Evaluation of thioacetamide Mediated Oxidative Stress Induced Hepatic Damage

Abstract

The aim of this study was to evaluate thioacetamide mediated oxidative stress induced hepatic damage. Fresh stem bark of *Cassia spectabilis* was chopped and subsequently dried. Dried stem bark was ground into fine powder. Exactly 500 g of powdered plant sample was extract with cold water. Twenty five adult male albino rats were divided into five groups of five rats per group. Group I was the normal control and was administered with 2 ml of distilled water, **Groups II** and IV were pretreated with 400 mg/kg of extract, while **Group V** was administered with 50 mg/kg of silymarin for 10 days after which animals were sacrificed and blood sample collected for analyses which were performed using standard procedures. Aqueous extract of *Cassia spectabilis* protected against hepatic damage and enhanced the activities of antioxidant enzymes. In conclusion, aqueous extract of *C. spectabilis* can protect against oxidative stress mediated hepatic damage.

Keywords: *Cassia spectabilis*, Silymarin, Thioacetamide, Liver, Enzyme

Introduction

Oxidative stress strikes when an imbalance ensues between the generation and elimination of reactive oxygen species (ROS). It is notably one of the major causes of hepatic damage and tissue injury [1][2]. ROS orchestrate cellular disruption owing to its ability to attack cellular components such as lipids, protein and nucleic acid [3]. Liver being rated as one of the most important organs of the body is saddled with the task of biotransforming xenobiotics into forms that can be tolerated by the body. More so, it is involved in numerous biochemical and physiological functions and has been largely implicated in the synthesis of biomolecules. Thus, damage on the liver could translate to life threatening consequences. Although a very resilient

organ, it is readily and easily collapsed by oxidative stress in the absence of mitigating factors such as sustainable endogenous and exogenous antioxidant systems [4]. Despite that, dietary supplements, diverse plants and foods can be relied upon as viable sources of antioxidants, there is still intense need to sustain efforts in the search for more sources as the certainty that all source would be confined to a given location is not affirmed. Thus, the imperativeness of this study is informed.

Cassia spectabilis commonly called Golden cassia is a tropical leguminous plant and a member of the family Fabaceae, and subfamily, *Caesalpinioideae* [5]. It is a roundly shaped tree which bears evergreen foliage and measures 15–20 ft. high [6]. The plant is known to function as a laxative and purgative and has been traditionally used in the treatment of flu and cold [7]. Research efforts have revealed that extracts derived from its leaf wield antioxidant properties (Jothy et al 2012), in addition to having antifungal as well as antimicrobial activities [7].

Collection of plant material

Stem bark of *Cassia spectablis* was harvested from a compound in Afikpo North Local Government, Ebonyi State. It was subsequently conveyed in a black polythene bag for identification at the herbarium unit of the Biological Sciences, Ahmadu Bello University Zaria, Kaduna

Processing of plant material

The stem bark of *Cassia spectablis* was washed with clean tap water and afterwards dried at room temperature for 14 days. The dried stem bark of the said plant was then ground into a fine powder with the aid pestle and mortar. Precisely 500g of the powdered plant sample was steeped

in 1000 ml of distilled water for 24 hr. The resulting extract was filtered using Whattman filter paper No. 1 and filtrate obtained was dried using rotatory evaporator, weighed and reconstituted.

Animals

Adult male albio rats weighing 120-140 g were bought from animal house of the Department of Pharmacy, University of Nigeria, Nsukka, Enugu State. The Rats were kept in adequately ventilated transparent plastic cages under standard laboratory conditions. The rats were allowed unhindered access to water and food. Acclimatization of rats lasted for a period of two weeks [8].

Median Lethal Dose 50% (LD50%)

A total of nine (9) adult male albino rats were used in the initial phase of LD_{50} determination. The three groups of three rats generated from the nine rats were labeled A, B, and C and administered with 10, 100 and 1000 mg/kg of aqueous stem bark *Cassia spectablis* extract orally respectively. Observation on the rats lasted for 24 h to possible identification of signs of toxicity. Being that mortality was not recorded in the first phase, the second phase which involved three rats shared into three groups was commenced and each rat, was separately administered with 1600, 2900 and 5000 mg/kg of ***CSassia spectablis stem bark*** extract and afterwards, animals were observed for 48 h for signs of toxicity according to Lorke [9].

