International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

International Journal of Pharmacology 4 (2): 67-77, 2008
ISSN 1811-7775

An Evaluation of Toxicity and Mutagenicity of *Sphenocentrum jollyanum*

[1]Amidu Nafiu, [2]Woode Eric, [1]Owiredu K.B.A. William, [3]Asare A. George,
[4]Boateng A. Kofi and [5]Opoku-Okrah Clement
[1]Department of Molecular Medicine, School of Medical Sciences,
[2]Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences,
College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana
[3]Medical School, MRC/University Molecular Hepatology Research Unit, Department of Medicine,
University of the Witwatersrand, 7 York Road, Parktown 2193, Johannesburg, South Africa
[4]Department of Pathology, School of Medical Sciences,
[5]Department of Medical Laboratory, Faculty of Allied Health Sciences, College of Health Sciences,
Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Abstract: This study was designed to evaluate the toxicity of *S. jollyanum* using Fischer 344 male rats and the genotoxic effect of the alcoholic extract of the roots. In experiment 1, haematological, serum biochemical and histopathological parameters were determined after 30, 60 and 90 days of oral administration. Experiment 2 involved determinations of total hepatic cytochrome P-450 content. Pentobarbitone induced sleeping times was measured in experiment 3. These are indices of organ specific toxicity or potential for drug interactions. The mutagenic potential was assessed by reverse mutation test using *Salmonella typhimurium* TA_{97}, TA_{98}, TA_{100} and TA_{102} tester strains in experiment 4. There were no significant differences found in most of the hematological, serum biochemical parameters and organ/body weight ratio. No abnormality of any organ was found during histopathological examination and no mutagenicity evidence was detected in any of the mutagenic tests. It, however, caused a significant increase in cytochrome P-450 which correlates well with the decreased pentobarbitone induced sleeping times. The results showed that the no-observed adverse- effect level (NOAEL) of *S. jollyanum* extract (SJE) was >1000 mg kg^{-1} body weight per day in rats, which can be regarded as virtually non-toxic. In conclusion, SJE had no overt organ specific toxicity but demonstrates a potential for drug interactions via cytochrome P-450-mediated metabolism in the rat.

Key words: *Sphenocentrum jollyanum*, chronic toxicity, Ames test, F344 rats

INTRODUCTION

The herbal and natural products of traditional medicine have been used by man since the advent of the human race. Traditional medicine probably evolved as our ancestors battled against natural calamity and disease (Baliga *et al*., 2004).

Sphenocentrum jollyanum Pierre (family Menispermaceae) popularly known as *Aduro kokoo* (red medicine) or *Okramankote* (dog's penis) in the Akan language of Ghana is a small erect sparsely branched shrub which grows up to 1.5 m in height. Different parts of the plant have been used extensively for the treatment of various ailments in the West African sub-region. Extracts from the root have been used for the relief of constipation, as stomachic, as a cough medicine, for sickle cell disease, rheumatism, aphrodisiac and other inflammatory conditions (Iwu, 1993; Moody *et al*., 2006). Decoctions prepared from the fruits, together with the fruits of *Piper guineense* and lime juice, are used for the relief of cough. The plant is reputed to possess exceptional wound healing properties (Raji *et al*., 2006). The fruits are used as an antifatigue snack (Raji *et al*., 2006). It is also perceived to have unusual hemostatic and stomachic properties as well as an emetic for poisonings by traditional medical practitioners in the Ivory Coast (Abbiw, 1990).

Some scientific research has been done on this plant in relation to its antiviral and anti-inflammatory activities (Moody *et al*., 2006), anti-oxidant and anti-angiogenic property (Nia *et al*., 2004), anxiogenic and aphrodisiac property (Owiredu *et al*., 2007; Woode *et al*., 2006) and

Corresponding Author: Dr. William K.B.A. Owiredu, Department of Molecular Medicine, School of Medical Sciences, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana Tel: +233 244 228 667

also, Raji *et al.* (2006) have shown that the methanolic extract of the root of *S. jollyanum* increased the testosterone levels in a dose-dependent manner and also reduced the count, motility and viability of spermatozoa in albino rats.

In spite of documented extensive usage of *S. jollyanum* for the treatment of various ailments, there is no information in the literature of any conventional safety study of this traditional medication. The study was therefore designed to investigate the acute and subacute toxicity of *S. jollyanum* using Fischer 344 rats and the genotoxic effect of the alcoholic extract of the roots.

MATERIALS AND METHODS

Plant material: The sun-dried roots of the *S. jollyanum* Pierre (family Menispermaceae) were bought from the Central Market, Kumasi, Ashanti Region, Ghana and identified by Dr. T.C. Fleischer, Department of Pharmacognosy, KNUST, Kumasi, Ghana and a voucher sample was deposited at the Department. This study was conducted between January and April, 2007.

Preparation of the root extract: The roots were pulverized with a hammer-mill to obtain a coarse powder and 5 kg of the powder was extracted with 70% (v/v) ethanol in a Soxhlet apparatus for 24 h. Using a vacuum rotary evaporator, the hydro-alcoholic filtrate was concentrated under reduced pressure to obtain a yellowish-brown syrupy mass which was then air-dried at room temperature (28°C) for 36 h. This yielded 478 g (9.56%) extract which was kept in a desiccator at room temperature and is subsequently referred to as extract or *Sphenocentrum jollyanum* extract (SJE).

Phytochemical screening: The ethanolic root extract of *S. jollyanum* was subjected to phytochemical screening reagents of tannins, phlobatanins, glycosides, steroids, terpenoids, flavonoids and alkaloids according to the methods of Trease and Evans (1989).

Animals: In Ghana, the root of *S. jollyanum* is chewed as a Central Nervous System (CNS) stimulant and aphrodisiac (Abbiw, 1990) by men, as such we tested its toxicity in male rats. Male Fischer 344 rats (250-300 g; 3 months old) were purchased from Noguchi Memorial Institute for Medical Research, University of Ghana, Accra and housed at the animal facility of the Department of Pharmacology, KNUST, Kumasi, Ghana. The animals were housed in groups of 6 in stainless steel cages (34×47×18 cm) with soft wood shavings as bedding, fed with normal commercial pellet diet (GAFCO, Tema), given water *ad libitum* and maintained under standard laboratory conditions (temperature 24-28°C and 12 h light-dark cycle). All animals used in these studies were treated humanely in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH, Department of Health and Human Services publication No. 85-23, revised 1985) and the study was approved by the Faculty Ethics Committee.

Experiment 1

Hematological assays, biochemical assays and histopathological study

Treatment and sample collection: Experiment 1 involved 72 rats. To measure the toxicological effect of the extract, several blood samples were collected at monthly intervals for three months during the study by exactly the same protocol: in vehicle-treated or SJE treated (100-1000 mg kg^{-1}, p.o.) animals. Male rats were placed in four groups of 18 animals each. Group A, the vehicle-treated control, received 10 mL kg^{-1} (p.o.) of saline daily. Group B, C and D were repeatedly dosed daily with SJE at 100, 300 and 1000 mg kg^{-1} (p.o.), respectively. At the end of each month, 6 rats per group were sacrificed and blood samples collected for biochemical and haematological assays. An amount of 1.5 mL of blood was collected in a vial containing 2.5 μg of Ethylene Diamine Tetraacetic Acid (EDTA) as an anticoagulant for haematological assay and the rest of the blood was collected into vacutainer® tubes. The blood was centrifuge at 500 g for 15 min and serum was collected and stored at -80°C until assayed for the biochemical parameters.

