Journal of Medicine, Physiology and Biophysics
ISSN 2422-8427 An International Peer-reviewed Journal
Vol.45, 2018

Antioxidant Profile of Graded Levels of Unprocessed Pigeon Pea (Cajanus cajan) Seeds Assayed from Liver and Kidneys of Male Wistar Strain Rats

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Abstract
The present study investigated the hepatic and renal antioxidant profiles in male Wistar rats fed with graded levels of unprocessed pigeon pea (*Cajanus cajan*) seeds for 21 days. The study consisted of six groups of 5 rats each. Group A was fed 10% pigeon pea inclusion diet (PPID), group B: 20% (PPID), Group C: 30% (PPID), Group D: 40% (PPID), Group E: 100% pigeon pea diet (PPD), while Group F rats were fed with commercial rat feed and served as the control. Endogenous activities of antioxidant enzymes namely catalase and superoxide dismutase as well oxidative stress markers including hydrogen peroxide and malondialdehyde were analyzed in the liver and kidney of the rats. Results showed that reduced glutathione (GSH) concentration was decreased in the kidneys whereas it increased significantly above the control in the liver tissues of rats in all the fed groups. Feeding of unprocessed *Cajanus cajan* caused a significant increase in the hydrogen peroxide (H_2O_2) level in the liver of 30% PPID and in 40% PPID group and in the kidney at 100% PPD group. Malondialdehyde (MDA) concentration was significantly ($p<0.05$) increased in the liver and kidney tissues of the rats with the highest value in the 40% PPID for the liver of the rats. Renal superoxide dismutase (SOD) activity was significantly decreased in 10% PPID and in 30% PPID rats whereas hepatic SOD activity increased in the 40% PPID and in 100% PPD group. Hepatic catalase activity increased significantly in all treatment groups whereas the increase in renal catalase activity was noted only in 40% PPID and in 100% PPD group. In conclusion, consumption of unprocessed pigeon pea (*Cajanus cajan*) seeds disrupted antioxidant system and induced oxidative stress in the liver and kidney of rats.
Keywords: Pigeon pea, antioxidant, liver, kidney, rats.

INTRODUCTION

Pigeon pea (*Cajanus cajan)*, is a leguminous plant belonging to the family Fabbaceae (Krishna and Bhatia, 1985). It is known as known as "*fio fio"* in Igbo, *otiili* in Yoruba and pigeon pea in English (Aiyeloja and Bello, 2006). It is native to India, the world's largest producer. Pigeon pea is also grown in Africa and the Americas, and it has been suggested to be one of Africa's drought-tolerant crops and is referred to as an 'orphan crop' because it falls into the group of least researched crops world-wide (Odeny, 2007).

Pigeon peas are domesticated and harvested as crops for human and as a forage for animal consumption (Odeny, 2007; Ojo, 2013). *C. cajan* extracts have several reported ethno-medicinical applications as therapeutic agents in diseases and disorders like sickle cell anemia (Egunyomi *et al*., 2009), GIT infections (Luo *et al*., 2010), hepatic disorder (Kundu *et al*, 2008), hyperlipidermia (Dai *et al*., 2013), bladder stone, jaundice and cardiac diseases (Lawal, 2012), hypertension (Ajaiyeoba *et al.*; 2005; Lawal, 2012), and both the seeds and leaves of *C. cajan* have hypoglycemic potentials (Jaiswal *et al*., 2008). Smokes from the burnt leaves of *C. cajan* is inhaled as a relief from cough and asthma (Lawal, 2012).

Pigeon pea is high in protein (21.5%) and the fiber content is approximately 2.5% (Akande *et al*., 2010). Pigeon-pea extracts have also been used for the treatment of dysentery, diabetes, hepatitis and measles and as a febrifuge to stabilize the menstrual period (Amalraj *et al.,* 1998). Other ethno-medical applications of pigeon peas are, to arrest blood, achieve analgesia and kill helminths (Tang *et al*., 1999). Protective effects of extracts from pigeon pea leaf against hypoxic-ischaemic brain damage and alcohol-induced liver damage have also been reported (Huang *et al.,* 2006). Evaluation of the antioxidant properties of natural substances has been of interest in recent years (Cardador-Martinez *et al*. 2002). Antioxidants scavenge free radicals and reactive oxygen species and can be very important in inhibiting the oxidative me-chanisms leading to several degenerative diseases (Cardador-Martinez *et al*. 2002).