Experimental design

Twenty-five adult albino rats were starved of food for 24 h prior to the commencement of experiment. The rats were divided into five groups of five rats per group.

Group 1: (Normal control) rats were administered 2 ml of distilled water

Group 2: Animals were pretreated with 400 mg/kg of AECSB.

Group 3: Rats were induced oxidative stress with a single dose of 100 mg/kg/ thioacetamide (TAA)

Group 4: (AECSB+TAA): Rats were pretreated with 400 mg/kg of AECSB, prior to administration of TAA.

Group 5: (Silymari+TAA): Rats were pretreated with 50 mg/kg of silymarin, prior to administration of TAA.

Pretreatment with extract and drug (silymarin) lasted for 10 days, while TAA was administered on the 10^{th} day by a single dose subcutaneous injection of 100 mg/kg of TAA. Animals were denied food overnight, and subsequently sacrificed by cervical dislocation (Kumar et al. 2004). Collected blood samples collected in plain bottles was analysed for the serum hepatomarkers. The liver was without delay, harvested and subsequently steeped in cold saline. Exactly 1 g of liver sample from each rat was separately homogenized and the resulting homogenate, centrifuged and the supernatant obtained used to evaluate the activities of the antioxidant enzymes (catalase and superoxide dismutase).

Biochemical Assays

The activities of serum hepatomarkers such as alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST) using Randox Laboratories kits (Randox Laboratories LTD, United Kingdom, BT294QY). SOD and CAT were measured in liver tissue homogenate based the method of Marklund et al [10] and Sinha [11] respectively.

Statistical Analysis

The data obtained were expressed as Mean ± Standard Deviation using SPSS (Ver. 23). Data were analysed using one way Analysis of Variance (ANOVA). Differences in mean were compared using Turkey Test. *p-values* less than 0.05 was considered statistically significant.

Table 1: Liver enzymes activities in Rats administered with Aqueous Stem Bark Extract of *Cassia spectablis*

Treatment	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Normal Ctrl (2 ml of distilled H_2O)	19.67±0.68^{a}	35.91±2.03^{a}	58.77±4.23^{a}
Negative control (induction without treatment)	25.46±0.96^{c}	48.76±2.87^{c}	85.24±4.92^{d}
AECSB $_{100}$ mg/kg	23.02±1.15^{b}	43.00±1.82^{b}	66.76±1.86^{c}
AECSB $_{200}$ mg/kg+TAA_{100} mg/kg	21.44±0.07^{ab}	42.23±2.25^{ab}	63.33±2.32^{bc}
$Silymari_{50}$ mg/kg+TAA_{100} mg/kg	20.00±1.05^{a}	40.68±0.08^{a}	61.02±3.06^{b}

Results are expressed as mean ± standard deviation from five determinations. Values with same superscripts are not significantly different at ($P<0.05$)

Table 2: Activity of Anti-oxidant enzymes in Rats administered with Aqueous Stem Bark Extract of *Cassia spectablis*

TREATMENT	SOD	CAT
Normal Ctrl (2 ml of distilled H_2O)	167.82 ± 0.08^{c}	45.66 ± 2.37^{c}
Negative control	90.02 ± 0.20^{a}	32.00 ± 3.80^{a}
$AECSB_{400}$ mg/kg only	166.23 ± 0.30^{c}	43.00 ± 2.26^{bc}
$AECSB_{400}$ mg/kg+TAA_{100} mg/kg	160.21 ± 0.09^{c}	41.23 ± 2.89^{b}
$Silymarin_{50}$ mg/kg+ TAA_{100} mg/kg	208.34 ± 0.07^{d}	49.33 ± 4.20^{d}

Results are expressed as mean ± standard deviation from five determinations. Values with same superscripts are not significantly different at ($P<0.05$)