Animal body weight and organ weight: Animals in all groups were observed twice daily for toxicological signs, morbidity and mortality. Weights of all the animals were recorded weekly and abnormalities in gross behavioral change or food and water intake were registered. Each organ (heart, lung, liver, kidney, spleen and testes) was excised quickly after sacrificed as described above, blotted and weighed to determine the organ-to-body weight index (OBI), which was calculated as the ratio of organ weight × 100 and the animal body weight.

Haematological variables: Various haematological parameters including white blood cell count (WBC), lymphocyte count (LYM), mid cell count (MID), granulocyte count (GRAN), red blood cell count (RBC), haemoglobin concentration (HGB), packed cell volume (PCV), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), red cell distribution width (RDW) and platelet concentration (PLT) were determined by automated blood analyzer (CELL-DYN 1700, version 1.08, USA).

Biochemical assays: Serum biochemistry was performed with an ATAC® 8000 Random Access Chemistry System (Elan Diagnostics, Smithfied, RI, USA). Parameters that were determined include aspartate aminotransferase (AST), alamine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyltranspeptidase (GGT), lactate dehydrogenase (LDH), total-bilirubin (T-BIL), direct bilirubin (D-BIL), indirect-bilirubin (I-BIL), total-protein (T-PROT), albumin, sodium (Na^+), potassium (K^+), chloride (Cl^-), carbon dioxide, blood urea nitrogen (BUN), creatinine, uric acid, globulin, Anion gap. Also lipid profile which include total cholesterol (T-CHO), triglycerides (TAG), high density lipoprotein (D-HDL), very low density lipoprotein (VLDL), low density lipoprotein (LDL) and coronary risk calculated as the ratio of cholesterol (mg dL^{-1}) and HDL (mg dL^{-1}) were determined. The methods adopted by the automated instrument for the determination of the above parameters are according to the reagent manufacturer's instruction-JAS™ diagnostics, Inc., (JAS Diagnostics, Ine. Miami Florida, USA).

Histology: The liver, kidney and testes removed from all the rats from each group described above on 30, 60 and 90 days post exposure were fixed in 10% buffered formaline and dehydrated in graded series of alcohol, cleared in xylene and embedded in paraffin wax. Multiple sections from each block were prepared at 5 μm and stained with haematoxylin and eosin (H and E) (McManus and Mowry, 1965).

Experiment 2

Total cytochrome P-450: In experiment 2, twenty four rats were divided into four groups of six animals each. Group 1 served as control and received the vehicle (normal saline), Group 2 was treated with 80 mg kg^{-1} ketoconazole (i.p.), Group 3 was treated with 100 mg kg^{-1} phenobarbitone (i.p.) and Group 4 received 300 mg kg^{-1} of the *S. jollyanum* root extract (p.o.). They were treated for seven days and on the 8th day sacrificed.

Preparation of rat liver microsomes: The livers were rapidly excised and immediately perfused with cold 0.25 M sucrose to wash off excess blood and to cool the liver. The livers were homogenized in four volumes of 0.25 M sucrose, i.e., a 20% (w/v) using a Potter-Elvehjem homogenizer. The homogenate was centrifuged in a refrigerated centrifuge to isolate subcellular fractions. Hepatic microsomes were prepared from the tissue homogenate (20% w/v) according to the method of Lake (1987) as modified by Anjum *et al.* (1992) by $CaCl_2$ precipitation of the post-mitochondrial fraction and ultracentrifugation at 27,000 g for 15 min in a high speed refrigerated centrifuge (Hitachi ZORR 52D, Japan).

Determination of protein and total cytochrome P-450 content: Protein content of liver microsomal preparation was determined by the method of Lowry *et al.* (1951). The amounts of total cytochrome P-450 in the hepatic microsomes were estimated by the conventional method of Omura and Sato (1964).

Experiment 3

Pentobarbital induced sleeping time: In experiment 3, another 24 rats were grouped and treated as described above for cytochrome P-450 determination. Thirty minutes after the last dose each animal was injected with sodium pentobarbital (SPB-40 mg kg^{-1}, i.p.). The time which elapsed from the injection to the loss of the rightness reflex (induction time) and the time from the loss of rightness reflex to awakening (duration of sleeping) were registered (in minutes) for each animal.

Experiment 4

Ames mutagenicity test: Ames mutagenicity test was performed according to the method of Maron and Ames (1983). The *Salmonella typhimurium* TA_{97}, TA_{98}, TA_{100} and TA_{102} tester strains were used in the Ames reversion test. Assays were carried out on samples of fractionated liver homogenate, namely, whole homogenate, S9 fraction, nucleosomes, microsomes and the cytosolic fraction. All samples were assayed according to the standard plate incorporation test and challenged with S9-Mix (Maron and Ames, 1983). Five percent dimethyl sulphoxide was used as a negative control whilst 5.0 μg $plate^{-1}$ daunomycin (daunorubicin hydrochloride), 1.5 μg $plate^{-1}$ of sodium azide were used as positive controls for TA_{98}, TA_{100} and TA_{102}, respectively and 3.0 μg $plate^{-1}$ of sodium azide were used as positive controls for TA_{97}.

Statistical analysis: The results were presented as mean±SEM. The presence of significant differences among means of the groups was determined by one-way ANOVA using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com. Significant differences between pairs of groups were calculated using the Tukey's Multiple Comparison Test. However, paired t-test was used for the analysis of total cytochrome P-450 and the sleeping time. In all statistical test, a value of $p<0.05$ was considered significant.

RESULTS

Phytochemical screening: SJE was found to contain terpenoids, flavonoids and alkaloids compound. The most dominant being the alkaloids.

Clinical signs and mortality: All animals survived until the end of the study period. Furthermore, no remarkable clinical signs of toxicity were observed either immediately or during the post-treatment period even at the highest dose of 1000 mg kg^{-1} b.wt. in any of the animals during the study period. There were also no changes noted during the duration of the study in behavior, activity, posture, gait, or external appearance that were considered to be test drug related (data not shown).

Animals body weight and organ weight: Overall, mean body weights versus time were comparable for SJE treated groups and control groups (Fig. 1) with no significant difference. Rats in all experimental groups gained weight over the course of this study.

The absolute organ weight shows some statistically significant differences between the SJE treated and control groups after two months (Table 1). None of the differences were considered to be test drug-related.

There were generally no statistically significant differences noted in relative organ weights between treated and control animals except for the liver [$F(3,19) = 6.391$, $p = 0.0035$]. Liver weight relative to body weights at the third month increased in a dose-dependent manner in all groups dosed with the test drug (Table 2) with the highest liver weights at 1000 mg kg^{-1}. However, the magnitudes of the alterations were small and were not considered treatment-related.

Clinical pathology parameters

Hematology: There were generally no significant difference between the treated and the control groups for most of the parameters measured. However, there were some statistically significant differences for the first month i.e., WBC ($p = 0.0089$), HGB ($p = 0.0001$), HCT ($p<0.0001$), MCH ($p<0.0001$) MCHC ($p<0.0001$) and PLT ($p = 0.0064$) (Table 3); for the second month i.e., WBC ($p = 0.0399$), HGB ($p = 0.0025$) and HCT ($p = 0.0011$) (Table 3) and for the third month HGB ($p = 0.0060$) HCT ($p = 0.0014$) and PLT ($p = 0.0026$) (Table 3). Because significant statistical differences of HGB and HCT occurred consistently for three months those are regarded as relevant and study related.

