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ERGOGENIC POTENTIAL AND HEPATOPROTECTIVE EFFECTS OF ETHANOL EXTRACTS OF *SPHENOCENTRUM JOLLYANUM* ON WISTAR RATS

U. E. Ekpoikong, M. O. Wegwu, C. U. Ogunka-Nnoka

Department of Biochemistry

Faculty of Science

University of Port Harcourt, Rivers State.

Abstract

This study investigated ergogenic and hepatoprotective effects of ethanol extracts of *Sphenocentrum jollyanum* on wistar rats. A total of 40 wistar rats were used in the study the rats were allocated on the basis of weight to eight (8) groups of five (5) rats each and administered extracts as follows: group 1 served as control while groups 2-8 were administered as follows: group 2-500g/kgbw, group 3-1000g/kgbw, group 4-1500g/kgbw, group 5-2000g/kgbw, group 6-2500g/kgbw, group 7-3000g/kgbw and group 8-3500g/kgbw. The rats were housed in cages according to groups with facilities for food and water given *ad libitum*. Lactate dehydrogenase (LDH) and creatine kinase (CK) activities were determined by kinetic method, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities were determined using Randox kits, total protein (TP), serum albumin and total bilirubin were determined by standard methods. There was significant difference ($p<0.05$) in both muscle and serum LDH concentration in some of the treated groups when compared with the control but however, there was no significant ($p<0.05$) difference in both muscle and serum CK levels. No significant difference ($p>0.05$) was observed in ALT activity between the experimental groups and control; ALP and AST also showed no significant ($p<0.05$) difference when the treated groups was compared to the control. However, the levels of TP, ALB and TBIL of some of the treated groups shows significant ($p<0.05$) reduction when compared to the control group. *S. jollyanum* has been shown to be a very potent herbal ergogenic aid and also exhibited significant hepatoprotective effects in Wistar rats as acclaimed by folklore medicine.

Key words: Ergogenic potential; hepatoprotective effect; ethanol extract; *S. jollyanum*

1.0 Introduction

An ergogenic substance is a physical, mechanical, nutritional, psychological, or pharmacological substance or treatment that either directly improves physiological variables associated with exercise performance or removes subjective restraints which may limit physiological capacity" (Robergs, 2010). A very popular class of ergogenic substances (which has been banned in sports all over the world) are the steroids. There is so much illegality surrounding use of certain substances to enhance performance in sports; athletes may face ban or stripping off of medals or both. Over the past decade, athletes all over the

world have been using different herbs and herbal mixtures/formulation to enhance their athlete performance without the threats of sanction, bans or negative side effects. Herbal products are extract from seeds, gums, roots, leaves, bark, berries, or flowers, and contain numbers of phytochemicals such as carotenoids and polyphenols, including phenolic acids, alkaloids, flavonoids, glycosides, saponins, and lignans which are thought to provide health benefits (Sellami *et al.*, 2018). Proximate and phytochemical screening of plants have shown they contain several metabolites such as lipids, carbohydrates and nucleic acids and alkaloids, phenolic compounds and terpenoids. These phytochemicals are widely sought for their biological properties: anti-allergic, anti-atherogenic, anti-inflammatory, hepato-protective, antimicrobial, antiviral, antibacterial, vasodilatory (Ksouri *et al.*, 2007). Based on these biological properties, herbs/herbal mixtures have been viewed as the best candidates to enhance muscle performance in sports (Sellami *et al.*, 2018). Common herbs/natural products such as ginseng, caffeine, ephedrine, *Tribulus terrestris*, *Ginkgo biloba*, *Rhodiolarosea*, *Cordyceps Sinensis* have demonstrated benefits on muscle growth and strength in active men (Sellami *et al.*, 2018).

Sphenocentrum jollyanum has several potentials and has been shown to contain high levels of phytochemicals hence, this wonder herb can as well become the new herbal medication for enhancement of performance in athletes.

S. jollyanum is a short evergreen shrub (dioecious in nature) found in the undergrowth of dense forest and it grows to a height of 1.5m. The plant is characterized by few branches, bright yellow roots, grey coloured bark, a stem that is thinly short-hairy when young and is widely distributed in West Africa from Sierra Leone to Nigeria (Nia *et al.*, 2004). It is commonly called *Burantashi*. *S. jollyanum* flowers and bears fruit either irregularly or continuously all through the year while it is pollinated by certain insects or ants and seeds are usually dispersed within a short distance from the parent plant. This herb has been extensively used in traditional medicine to cure a wide range of ailments and diseases such as constipation, treatment of cough, treatment of sickle cell disease, rheumatism, aphrodisiac and other inflammatory conditions (Olorunnisola, *et al.*, 2011).

