

Effects of Saponin from *Solanum anguivi* Lam Fruit on Heart and Kidney Superoxide Dismutase, Catalase and Malondialdehyde in Rat

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Abstract: Reactive Oxygen Species (ROS) are generated via normal metabolic processes or as the products of exogenous insults. They are capable of damaging essential biomolecules and accelerating cancer, cardiovascular diseases and neurodegenerative diseases. In this study, the effect saponin from *Solanum anguivi* (SAS) fruits on Superoxide Dismutase (SOD), catalase (CAT) and Lipid Peroxidation (LPO) in the homogenates of the hearts and kidney was evaluated. Thirty six male Wister rats of average weight 125 ± 12 g were divided into six groups of six animals each. Five treated groups received a daily dose of saponin at 20, 40, 60, 80 and 100 mg/kg respectively, while distilled water was administered to the control group for 3 weeks. *Solanum anguivi* saponin significantly increased ($p < 0.05$) both catalase and SOD activities in the heart, There was also corresponding increase in activities of both enzymes in the kidney but was not significant. MDA concentration was reduced significantly ($p < 0.05$) in both tissues. SAS exhibit both antioxidant and antiperoxidative properties. Saponin from *Solanum anguivi* could therefore be employed as sources of natural antioxidant boosters and for the treatment of some oxidative stress disorders in which free radicals are implicated.

Keywords: Antioxidant, antiperoxidative, heart, kidney, oxidative stress

INTRODUCTION

Oxidative stress and its related biological damage have been proposed to be involved in the development and maintenance of disease (Navarro-Arevalo *et al.*, 1999). Consequently; the use of medicinal plants exhibiting antioxidative activity in the treatment and management of disease has been on the increase in recent times (Poumorad *et al.*, 2006). In the living organisms the first line of defense against free radical is the oxidative stress enzymes Superoxide Dismutase (SOD). Endogenous antioxidant enzymes such as SOD and catalase have been reported to reduce the free radical formation and prevent oxidative damage (Yang *et al.*, 2008).

Plants are generally believed to be rich in wide variety of secondary metabolites such as alkaloids, flavonoids, terpenoids and saponins of these metabolite plant antioxidants such as numerous phenolic compounds have received increased attention as useful nutraceuticals in the management of diseases (Wan and Diaz-Sanchez, 2007). Recently, there have been a tremendous commercially driven promotion of saponins as dietary supplement and nutraceuticals and there is evidence of the presence of saponins in traditional medicine preparation (Repetto and Llesuy, 2002). Saponin from different plant sources vary widely in

their toxicity (Aksoy *et al.*, 2004). Despite the high toxicity of many saponins when given intravenously to higher animals, their toxic effect are greatly reduced when administered orally (Halliwell, 1987). Ability of saponin to form pores in membranes have been reported (Ellzzi *et al.*, 1992; Authi *et al.*, 1988; Choi *et al.*, 2001). Cholesterol lowering properties of saponin from *S. anguivi* has also been reported (Adanlawo and Akanji, 2008).

Solanum anguivi lam. belong to the family *Solanaceae* and can be found in many places throughout the non arid parts of Africa. It is highly polymorphic and variable in its plant structure, fruits and leaf characters (Adanlawo and Akanji, 2003). It is a nourishing vegetable commonly used in soups and medically to control high blood pressure (Schipper, 2000). The roots are carminative and expectorant useful in coughs, catarrhal, colic, nasal ulcers, asthma, tooth ache, nervous disorder and fever (Zhu *et al.*, 2000).

The fruit of *Solanum anguivi* is eaten in south western part of Nigeria because of the traditional believe that it cures hypertension. The study therefore is aimed at providing information on the effects of the saponin from *Solanum anguivi* on the antioxidant enzymes and lipid peroxidation in heart and kidney of rats.