Clinical chemistry: There were occasional statistically significant observations noted (e.g., Na^+ ($p = 0.0001$), BUN ($p = 0.0139$), alkaline phosphatase ($p = 0.0078$) and coronary risk ($p = 0.0009$) for the first month; Na^+ ($p = 0.0028$), BUN ($p = 0.0091$), Anion gap ($p = 0.0113$), Protein-total ($p = 0.0210$), Globulin ($p = 0.0049$), A/G ($p = 0.0025$), D-HDL ($p<0.0001$) and coronary risk ($p<0.0001$) for the second month and K^+ ($p = 0.0367$) and coronary risk ($p = 0.0012$) for the third month) as shown in Table 4-6, respectively. Even though coronary risk was significant at a particular dose for each of the months, it was not judged to be test drug-related because it was not

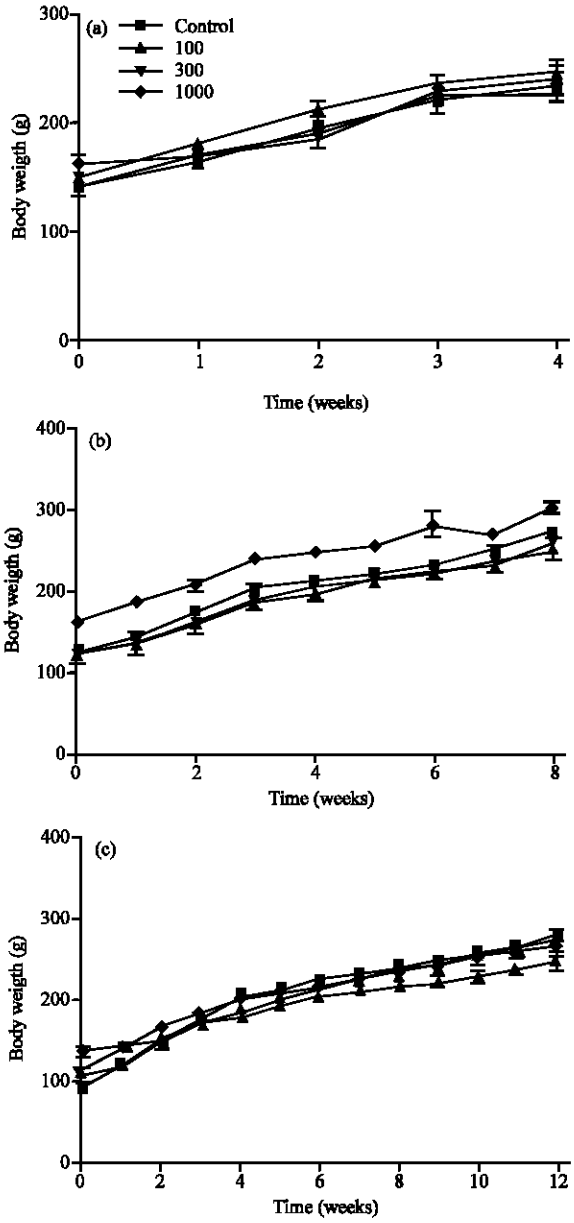

Fig. 1: Mean body weight (mean±SEM) for (a) one month, (b) two month and (c) three months (n = 6)

Table 1: Absolute organ weight (mean±SEM) for one, two and three months of exposure to SJE (mg kg^{-1})

		Absolute organ weight		
Parameters (g)	Control	100	300	1000
One month				
Lungs weight	1.01±0.16	1.38±0.11	1.38±0.09	1.44±0.11
Heart weight	0.71±0.03	0.82±0.05	0.78±0.03	0.78±0.05
Liver weight	6.00±0.28	5.95±0.34	5.85±0.12	6.61±0.37
Kidney weight	1.29±0.09	1.35±0.09	1.21±0.01	1.34±0.07
Spleen weight	0.73±0.06	0.75±0.10	0.75±0.07	0.81±0.06
Testes weight	2.37±0.07	2.09±0.38	2.07±0.21	2.45±0.09
Two months				
Lungs weight	1.65±0.10	1.58±0.11	1.68±0.08	2.20±0.15*
Heart weight	0.82±0.02	0.76±0.03	0.81±0.02	0.78±0.05
Liver weight	8.65±0.46	8.03±0.44	7.61±0.27	10.44±0.60
Kidney weight	1.44±0.06	1.38±0.07	1.35±0.03	1.76±0.11*
Spleen weight	1.04±0.09	0.90±0.04	0.95±0.05	1.48±0.21
Testes weight	2.57±0.13	2.56±0.08	2.66±0.14	2.45±0.09
Three months				
Lungs weight	1.70±0.15	1.59±0.10	2.07±0.22	1.64±0.18
Heart weight	0.80±0.03	0.81±0.03	0.93±0.03	0.85±0.05
Liver weight	6.49±0.32	6.65±0.29	7.46±0.16	7.40±0.24
Kidney weight	1.46±0.05	1.31±0.06	1.60±0.04	1.49±0.03
Spleen weight	0.86±0.04	0.90±0.07	1.08±0.09	1.08±0.06
Testes weight	2.92±0.066	2.41±0.22	2.75±0.11	2.75±0.05

Statistically significant difference *: Indicates ($p<0.05$) compared to the control group by one-way ANOVA followed by Tukey's Multiples Comparison Test, Values are presented as mean±SEM (n = 6)

Table 2: Relative organ weight (mean±SEM) for one, two and three months of exposure to SJE (mg kg^{-1})

		Relative organ weight		
Parameters (g)	Control	100	300	1000
One month				
Lungs weight	0.44±0.08	0.55±0.03	0.61±0.04	0.61±0.05
Heart weight	0.31±0.01	0.33±0.01	0.34±0.02	0.33±0.02
Liver weight	2.58±0.09	2.40±0.05	2.58±0.06	2.77±0.05
Kidney weight	0.55±0.03	0.55±0.03	0.53±0.01	0.56±0.01
Spleen weight	0.32±0.03	0.30±0.03	0.33±0.03	0.34±0.03
Testes weight	1.03±0.05	0.85±0.15	0.91±0.09	1.04±0.06
Two months				
Lungs weight	0.62±0.04	0.66±0.07	0.67±0.04	0.75±0.06
Heart weight	0.31±0.01	0.31±0.02	0.32±0.01	0.26±0.02
Liver weight	3.27±0.19	3.29±0.23	3.04±0.17	3.55±0.25
Kidney weight	0.55±0.02	0.56±0.03	0.54±0.02	0.59±0.03
Spleen weight	0.39±0.03	0.37±0.01	0.38±0.02	0.49±0.06
Testes weight	0.97±0.05	1.06±0.08	1.06±0.06	0.83±0.03
Three months				
Lungs weight	0.63±0.07	0.65±0.05	0.76±0.10	0.63±0.08
Heart weight	0.30±0.01	0.33±0.02	0.34±0.01	0.32±0.02
Liver weight	2.38±0.06	2.70±0.06*	2.68±0.08*	2.81±0.07**
Kidney weight	0.54±0.02	0.53±0.02	0.58±0.03	0.57±0.01
Spleen weight	0.32±0.02	0.36±0.01	0.39±0.03	0.41±0.01
Testes weight	1.08±0.04	0.97±0.06	0.98±0.02	1.05±0.04

Statistically significant difference *: Indicates ($p<0.05$) and **: Indicates ($p<0.01$) compared to the control group by one-way ANOVA followed by Tukey's Multiples Comparison Test, Values are presented as mean±SEM (n = 6)

consistent, not related to dose and it is a calculated value and as such will depend on the degree of deviation of cholesterol and HDL from the control value. The cholesterol and HDL did not show any significant difference from the control value. Therefore, none of these changes was judged to be of toxicological significance.