Fig. 1: *Sphenocentrum jollyanum*

2.0 Materials and methods

Collection and preparation of root extracts

S. jollyanum was obtained from Ikata, Ahoada East Local Government Area, Rivers State. The samples were identified at the department of Plant Science and Biotechnology, Faculty of science, University of Port Harcourt, Rivers State.

The plant roots were air-dried at room temperature and pulverized with an electric mill to obtain the course powder and 5kg of the powder was extracted with 70%(v/v) ethanol in a conical flask for 72 hrs. Using a rotary evaporator, the filtrate was concentrated under reduced pressure to obtain the crude ethanolic extract. It was further air dried at room temperature and kept in a desiccator and used for analysis.

3.0 Experimental design

A total of 40 wistar rats were used in the study; they were purchased from the animal house of the Department of Biochemistry, University of Port Harcourt. The rats were weighed and acclimatized for four days prior to feeding then allocated on the basis of weight and litter origin to eight (8) groups of five (5) rats each and administered extracts as follows: group 1 served as control while groups 2-8 were administered as follows: group 2-500g/kgbw, group 3-1000g/kgbw, group 4-1500g/kgbw, group 5-2000g/kgbw, group 6-2500g/kgbw, group 7-3000g/kgbw and group 8-3500g/kgbw. The rats were housed in cages according to groups with facilities for food and water given *ad libitum*. At the end of the feeding period the rats were sacrificed and blood and tissue (testis) taken for laboratory analyses.

Enzyme assays

Determination of Lactate dehydrogenase (LDH-L) activity (kinetic procedure): The reagents were first reconstituted according to instruction. Next, 1.0 ml of reagent was pipetted into appropriate tubes and pre-warmed at 37°C for three (3) minutes. The spectrophotometer was zeroed with water at 340 nm and 0.025 ml (25 ul) of sample added to reagent, mixed and incubated at 37°C for one (1) minute. After one (1) minute, the absorbance is read and recorded and the tube returned to 37°C. Readings were repeated every minute for the next two (2) minutes. The average absorbance difference was calculated per minute (ΔAbs/min.). The absorbance ΔAbs/min. multiplied by 6592 will yield results in IU/L.

Calculation:

$$\text{LDH activity (IU/L)} = \frac{\Delta \text{ Abs/min} \times TV \times 100}{d \times € \times SV}$$

$$= \frac{\Delta\text{Abs/min} \times 1.025 \times 1000}{1 \times 6.22\ 0.025}$$

$$= \Delta\text{Abs/min.} \times 6592$$

Where: A (Abs/min); ΔAbs/min = Average absorbance change per minute; TV = Total reaction volume (1,025); 1000 = Conversion of in to IU/L Light path in cm (1.0); € = Millimolar absorptivity of NADH (6.22); SV = Sample volume in ml (0.025).

Determination of creatine kinase (CK) activity: Kinetic determination of creatine Kinase is based on following reactions:

$$\text{Creatine Phosphate} + \text{ADP} \xrightarrow{CK} \text{Creatine} + \text{ATP}$$

$$\text{ATP} + \text{Glucose} \xrightarrow{HK} \text{ADP} + \text{Glucose-6-phosphate}$$

$$\text{G-6-P} + \text{NADP} \xrightarrow{G6P\text{-}ĐH} \text{D-Gluconate 6-phosphate} + \text{NADPH} + H^+$$

Activity of creatine kinase was determined by pipetting 1000µL of working reagent and 40µL of sample into a cuvette. This was then mixed and incubated at 37˚C for 100 seconds. The change in absorbance per minute (ΔAD/min) is now read during 3 minutes.

Creatine Kinase Activity (U/L) = (ΔOD /min) × 4127

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT): The procedure for ALT and AST are the same and have thus been described together. Three test tubes were labelled as test, standard and blank. 0.5ml of reagent 1 (R_1) was pipetted into all the test tubes followed by addition of 0.1ml of the sample to the test tube labelled sample. Each test tubes mixed and incubated for exactly 30mins at 37˚C. 0.5ml of reagent 2 (R_2) was then pipetted to all the test tubes and 0.1ml of the sample added to the tube labeled sample blank. Each test tube was mixed properly and allowed to stand for exactly 20mins at 25˚C and pipette 5.0ml of NaOH to all the test tubes. The test tubes were mixed properly and the absorbance of the sample against the sample blank after 5mins at 540mm.