MATERIALS AND METHODS

Plant materials: The fruits of *Solanum anguivi* were collected from Adekunle Ajasin University, Akungba Akoko horticultural garden. They were identified and authenticated at the herbarium of plant science and Forestry department, University of Ado Ekiti, Nigeria. The fruits were air dried and grounded into a powdery fine texture and stored at room temperature in air tight polythene bag prior to use.

Preparation Saponin extract: One hundred grams of ground sample was extracted with 500 ml of petroleum ether (40-60°C) in a soxhlet extractor for 12 h. The air-dried, defatted sample was extracted with methanol (500 ml) for 12 h. The methanolic extract was partitioned between mixture of n-butanol and water (1:1 v/v). After a thorough shaking and allowing to stand overnight, the n-butanol layer was separated. The aqueous layer was washed five times with aliquots of n-butanol until it became colourless. The pooled butanolic layer was evaporated in vacuo to give a residue which was dissolved in 100 ml methanol and precipitated by adding a large amount of diethyl ether to obtain a solid crystalline dark brown compound (Adanlawo and Akanji, 2003).

Qualitative thin layer chromatography: The crude saponin fraction was spotted onto pre-coated silica gel TLC plate (Merck, Kieselgel 60F-254). The plates were developed with n-butanol: acetic acid: water (60:10:30 v/v/v). The spots on the chromatograms which were due to saponins were identified by spraying with Lieberman-Burchard reagent (methanol: sulphuric acid: acetic acid (50:5:5 v/v/v). *Solanum anguivi* saponin extract was spotted alongside a standard solution (5g/litre) of saponin white as a reference (Adanlawo and Akanji, 2003).

Saponin extract purification: Concentrated crude saponin extract was applied to a silica gel column of (60-120 mesh). The impurities were washed with n-hexane through a 2.4x50 cm bed of silica gel. The column was eluted with n-butanol: acetic acid: water (1:1:1 v/v/v). The fractions were collected and aliquots applied as a series of spots to a strip of TLC plate, dried, sprayed with Lieberman-Burchard reagent and heated. Positive fractions were pooled together and used for the experiment.

Animal grouping: Thirty six albino rats of average weight 125 ± 12 g were obtained from Animal unit of Federal university of Technology, Akure Ondo state. They were divided into six groups of four animals each and allowed to acclimatize to experimental condition for two weeks. They were housed in clean cages and maintained under standard laboratory conditions (temperature $25 \pm 2^\circ\text{C}$ with dark/light cycle 12/12 h). They were fed *ad libitum* on rat pellets by (Top Feeds, Nigeria) and water. Groups A (control) was given distilled, B, C, D, E and F were given daily oral dose of 20, 40, 60, 80, 100 mg/kg body weight of saponin respectively as previously reported by (Adanlawo and Akanji, 2008) for 21 days. The rats were sacrificed by cervical dislocation and dissected. The heart and kidney were removed into 0.25 M ice cold sucrose solution in ratio 1:5 w/v. Using the supernatant of the centrifuged homogenate of the liver tissue, the SOD level (Sun and Zigman, 1978), Catalase level (Aebi, 1984) and MDA level (Okhawa and Ohishiand, 1979) were determined.

Statistical analysis: The data are expressed as mean \pm SEM. Statistical analysis was carried out by one-way analysis of variance (ANOVA). Differences were considered to be statistically significant when ($p < 0.05$).

RESULTS AND DISCUSSION

The administration of saponin from *Solanum anguivi* fruit at test doses significantly ($p < 0.05$) increase both catalase and SOD activities in the heart while the concentration of MDA decreased significantly as depicted in Table 1.

However, in the kidney, SAS increased both catalase and SOD activities. The increase was not significant ($p < 0.05$) but at 100 mg/kg SAS there was a significant difference. While the concentration of MDA was decreased significantly ($p < 0.05$) with increasing dose of SAS as shown in Table 2.

Cells maintain a variety of defenses against oxygen toxicity. Among these are an array of enzymes that have evolved to deal with oxidative stress, including Superoxide Dismutase (SOD), catalase and malondialdehyde (Omaga *et al.*, 2011). Catalase works closely with superoxide dismutase to prevent free radical damage to the body. SOD converts the dangerous superoxide radical to hydrogen peroxide, which catalase converts to harmless water and oxygen.