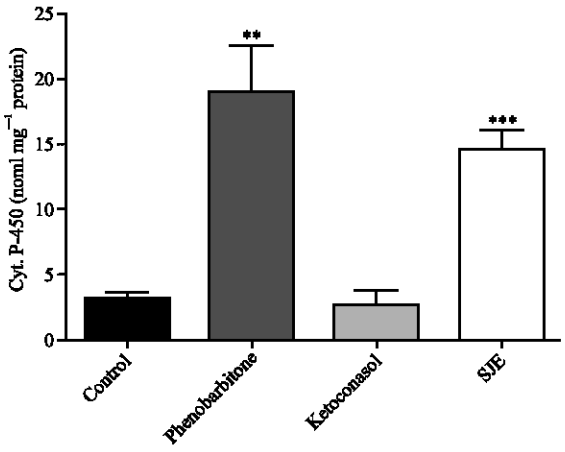

Fig. 2: Effect of SJE (300 mg kg^{-1} p.o), phenobarbitone (80 mg kg^{-1} p.o) and ketoconazole (100 mg kg^{-1} p.o) on the levels of total cytochrome P-450 of rats after 8 days treatment period. Values are mean±SEM and **$p<0.01$ and ***$p<0.001$ (n = 10)

Total cytochrome P-450: The typical enzyme inducer phenobarbitone significantly increased the levels of the total cytochrome P-450 ($p = 0.0026$) where as the enzyme inhibitor ketoconazole reduced the levels of Cytochrome P-450 compared to the control group though the reduction was not statistically significant ($p = 0.7355$). SJE like phenobarbitone significantly induced the drug metabolizing enzyme (cytochrome P-450) as compared to the control groups ($p = 0.0001$) as shown in Fig 2.

Pentobarbitone induced sleeping time: Phenobarbitone caused a profound decrease in pentobarbitone-induced sleeping time ($p<0.0001$) as compared to the control group. SJE also produced a statistically significant decrease in the duration of pentobarbitone-induced sleeping time ($p = 0.0085$) as compared to the control group. However, ketoconazole did not have any significant effect on the pentobarbitone induced sleeping time ($p = 0.2739$) when compared to the control group. None of the test drugs had any significant effect on the onset of pentobarbitone induced sleeping time (Fig. 3).

Histopathology: No test drug-related changes were observed in this study. All changes were considered normal background lesions in this strain and age of rat. For all the rats in the treated and control groups, the morphological structure of the liver, capsule and hepatic lobule were normal and no necrosis or denaturation was found. No infiltrations of inflammatory cells were observed in the portal area and no hyperplasia was found in the connective tissues. The morphological structure of

Table 3: Haematology mean±SEM following one, two and three months of exposure to SJE, respectively

		SJE (mg kg^{-1})		
Parameters	Control	100	300	1000
One month				
WBC (k μL^{-1})	6.57±0.67	2.92±0.49**	5.00±0.53	5.30±0.91
LYM (%)	90.93±0.50	91.22±0.81	91.52±0.83	92.83±0.35
MID (%)	7.18±0.45	6.05±0.63	6.60±0.57	5.47±0.35
GRAN (%)	1.88±0.12	2.73±0.38	1.88±0.29	1.70±0.13
RBC (M μL^{-1})	4.39±0.12	3.82±0.23	4.35±0.17	4.11±0.18
HGB (g dL^{-1})	8.48±0.38	7.27±0.48	10.82±0.47*	9.97±0.51
HCT (%)	24.25±0.95	20.78±1.28	31.77±1.59**	28.67±1.57
MCV (fL)	55.27±1.15	54.37±0.73	56.47±0.27	54.45±1.15
MCH (pg)	19.32±0.48	19.07±0.84	24.85±0.16**	24.27±0.56**
MCHC (g dL^{-1})	34.95±0.24	35.05±1.40	44.03±0.31**	44.53±0.25**
RDW (%)	16.62±0.56	15.57±0.40	16.05±0.22	15.95±0.64
PLT (k μL^{-1})	295.70±30.62	159.50±26.55**	232.80±19.77	210.70±16.80
Two month				
WBC (k μL^{-1})	9.85±0.54	6.77±0.27*	8.13±1.07	9.20±0.82
LYM (%)	93.57±0.64	92.85±0.82	94.45±0.48	90.02±1.70
MID (%)	5.23±0.52	5.45±0.66	4.18±0.38	7.55±1.24
GRAN (%)	2.41±0.50	1.70±0.16	1.37±0.18	2.42±0.50
RBC (M μL^{-1})	5.00±0.27	4.90±0.18	5.08±0.2319	5.51±0.24
HGB (g dL^{-1})	8.82±0.29	9.62±0.46	10.25±0.31	11.12±0.41**
HCT (%)	25.33±0.73	27.97±1.29	29.62±1.01	32.47±1.12**
MCV (fL)	52.75±0.99	53.77±0.53	52.53±0.63	54.18±0.52
MCH (pg)	18.78±0.42	19.62±0.27	18.82±0.26	19.88±0.22
MCHC (g dL^{-1})	35.32±0.37	36.45±0.18	35.88±0.37	36.60±0.13
RDW (%)	14.83±0.48	14.62±0.15	13.98±0.39	15.58±1.25
PLT (k μL^{-1})	217.50±6.99	187.50±10.51	180.20±5.05	241.70±12.95
Three month				
WBC (k μL^{-1})	5.98±0.87	6.67±0.87	9.38±0.73	10.32±1.90
LYM (%)	86.98±1.20	86.05±1.14	85.08±1.38	86.80±0.57
MID (%)	9.60±0.62	10.22±0.72	10.63±0.76	9.82±0.59
GRAN (%)	3.42±0.60	3.73±0.52	4.28±0.83	3.38±0.22
RBC (M μL^{-1})	4.87±0.17	5.51±0.31	5.46±0.17	5.48±0.13
HGB (g dL^{-1})	9.90±0.33	11.20±0.47	11.52±0.28*	11.78±0.24**
HCT (%)	27.10±0.96	31.45±1.63	32.97±0.94 **	34.40±0.53**
MCV (fL)	51.63±0.78	51.23±0.91	53.80±0.74	54.14±1.02
MCH (pg)	20.35±0.35	20.07±0.34	21.12±0.30	20.80±0.56
MCHC (g dL^{-1})	39.42±0.13	39.22±0.32	39.30±0.20	38.38±0.52
RDW (%)	14.63±0.31	15.27±0.31	14.82±0.28	14.60±0.23
PLT (k μL^{-1})	145.80±13.08	222.00±26.77	278.20±24.96**	232.00±15.48

Statistically significant difference *: Indicates ($p<0.05$) and **: Indicates ($p<0.01$) compared to the control group by one-way ANOVA followed by Tukey's Multiples Comparison Test, Values are presented as mean±SEM (n = 6)