Alkaline phosphatase (ALP): Three test tubes were labelled as test, standard and blank. 0.5ml of ALP substrate was pipetted into all the test tubes and incubated at 37 ˚C for 3mins followed by addition of 50µl of the standard, sample and distilled water into appropriate tubes. The test tubes were then incubated for exactly 10mm at 37˚C and 2.5ml of ALP colour developer pipetted into all the test tubes & mix well. The spectrophotometer was then zeroed with the blank at 590nm and the absorbances of the standard and sample was read and recorded. ALP concentration was derived by the equation below:

$$ALP\ activity\ (U/L) = \frac{Abs\ of\ test\ \times conc\ of\ standard}{Abs\ of\ standard}$$

Liver function tests

Total protein concentration (Biuret method): Three test tubes were labelled as standard, blank and sample and 1ml of reagent was pipetted into all the test tubes and add 0.02ml of the standard, sample and distilled into appropriate tubes. The test tubes were then mixed and incubated for 30mins at 25˚C. The spectrophotometer was then zeroed at 540nm with the blank; the absorbance was then read and recorded.

Serum albumin concentration (Bromocresol Green method): Three test tubes were labelled as blank, sample and standard and pipette 3ml of albumin reagent into all the tubes. 0.01ml of sample was pipetted into appropriate test tubes, mix properly and incubated for 10mins at 25˚C. Using the blank, the spectrophotometer was zeroed at 578nm, the absorbances were read and recorded.

Total bilirubin concentration: Three test tubes were labelled as blank, sample and standard and 0.2ml of the reagent pipetted into all the test tubes and addition of 1 drop of sodium nitrate to the tube labelled

sample.1ml of caffeine was then pipetted into all the test tubes followed by 0.02ml of the sample to all the test tubes. The contents of the test tubes were mixed adequately and incubated for 10mms at 25˚C. 1ml of tartarate was then pipetted into all the tubes, mixed and incubated for 30mins at 25˚C. This was followed by zeroing the spectrophotometer at 590nm and read the absorbance.

Total Bilirubin conc. (Umol/L) = Absorbance x 185

4.0 Results and Discussion

Serum and muscle tissue enzymes were analyzed to determine the concentration of lactate dehydrogenase (LDH) and creatine kinase (CK). These enzymes have been found to be involved in ergogenic activities in skeletal muscle tissues (Xijun *et al*,2016). LDH catalyzes the interconversion of pyruvate and lactate, which are critical fuel metabolites of skeletal muscles and CK plays a pivotal role in maintaining ATP homeostasis by catalyzing the reversible transfer of high-energy phosphate from ATP to creatine, facilitating storage of energy in the form of phosphocreatine (Xijun *et al*, 2016). There was significant difference ($p<0.05$) in muscle and serum LDH concentration when compared with the control, in the muscle tissue of group 4(35.30±1.45) and in group 2(51.33±12.46) and group 5(51.47±12.46) of the serum. There was however no significant ($p<0.05$) difference in serum and muscle CK level when control was compared to the groups. These values are in agreement with previous report (Laposy *et al.*, 2012; Mehdizadeh *et al.*, 2013). In a study by Olorunnisola, *et al.*, (2011) on hepatoprotective and antioxidant effect of *Sphenocentrum jollyanum* (Menispermaceae) stem bark extract against CCl4- induced oxidative stress in rats, significant reduction ($P<0.05$) was observed in serum marker enzymes, aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP) and total bilirubin. AST, ALT, ALP and bilirubin values in this study differed from those earlier reported (Olorunnisola *et al.*, 2011); no significant difference ($p>0.05$) was observed in the ALT levels between the experimental groups and control; ALP and AST values for experimental groups were higher than control however only group 3 (1000g/kgbw) showed significant ($p>0.05$) increase. In another study, there were no appreciable increase in serum AST and ALT in the extract-administered animals and it was thus concluded that the extract had no deleterious effects on the liver (Mbaka & Adeyemi, 2010; Mbaka & Owolabi, 2011).