Table 1: Measure of heart; SOD, CAT, MDA in all experimental and control group (n = 6, Mean±SEM)

Heart	Catalase nmols mg of protein/min	SOD u/mg of protein	MDA nmols/mg of protein
Control	0.062±0.002 ^a	2.978±1.279 ^a	17.31±0.75 ^c
20 mg/kg	0.110±0.023 ^{ab}	4.465±1.487 ^{ab}	15.64±1.76 ^c
40 mg/kg	0.132±0.019 ^{ab}	5.952±1.144 ^{ab}	12.66±0.21 ^b
60 mg/kg	0.198±0.084 ^{ab}	7.439±2.478 ^{ab}	6.56±1.63 ^a
80 mg/kg	0.207±0.083 ^{ab}	7.935±1.402 ^{ab}	4.47±0.70 ^a
100 mg/kg	0.368±0.174 ^b	10.413±2.847 ^b	4.27±1.12 ^a

Values are expressed as: mean±Standard Error Mean (SEM); Values with different superscript are significantly different (p<0.05)

Table 2: Measure of kidney; SOD, CAT, MDA in all experimental and control group (n = 6, Mean±SEM)

Kidney	Catalase nmols mg of protein/min	SOD u/mg of protein	MDA nmols/mg of protein
Control	0.084±0.011 ^a	2.978±1.279 ^a	43.37±0.99 ^d
20 mg/kg	0.104±0.030 ^a	5.952±1.402 ^a	35.65±10.73 ^{cd}
40 mg/kg	0.149±0.039 ^a	7.935±3.337 ^a	22.48±1.18 ^{bc}
60 mg/kg	0.215±0.550 ^a	8.430±2.607 ^a	20.35±2.70 ^{ab}
80 mg/kg	0.258±0.113 ^a	9.422±2.043 ^a	13.48±2.23 ^{ab}
100 mg/kg	0.855±0.737 ^a	20.327±4.235 ^b	6.18±1.40 ^a

Values are expressed as: mean±Standard Error Mean (SEM); Values with different superscript are significantly different (p<0.05)

Exposure of rats to SAS caused increased activities of both catalase and SOD and the increase was dose dependent in both kidney and heart of the animals. Hence Saponin increased antioxidant defense system of the rats in both kidney and heart. The activation of catalase and SOD observed with increase administration of *Solanum anguivi* saponin could be due to the fact that saponin has hydrogen donating abilities and chelate metal ions (Bravo, 1998).

Reactive oxygen species degrade polyunsaturated lipids, forming malondialdehyde (Pryor and Stanley, 1975). This compound is a reactive aldehyde and is one of the many reactive electrophile species that cause toxic stress in cells and form covalent protein adducts referred to as Advanced Lipoxidation End-products (ALE), in analogy to Advanced Glycation Endproducts (AGE) (Farmer and Davoine, 2007). The production of this aldehyde is used as a biomarker to measure the level of oxidative stress in an organism (Moore and Roberts, 1998; Del *et al.*, 2005).

MDA is a product of lipid peroxidation (Devaki *et al.*, 2004) Extensive lipid peroxidation leads to disorganization of membrane by peroxidation of unsaturated fatty acids which also alters the ratio of polyunsaturated to other fatty acids. This would lead to a decrease in the membrane fluidity and cell death of the cell (Onyeka *et al.*, 2012). From this study, a marked decrease in the levels of lipid peroxides were recorded both in kidney and heart of rats treated with saponin from *Solanum anguivi*. Hence, saponin from *Solanum anguivi* has the potential to prevent lipid peroxidation by inhibition of the lipid peroxidation process.

The present study results show that saponin from *Solanum anguivi* fruits has antioxidant and antiperoxidative properties. Investigation on the type and structure of saponin is still ongoing.

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