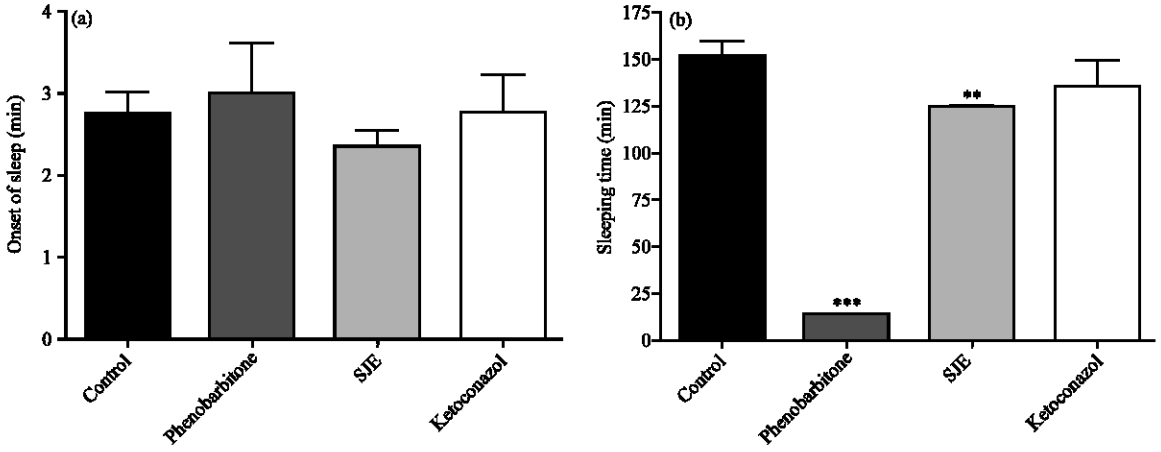

Fig. 3: Effect of SJE (300 mg kg^{-1} p.o), phenobarbitone (80 mg kg^{-1} p.o) and ketoconazole (100 mg kg^{-1} p.o) on pentobarbitone induced sleeping time after 8 days treatment period in rats. Values are mean±SEM and **$p<0.01$ and ***$p<0.001$ (n = 10)

Table 4: Serum chemistry mean±SEM following 30 days of exposure to SJE

		SJE (mg kg^{-1})		
Parameters	Control	100	300	1000
Sodium (mmol L^{-1})	151.00±0.97	143.80±1.67*	142.70±1.45**	152.70±1.69
Potassium (mmol L^{-1})	7.12±0.45	7.22±0.37	7.05±0.25	8.55±0.57
BUN (SI) (mmol L^{-1})	7.57±0.22	7.32±0.40	9.27±0.48*	7.90±0.48
Creatinine (SI) (µmol L^{-1})	47.13±1.86	47.17±6.31	50.08±2.95	47.13±3.71
Anion Gap (mmol L^{-1})	30.12±1.04	28.72±2.46	28.38±0.78	32.38±1.04
GOT (AST) (U L^{-1})	304.50±60.02	405.70±74.76	293.00±31.14	347.50±49.93
GPT (ALT) (U L^{-1})	112.30±17.41	115.80±16.46	111.30±6.71	114.50±13.32
Alkaline Phos (ALP) (U L^{-1})	512.70±37.60	327.20±39.29**	421.50±29.56	370.30±31.81
GGT (U L^{-1})	2.50±0.34	1.50±0.22	2.00±0.52	1.67±0.33
Bilirubin-Total (SI) (µmol L^{-1})	5.10±0.62	6.83±0.78	4.82±0.52	6.55±0.83
Bilirubin-Direct (SI) (µmol L^{-1})	3.40±0.62	3.97±0.84	2.83±0.57	3.68±0.68
Bilirubin-Indirect (SI) (µmol L^{-1})	1.70±0.00	2.87±0.85	1.98±0.28	2.88±0.85
Protein-Total (SI) (g L^{-1})	79.50±2.05	78.83±2.39	81.67±1.48	82.67±4.51
Albumin (SI) (g L^{-1})	39.17±2.07	36.83±1.20	41.00±0.52	40.67±1.45
Globulin (g dL^{-1})	4.03±0.20	4.20±0.13	4.07±0.16	4.20±0.36
A/G (Ratio)	0.98±0.08	0.88±0.03	1.02±0.05	1.02±0.10
Bun/Crt (Ratio)	40.05±1.35	41.47±4.80	46.55±2.75	42.88±3.96
Cholesterol-Total (SI) (mmol L^{-1})	1.92±0.15	2.08±0.16	1.95±0.10	2.03±0.14
Triglyceride (SI) (mmol L^{-1})	0.63±0.07	0.55±0.08	0.60±0.05	0.55±0.04
D-HDL (SI) (mmol L^{-1})	1.03±0.06	0.86±0.05	1.09±0.06	0.94±0.07
VLDL (mg dL^{-1})	11.17±1.25	9.50±1.41	10.50±0.92	9.83±0.83
LDL (mg dL^{-1})	33.50±3.97	46.50±7.00	34.33±2.61	41.67±3.95
Coronary Risk (Ratio)	2.53±0.12	3.37±0.24**	2.48±0.09	2.97±0.07
Uric acid (SI) (µmol L^{-1})	118.80±30.73	119.00±21.73	89.25±13.30	119.00±0.00
Calcium (SI) (mmol L^{-1})	6.10±0.09	2.65±0.08	4.57±0.40	7.03±0.11

Statistically significant difference *: Indicates ($p<0.05$) and **: Indicates ($p<0.01$) compared to the control group by one-way ANOVA followed by Tukey's Multiples Comparison Test, Values are presented as mean±SEM (n = 6)

Table 5: Serum chemistry mean±SEM following 60 days of exposure to SJE

		SJE (mg kg^{-1})		
Parameters	Control	100	300	1000
Sodium (mmol L^{-1})	158.70±1.67	150.50±1.43**	150.50±1.63**	153.70±1.23
Potassium (mmol L^{-1})	7.27±0.20	6.52±0.24	6.43±0.31	7.30±0.34
BUN (SI) (mmol L^{-1})	8.28±0.25	7.15±0.34	6.55±0.27**	7.40±0.40
Creatinine (SI) (µmol L^{-1})	22.10±1.97	25.05±5.32	20.62±2.95	17.68±2.29
Anion Gap (mmol L^{-1})	41.15±1.88	33.68±1.03*	35.27±1.01	34.13±2.10*
GOT (AST) (U L^{-1})	226.20±29.01	190.00±10.12	177.50±17.14	191.30±8.87
GPT (ALT) (U L^{-1})	158.00±17.94	134.20±4.25	127.20±13.50	139.00±5.97
Alkaline Phos (ALP) (U L^{-1})	492.70±31.46	430.70±50.34	349.50±32.07	469.80±29.62
GGT (U L^{-1})	2.67±0.92	2.00±0.29	1.67±0.33	2.17±0.65
LDH (U L^{-1})	2012.00±41.53	1660.00±140.30	1750.00±153.70	1604.00±82.54
Bilirubin-Total (SI) (µmol L^{-1})	4.25±0.58	4.53±0.36	4.53±0.36	4.53±0.36
Bilirubin-Direct (SI) (µmol L^{-1})	1.82±0.36	2.82±0.35	2.27±0.36	2.55±0.38
Bilirubin-Indirect (SI) (µmol L^{-1})	2.27±0.57	1.70±0.00	2.27±0.36	1.98±0.28
Protein-Total (SI) (g L^{-1})	79.33±2.06	74.33±1.73	71.00±1.10*	73.67±1.87
Albumin (SI) (g L^{-1})	37.17±0.79	36.50±0.99	37.67±0.76	37.50±0.96
Globulin (g dL^{-1})	4.10±0.21	3.78±0.10	3.33±0.08**	3.62±0.10
A/G (Ratio)	0.92±0.06	0.95±0.02	1.13±0.03**	1.05±0.02
Bun/Crt (Ratio)	96.88±8.99	92.35±22.68	97.72±19.52	118.80±21.36
Cholesterol-Total (SI) (mmol L^{-1})	2.08±0.19	2.15±0.11	5.25±3.15	1.55±0.11
Triglyceride (SI) (mmol L^{-1})	1.47±0.09	1.82±0.20	1.72±0.28	1.28±0.11
D-HDL (SI) (mmol L^{-1})	0.63±0.07	0.90±0.07*	0.67±0.04	0.34±0.05*
VLDL (mg dL^{-1})	26.17±1.72	31.50±3.59	29.67±4.94	22.50±1.84
LDL (mg dL^{-1})	37.00±4.26	29.00±2.48	32.50±7.25	28.67±3.51
Coronary Risk (Ratio)	4.63±0.25	3.38±0.26	4.42±0.32	6.80±0.53**
Uric acid (SI) (µmol L^{-1})	89.25±13.30	59.50±0.00	79.33±12.54	59.50±0.00

