract of *Strychnos henigsii* Gilg in Wistar rats. J. Nat Pharm. 2009; 1:33-39

Sharma R. "Nutraceuticals and nutraceutical supplementation criteria in cancer: literature survey." Open Nutraceuticals J. 2009; 2(2): 92-106.

Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. free radicals, metals and antioxidants in oxidative stress induced cancer. Chemico-Biological Interactions. 2006; 160: 1-40.

Yakubu MT, Bilbis LS, Lawal M, Akanji MA. Effect of Repeated administration of sildenafl citrate on selected enzyme activities of liver and kidney of male albino rats. Nig. J. Pure & Appl. Sci. 2003; 18: 1395-1400.