Total protein and total bilirubin values in this study follow a similar trend as those reported by (Raji *et al.*, 2006).

Table 3: Liver function parameters of Wistar rats

Groups	TP(g/L)	ALB(g/L)	TBIL(µmol/L)
Group 1 (Control)	72.89±1.51[a]	8.95±1.22[a]	0.97±0.11[a]
Group 2(500g/kgbw)	56.65±1.13[b]	8.27±1.25[a]	0.99±0.04[a]
Group 3(1000g/kgbw)	54.34±1.18[b]	8.71±0.57[a]	1.22±0.14[b]
Group 4(1500g/kgbw)	58.56±3.93[b]	8.27±1.53[a]	1.15±0.11[a]
Group 5(2000g/kgbw)	69.43±1.11[a]	7.71±0.64[a]	0.90±0.02[a]
Group 6(2500g/kgbw)	52.07±0.53[b]	7.09±0.35[b]	0.85±0.13[a]
Group 7(3000g/kgbw)	70.68±2.70[a]	8.48±0.70[a]	0.92±0.14[a]
Group 8(3000g/kgbw)	55.23±6.86[b]	7.16±1.20[b]	1.02±0.02[a]

Values are presented as mean± standard deviation (n=5)
Values in the same column bearing different superscripts letters differ significantly($p<0.05$)

Table 1: Ergogenic enzyme levels of Wistar rats

Groups	SERUM		MUSCLE	
	LDH (u/l)	CK (u/l)	LDH (u/l)	CK (u/l)
Group 1 (Control)	23.89±15.98[a]	14.67±4.37[a]	30.20±3.80[a]	3.33±1.81[a]
Group 2(500g/kgbw)	51.33±12.46[b]	14.47±0.25[a]	30.90±1.42[a]	4.10±0.56[a]
Group 3(1000g/kgbw)	47.17±0.40[a]	15.83±2.61[a]	34.10±1.85[a]	4.87±1.05[a]
Group 4(1500g/kgbw)	41.87±1.31[a]	10.67±2.95[a]	35.30±1.45[b]	4.53±2.21[a]
Group 5(2000g/kgbw)	51.47±3.35[b]	15.03±4.36[a]	32.03±0.89[a]	3.30±0.36[a]
Group 6(2500g/kgbw)	46.23±4.37[a]	12.27±3.92[a]	32.20±1.56[a]	3.63±1.53[a]
Group 7(3000g/kgbw)	54.20±32.39[b]	15.30±2.54[a]	32.00±3.11[a]	4.13±2.15[a]
Group 8(3000g/kgbw)	60.07±15.02[b]	16.90±1.31[a]	32.33±3.13[a]	3.53±1.74[a]

Values are presented as mean± standard deviation (n=5)
Values in the same column bearing different superscripts letters differ significantly($p<0.05$)

Table 2: Serum enzymes activity of Wistar rats

Groups	ALT(µ/L)	ALP(µ/L)	AST(µ/L)
Group 1 (Control)	29.74±6.05[a]	102.74±18.79[a]	55.69±4.69[a]
Group 2(500g/kgbw)	24.34±9.99[a]	107.44±7.01[a]	56.86±1.75[a]
Group 3(1000g/kgbw)	30.49±4.51[a]	145.44±22.66[b]	66.36±5.66[b]
Group 4(1500g/kgbw)	29.70±3.89[a]	133.70±18.62[a]	63.43±4.66[b]
Group 5(2000g/kgbw)	20.12±4.66[a]	92.38±3.59[a]	53.09±0.89[a]
Group 6(2500g/kgbw)	23.69±9.13[a]	81.92±20.84[a]	50.48±5.21[a]
Group 7(3000g/kgbw)	28.03±3.83[a]	93.82±24.26[a]	53.46±6.07[a]
Group 8(3000g/kgbw)	28.04±3.62[a]	111.02±4.37[a]	55.68±3.41[a]

Values are presented as mean± standard deviation (n=5)
Values in the same column bearing different superscripts letters differ significantly($p<0.05$)

5.0 Conclusion

The findings from this investigation provided more information on the ergogenic and hepatoprotective effects. *S. jollyanum* ethanol root extract was observed not to exert hepato-toxic effect that would lead to liver damage. This conform with earlier report of the plant to be a very useful herb in sports, which is also used extensively in treatment and management of different medical conditions.

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