Statistically significant difference *: Indicates ($p<0.05$) and **: Indicates ($p<0.01$) compared to the control group by one-way ANOVA followed by Tukey's Multiples Comparison Test, Values are presented as mean±SEM (n = 6)

renal glomerulus was also found normal for each rat in the study. Neither renal glomeruli nor epithelia of renal capsule were thickened. There were no pathological changes observed for testis.

Ames test: The reverse mutation test showed that no significant increase in the number of revertant colonies occurred in the four *S. typhimurium* strains TA_{97}, TA_{98}, TA_{100} and TA_{102} at any tested concentrations of SJE, in

Table 6: Serum chemistry mean±SEM following 90 days of exposure to SJE

		SJE (mg kg^{-1})		
Parameters	Control	100	300	1000
Sodium (mmol L^{-1})	151.80±2.23	150.50±1.61	147.70±1.50	149.60±5.18
Potassium (mmol L^{-1})	6.20±0.28	6.38±0.25	6.67±0.24	7.36±0.28*
BUN (SI) (mmol L^{-1})	7.73±0.32	7.70±0.46	7.90±0.63	6.90±1.34
Creatinine (SI) (µmol L^{-1})	22.10±4.98	14.73±1.88	23.58±7.80	30.06±12.70
Anion Gap (mmol L^{-1})	37.20±2.16	37.72±2.22	36.67±1.52	38.56±1.59
GOT (AST) (U L^{-1})	206.00±8.91	213.00±16.48	200.50±15.68	224.20±26.79
GPT (ALT) (U L^{-1})	129.50±11.56	141.50±9.29	114.00±6.56	130.20±11.20
Alkaline Phos (ALP) (U L^{-1})	452.30±19.50	486.50±68.13	381.30±52.82	371.60±53.43
GGT (U L^{-1})	1.33±0.21	1.67±0.21	2.33±0.21	3.20±1.07
Bilirubin-Total (SI) (µmol L^{-1})	4.53±0.57	4.53±0.36	4.82±0.52	5.44±0.64
Bilirubin-Direct (SI) (µmol L^{-1})	2.27±0.36	2.27±0.36	2.27±0.56	3.40±0.54
Bilirubin-Indirect (SI) (µmol L^{-1})	2.27±0.36	2.27±0.36	2.55±0.38	2.04±0.34
Protein-Total (SI) (g L^{-1})	75.67±2.73	79.83±1.87	74.83±2.73	76.00±2.95
Albumin (SI) (g L^{-1})	42.00±1.24	45.67±2.26	41.50±1.26	44.60±2.18
Globulin (g dL^{-1})	3.37±0.23	3.42±0.19	3.33±0.20	3.14±0.27
A/G (Ratio)	1.27±0.11	1.38±0.12	1.27±0.07	1.46±0.15
Bun/Crt (Ratio)	111.70±25.53	138.90±15.05	117.50±29.65	112.70±46.82
Cholesterol-Total (SI) (mmol L^{-1})	2.03±0.11	1.95±0.15	5.10±3.18	1.72±0.19
Triglyceride (SI) (mmol L^{-1})	1.03±0.19	1.33±0.30	1.30±0.37	0.98±0.20
D-HDL (SI) (mmol L^{-1})	0.73±0.09	0.80±0.11	0.50±0.06	0.39±0.07
VLDL (mg dL^{-1})	18.33±3.51	22.83±5.21	22.67±6.40	16.80±3.38
LDL (mg dL^{-1})	39.00±4.60	32.83±3.96	37.33±7.07	38.60±7.58
Coronary Risk (Ratio)	4.02±0.34	3.67±0.42	5.50±0.46	6.42±0.58**
Uric acid (SI) (µmol L^{-1})	79.33±12.54	89.25±13.30	89.25±13.30	119.00±0.00
Calcium (SI) (mmol L^{-1})	1.93±0.04	1.88±0.05	1.87±0.04	2.04±0.10

Statistically significant difference *: Indicates ($p<0.05$) and **: Indicates ($p<0.01$) compared to the control group by one-way ANOVA followed by Tukey's Multiples Comparison Test, Values are presented as mean±SEM (n = 6)

the presence of S9 mix (Fig. 4). However, the colonies for the positive controls were five to eleven times more than those for the negative control samples. Similar results were obtained from the duplicate test. No obvious dose-response relationship for SJE was found.

DISCUSSION

S. jollyanum has been used since time immemorial in traditional medicine in Ghana to treat several diseases including male erectile dysfunction (Abbiw, 1990; Owiredu *et al*., 2007). However, a survey of available literature has indicated that the toxicity of its extract has not been thoroughly evaluated. The assessment of toxicity is indispensable, if the extract has to be used for human applications, for the reasons of safe dose management. Therefore, the present study was carried out to evaluate the toxicity and genotoxic effects of the ethanolic root extract of *S. jollyanum* in laboratory animals.

When SJE was administered to Fischer 344 rats at 100-1000 mg kg^{-1} (p.o.) body weight per day for 90 days, there were no significant adverse toxicological effects attributable to the treatment. Clinical signs were unremarkable and there were no ocular findings in any animal. Monitoring of body weight gain and food consumption in drug studies can be a sensitive indicator of overall animal health (Borzelleca, 1996). The absence of any significant differences in these measured parameters across the studied groups therefore provides support for the safety of the extract.

Analysis of clinical pathologies, including blood biochemistries and hematology, revealed only random statistically significant effects. There were also occasional effects noted on absolute and relative organ weights. There were no findings at the end of the study in either macroscopic or histopathologic examinations that indicated that any of these effects were related to treatment with the test material.

The no-observable- adverse-effect level (NOAEL) for SJE was found to be more than 1000 mg kg^{-1} b.wt. day^{-1} when administered orally for 90 consecutive days. This finding is contrary to the study of Raji *et al*. (2006) who reported an LD_{50} of 136.5 mg kg^{-1} using methanolic extract of the root. Probably, the disparity may be related to the medium of extraction; since the methanolic extract and ethanolic extract of the root might contain different component. This observation may justify why the roots are chewed raw or cut into pieces and infused in gin for 3 days to be taken as bitters in the traditional methods of drug preparation.