Results and Discussion

Diverse diseases of human and animals are orchestrated by the reactive oxygen specie induced oxidative stress. Abnormally high levels of reactive oxygen species depletes the endogenous antioxidant and subsequently fail to counteract all the ROS, thereby leading to cellular damage. The roles of antioxidants in disease prevention depend on its ability to scavenge reactive oxygen species in the biological system. *C spectabilis* processes impressive hydrogen donating and radical scavenging activity [5]. Elevation of cytoplasmic AST, ALT and ALP is considered an indicator for the release enzymes from damaged hepatocytes. Table 1 shows the activities of the liver enzymes in rats administered with aqueous stem bark extract of *C. spectabilis* showing that the activities of aspartate aminotransferase (AST), alanine transaminase (ALT) and alkaline phospatase (ALP) were significantly ($P<0.05$) higher than those reported for the normal control. However, oral administration of *C. spectablis* stem bark extract caused a dose dependent reduction in the activities of the aforementioned enzymes to levels which were not significantly ($P>0.05$) different from those reported for the normal control. The decreased serum liver enzyme activities following oral administration of extract could be attributed to the presence of antioxidants notably phenolic compounds. This is finding is consistent with Jothy et al. [5] which established that leaf of *Cassia spectablis* could protect against paracetamol induced hepatic damage. Oxidative stress manifests when there is an imbalance between the reactive oxygen species (ROS) and the endogenous antioxidant system. Distortions in a cell's normal redox state can result to the generation of

free radicals, which wield the potential to harm cellular components such as proteins, lipids, and DNA [12]. Table 2 shows the activities of antioxidant enzymes in rats administered with aqueous extract of stem bark of *Cassia spectabilis* indicating that group II administered with TAA manifested markedly low levels of superoxide dismutase and catalase. TAA is rapidly bio-transformed into free radical derivatives such as TAA sulphoxide and TAA-S-S-dioxide which leads to lipid perioxidation [13]. However, oral administration of *C. spectabilis* stem bark extract significantly ($P<0.05$) increased the activities of superoxide dismutase (SOD) and catalase (CAT) to levels which were not significantly ($P>0.05$) different from those reported for the normal control.

Conclusions

The outcome of this study clearly reveals that aqueous extract of *Cassia spectabilis* can offer protection against oxidative stress mediated hepatic damage.

References

[1] Gill JG, Piskounova E, Morrison SJ. Cancer, oxidative stress, and metastasis. In: Cold Spring Harb Symp Quant Biol. Cold Spring Harbor: Cold Spring Harbor Laboratory Press; 2016.

[2] Ganesan, K.; Sukalingam, K.; Xu, B. Solanum trilobatum L. ameliorate thioacetamide-induced oxidative stress and hepatic damage in albino rats. Antioxidants **2017**, 6, 68.

[3] Sies H. Oxidative stress. London: Academic Press Inc.; 2013.

[4] Friedman, L. S. (2014). Liver, biliary tract, and pancreas disorders. In: Current medical diagnosis and treatment. 53rd ed. San Francisco(California): Mcgraw-Hill, pp. 13-15.

[5] Jothy SL, Aziz A, Chen Y, Sasidharan S. Antioxidant activity and hepatoprotective potential of Polyalthia longifolia and Cassia spectabilis leaves against paracetamol-induced liver injury. Evid Based Complement Altern Med. 2012; 2012:1–10. https://doi.org/10.1155/2012/561284.

[6] Omotayo FO. Plants of south western Nigeria. Ibadan: University of Ibadan press; 1999

[7] Sangetha S, Zuraini Z, Sasidharan S, Suryani S. Fungicidal effect and oral acute toxicity of Cassia spectabilis leaf extract. Jap J Medic Mycol. 2008;49: 299–304.

[8] National Institute of Health (NIH) revised guide for the care and use of laboratory animals NIH guide 1996.

[9] Lorke D. A new approach to practical acute toxicity testing. Arch Toxicol 1983; 54:275-89.

[10] Marklund, S.; Marklund, G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem. **1974**, 47, 469–474.

[11] Sinha, A.K. Colorimetric assay of catalase. Anal. Biochem. **1972**, 47, 389–394.

[12] Birnboin, H. C. (1986). DNA strand breaks in human leukocytes induced by super-oxide anion, hydrogen peroxide and tumor promoters are repaired slowly compared to breaks induced by ionizing radiation. Carcinogenesis, 7(9), 1511–1517.

[13] Bashandy, S.A.; Alaamer, A.; Moussa, S.A.; Omara, E. Role of zinc oxide nanoparticles in alleviating hepatic fibrosis and nephrotoxicity induced by thioacetamide in rats. Can. J. Physiol. Pharmacol. **2017**, 16, 1–8.