Liver function was determined in order to detect possible hepatic dysfunction, tissue damage or changes in biliary excretion evoked by prolonged exposure to SJE. Well known markers of liver function such as levels of serum albumin, lipoproteins, cholesterol and triglycerides

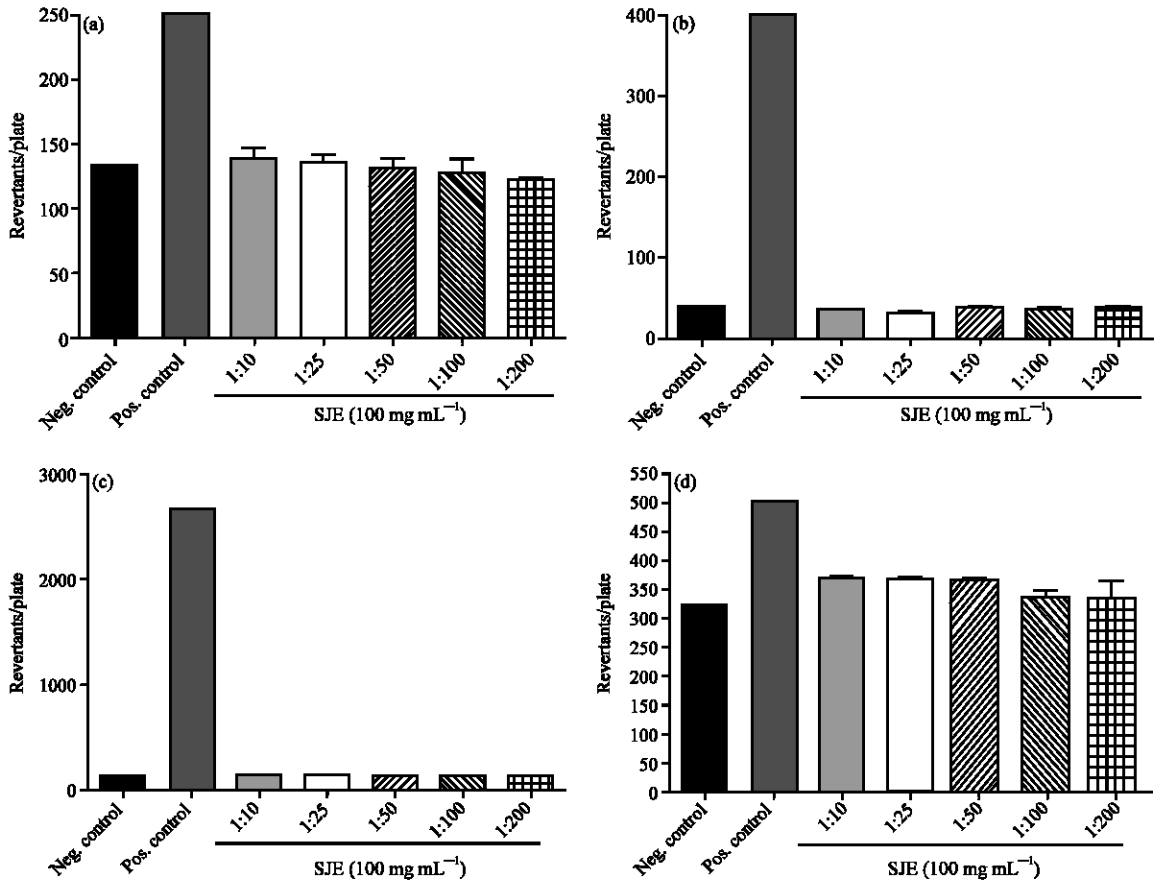

Fig. 4: Mutagenicity of SJE tested in the presence of rat S9 fractions using the Ames test with *S. typhimurium* (A) TA97, (B) TA98, (C) TA100 or (D) TA102. The positive control in A is 3.0 μg, B and C is 1.5 μg of Sodium azide per plate and D is 5.0 g of daunomycin per plate

were unchanged in animals chronically exposed to SJE, clearly demonstrating that liver function was preserved in these animals. Frequently, when hepatic injury occurs, serum aminotransferase (AST and ALT) activity increases. Chronic treatment of the male animals did not change these parameters indicating that the SJE treatment did not induce liver tissue damage. Similarly, since there were no significant changes in direct, indirect and total bilirubin fractions after treatment with the SJE it indicates that the extract does not alter hepatic metabolism or biliary excretion. Alterations observed in relative liver weight were not correlated with clinical biochemical changes and could not be characterized as a hepatotoxic effect of the SJE.

Similarly, renal function tests were performed to assess the possible nephrotoxicity of chronic treatment with *S. jollyanum* extract. Besides a slight change in BUN, sodium and potassium levels in blood samples from animals exposed to SJE, none of the other parameters evaluated were changed, indicating that renal function was unaffected by SJE treatment. These differences were not considered test drug-related since they were of small magnitude and within ±2SD of the mean for the population of control groups and not dose related (observed at the low dose but not at the high dose), and/or occurred only after a particular month and not others months of the study.

Some alterations in hematological parameters were found in male rats subjected to the 30, 60 and 90 day treatment period with SJE. Besides the significant increase in HGB, HCT, MCH and MCHC in a dose dependent manner in blood samples from animals exposed to SJE, other parameters evaluated showed differences that were not considered test drug related since they were of a small magnitude and within ±2SD of the mean for the population of control groups and not dose related (observed at the low dose but not at the high dose), and/or occurred only after a particular month and not

other months of the study. These imply that the extract probably has a haemopoietic effect. An increase in the HGB and HCT concentrations indicates that the extract enhances the oxygen-transport capacity of the blood (Massey, 1992), where as the increase in the MCH and MCHC may be further evidence of the haemopoietic effect of the extract. This is probably the basis for the relief experienced by sickle cell patients who are treated with the extract in the West African sub-region (Abbiw, 1990; Iwu, 1993).

The *Salmonella* mutagenicity test (Ames test) is used worldwide as an initial screening to determine the mutagenic potential of new drugs for hazard identification and in documents for the registration or acceptance of new drugs by regulatory agencies (Maron and Ames, 1983; Mortelmans and Zeiger, 2000).

The supposed 2-fold rule (Mortelmans and Zeiger, 2000) was used to evaluate the mutagenicity test. Thus, drugs were concluded to be mutagenic if the following factors were satisfied: (1) the greatest number of revertants was 2-fold or more relative to the negative control, (2) a dose-dependent increase in the number of revertants was observed and (3) the dose-finding and main assays produced reproducible results. If no increase in the number of revertants was observed, the drugs were concluded to have a negative result. If the mutagenic and negative criteria were not met, then the drugs were judged to have exhibited an equivocal response. Mutagenic activity (equal to the number of induced revertants/mg/plate) was calculated as a measure of mutagenicity (Fig. 4). The Ames microbial mutagenicity test also found no evidence of point mutations *in vitro*.

The result of this study demonstrates that, the extract has no apparent mutagenic effect on the four mutant *Salmonella* strains in the presence or absence of a metabolism activation system. This indicates that the SJE had no mutagenic effect *in vitro* within the dosage range applied.

S. jollyanum is rich in alkaloids, particularly in the leaves and roots (Odebiyi and Sofowora, 1978; Philardeau and Debray, 1965; Smolenski *et al*., 1975). Saponins were not present (Odebiyi and Sofowora, 1978) as confirmed by this study. Philardeau and Debray (1965) confirmed the presence of an alkaloid that corresponded to berberine iodide (S.P and colour reaction) and In 1967, columbine was isolated as the bitter principle of the seed (Gilbert *et al*., 1967). Moody *et al*. (2006) also confirmed the presence of columbine and isocolumbine in the stem back.

Columbine has been found to have no toxic effect in cats and dogs up to 200 mg kg^{-1} s.c and internally administered (Husemann, 1871). The alkaloid berberine has a variety of unique pharmacological effects: antimicrobial, diuretic, smooth muscle relaxant and cardiac depressant activities (Bruneton, 1995). More recently, berberine has been found to inhibit cell death (apoptosis) of thymocytes which are critical immune effector cells. It is well known that the effectiveness of cancer chemotherapy is limited by toxicity to immune effector cells. Hence this alkaloid has become of interest for new therapeutic modalities for cancer.

Cytochrome P-450s (CYP) comprises a superfamily of enzymes that catalyze the oxidation of a wide variety of xenobiotic chemicals, including drugs and carcinogens (Gonzalez, 1988, 1990; Guengerich, 1992). Multiple drug therapy is a common therapeutic practice, particularly in patients with several diseases or conditions and many drug-drug interactions involving metabolic induction are being reported. The possibility of interactions between SJE and other drugs exists as it is an enzyme inducer. This warrants a careful use of SJE as a remedy for male impotence and/or erectile dysfunction because toxicity and therapeutic failure of drugs have long been recognized as possible consequences of the interactions among drugs. The effect of inducers phenobarbitone and SJE as well as inhibitor ketoconasol on the liver drug metabolizing enzymes (cytochrome P-450) correlates well with the *in vivo* duration of action of pentobarbitone on the sleeping time in this study. Because pentobarbitone is metabolized by the hepatic microsomal cytochrome P-450 enzyme system, its duration of action was significantly reduced by phenobarbitone and SJE. However, ketoconasol has no effect on the pentobarbitone action probably because it was not able to inhibit the drug metabolizing enzyme significantly at the dose used.

CONCLUSION

The findings of this study indicates that the ethanolic roots extract of *S. jollyanum* is safe for consumption. However, SJE induces drug metabolizing enzyme which warrant its careful use as a remedy for male impotence and many other illnesses. This calls for further clinical studies to identify its effect in human.

ACKNOWLEDGMENTS

The authors are grateful to Thomas Ansah, George Ofei, Prosper Akortia of the Department of Pharmacology and Lawrence Quaye of the Regional Hospital, Bolgatanga for their technical assistance. We are also grateful to the Gates Foundation who partly funded this research.

REFERENCES

Abbiw, D.K., 1990. Useful Plants of Ghana. Intermediate Technology Publications and Royal Botanic Gardens Kew.

Anjum, F., A. Raman, A.R. Shakoori and J.W. Gorrod, 1992. An assessment of cadmium toxicity on cytochrome P-450 and flavin monooxygenase-mediated metabolic pathways of dimethylaniline in male rabbits. J. Environ. Pathol. Toxicol. Oncol., 11(4): 191-195.

Baliga, M.S., G.C. Jagetia, J.N. Ulloor, M.P. Baliga, P. Venkatesh, R. Reddy, K.V. Rao, B.S. Baliga, S. Devi, S.K. Raju, V. Veeresh, T.K. Reddy and K.L. Bairy, 2004. The evaluation of the acute toxicity and long term safety of hydroalcoholic extract of Sapthaparna (*Alstonia scholaris*) in mice and rats. Toxicol. Lett., 151 (2): 317-326.

Borzelleca, J.F., 1996. A proposed model for safety assessment of macronutrient substitutes. Regul. Toxicol. Pharmacol., 23 (1 Pt 2): S15-S18.

Bruneton, J., 1995. Pharmacognosy, Phytochemistry and Medicinal Plants. Intercept, Ltd., England, UK.

Gilbert, J.N.T., D.W. Mathieson and M.B. Patel, 1967. The bitter principle of *Sphenocentrum jollyanum*. Phytochemistry, 6 (1): 135-136.

Gonzalez, F.J., 1988. The molecular biology of cytochrome P-450s. Pharmacol. Rev., 40 (4): 243-288.

Gonzalez, F.J., 1990. Molecular genetics of the P-450 superfamily. Pharmacol. Ther., 45 (1): 1-38.

Guengerich, F.P., 1992. Characterization of human cytochrome P-450 enzymes. FASEB. J., 6 (2): 745-748.

Husemann, A., 1871. Die Pflanzenstoffe. Berlin.

Iwu, M.M., 1993. Handbook of African Medicinal Plants. CRC Press Inc., pp: 239.

Lake, B.G., 1987. Preparation and Characterization of Microsomal Fractions for Studies on Xenobiotic Metabolism. In: Biochemical Toxicology: A Practical Approach, Snell, K. and B. Mullock (Eds.). IRL Press, Washington DC., pp: 183-215.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem., 193 (1): 265-275.

Maron, D.M. and B.N. Ames, 1983. Revised methods for the Salmonella mutagenicity test. Mutat. Res., 113 (3-4): 173-215.

Massey, A.C., 1992. Microcytic anemia: Differential diagnosis and management of iron deficiency anemia. Med. Clin. North Am., 76: 549-566.

McManus, J.F.A. and R.W. Mowry, 1965. General Methods for Study of Cell and its Structure. In: Staining Methods: Histologic and Histochemical, McManus, J.F.A. and R.W. Mowry (Eds.). Harper Bros, New York, pp: 73-90.

Moody, J.O., V.A. Robert, J.D. Connolly and P.J. Houghton, 2006. Anti-inflammatory activities of the methanol extracts and an isolated furanoditerpene constituent of *Sphenocentrum jollyanum* Pierre (Menispermaceae). J. Ethnopharmacol., 104 (1-2): 87-91.

Mortelmans, K. and E. Zeiger, 2000. The Ames Salmonella/microsome mutagenicity assay. Mutat. Res., 455 (1-2): 29-60.

Nia, R., D.H. Paper, E.E. Essien, K.C. Iyadi, A.I.L. Bassey, A.B. Antai and G. Franz, 2004. Evaluation of the anti-oxidant and anti-angiogenic effects of *Sphenocentrum jollyanum* Pierre. Afr. J. Biomed. Res., 7: 129-132.

Odebiyi, O.O. and E.A. Sofowora, 1978. Phytochemical screening of Nigerian medicinal plants II. Lloydia, 41 (3): 234-246.

Omura, T. and R. Sato, 1964. The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. J. Biol. Chem., 239: 2370-2378.

Owiredu, W.K.B.A., N. Amidu, F. Amissah and E. Woode, 2007. The effects of ethanolic extract of root of *Sphenocentrum jollyanum* pierre on sexual behaviour and hormonal levels in rodents. J. Sci. Technol., 27 (2): 9-21.

Philardeau, Y. and M.M. Debray, 1965. Note Preliminaire Sur Les Alcaloides De *Sphenocentrum jollyanum*. Rapport Roneeo ORSTOM. In: Plantes Medicinales De La Cote D'lvoire, Bouquet, A. and M. Debray (Eds.). Paris, pp: 115.

Raji, Y., O.O. Fadare, R.A. Adisa and S.A. Salami, 2006. Comprehensive assessment of the effect of *Sphenocentrum jollyanum* root extract on male reproductive activity in albino rats. Reprod. Med. Biol., 5 (4): 283-292.

Smolenski, S.J., H. Silinis and N.R. Farnsworth, 1975. Alkaloid screening. VI. Lloydia, 38 (3): 225-255.

Trease, G.E. and W.C. Evans, 1989. A Textbook of Pharmacognosy. 13th Edn. Bailliere Tindall Ltd., London.

Woode, E., M. Duwiejua, C. Ansah, G.A. Kuffour, D.D. Obiri and N. Amidu, 2006. Effect of *Sphenocentrum jollyanum* in experimental mouse models of anxiety. In: Proceedings of the 2nd Western Africa Network of Natural Products Research Scientists (WANNPRES), Vol. 32. Elmina, Ghana, pp: 32.