Antioxidants are extensively used as food additives to act against oxidative stress caused by free radicals (Gulcin *et al.,* 2002). The antioxidant properties of aqueous and ethanol extracts of pigeon-pea leaves, different fractions of the ethanol extract with its four main components, namely cajaninstilbene acid, pinostrobin, vitexin and orientin by DPPH and β-carotene-linoleic acid assays have been reported (Nan *et. al.,* 2009).

In spite of the several documentations on the ethno-medicinal applications of pigeon pea, there is dearth of information in the literature on the effects of pigeon pea seeds on the antioxidant status of the liver and kidney. The present study determined the hepatic and renal antioxidant enzymes activities and lipid peroxidation level in

rats following consumption of graded levels of unprocessed pigeon pea seeds for twenty one days.

MATERIALS AND METHODS

Experimental Protocols

Thirty male Wistar rats weighing between 100 g and 120 g were purchased from the animal house of the Department of Physiology, University of Ibadan, Ibadan. The rats were kept individually in Allentown stainless steel metabolism cages located at the Department of Animal Science, University of Ibadan, Ibadan. They were allowed to acclimatize for a period of two weeks. The animals were weighed and the weights recorded before commencement of experiment**.** The rats were assigned into six groups (A-F) of 5 rats each. Group A was fed 10% pigeon pea, group B: .20% pigeon pea, Group C: 30% pigeon pea, Group D: 40% pigeon pea, Group E: 100% pigeon pea, while Group F rats served as the control being fed with commercial rat concentrates feed. All the rats were given 30g of feeds per day for 21 days with water *ad libitum*. Ethical approval was given by the Institutional Animal Care Use in Research Ethical Committee (ACUREC).

Feed Preparation

The *C. cajan* seeds and the rat concentrate feed (3kg each) was ground into powdery form using an electric miller. The feed was then be reconstituted into different percentage inclusion of pigeon pea seeds (10%, 20%, 30%, 40%, 100%) and normal concentrate feed as control.

Table 1 shows the composition of normal rat feed and Table 2 shows the graded levels of pigeon pea (*Cajanus cajan*) inclusion diets,

Table 1: Composition of Normal Feed

INGREDIENTS	PERCENTAGE (%)
Carbohydrate (Maize)	40
Protein (GNC, PKC, SB)	32 (8,16,8)
Fiber Content (Wheat bran, Rice bran)	18 (16,2)
Bone Meal	6
Oyster shell	3.8
Premix (Methionine and Lysine)	0.08
Salt	0.12

* *GNC- Groundnut cake, PKC- Palm kernel cake, SB- SOYA BEAN*

Source: Soetan *et al*. (2017).

Table 2: Graded levels of Pigeon pea (*Cajanus cajan*) Inclusion Diets

GROUPS	NORMAL FEED(kg)	PIGEON PEA INCLUSION(kg)	TOTAL(Kg)
A (Control, Normal feed)	3	-	3
B (10% inclusion)	2.7	0.3	3
C(20% Inclusion)	2.4	0.6	3
D(30% inclusion)	2.1	0.9	3
E(40% inclusion)	1.8	1.2	3
F(100%)	-	3	3

Source: Soetan *et al*. (2018).

Chemicals

Hydrogen peroxide (H_2O_2), hydrochloric acid, sulphuric acid, xylenol orange, sodium hydroxide, potassium iodide, reduced glutathione (GSH), potassium dichromate, O-dianisidine, sodium potassium tartrate, copper sulphate, glacial acetic acid, ethanol, sodium azide, 2-dichloro-4-nitrobenzene (CDNB), thiobarbituric acid (TBA), Trichloroacetic acid, Ellman's reagent (DTNB), ammonium ferrous sulphate, sorbitol were purchased from Sigma (St Louis, MO, USA). All other chemicals used were of analytical grade and obtained from British Drug Houses (Poole, Dorset, UK).

Determination of liver and kidney antioxidant profile

The post-mitochondrial fraction of the liver and kidneys of the rats were prepared by homogenizing in Tris-HCl Buffer (50 mM) (pH 7.4), containing 1.15% potassium chloride. The homogenate was then centrifuged at 12,000 g for 15 min at 4°C and the supernatant was collected for biochemical parameters.

Reduced glutathione (GSH) level was determined at 412 nm by the method of Jollow *et al*. (1974). Hydrogen peroxide (H_2O_2) generation was determined using the method of Wolff, (1994). Lipid peroxidation, quantified as malondialdehyde (MDA) was determined according to the method of Farombi *et al*. (2000) and the result expressed as micromoles of MDA per milligram protein. Superoxide dismutase (SOD) activity was

assayed according to the method of Misra and Fridovich, (1972). Catalase (CAT) activity was assayed using hydrogen peroxide as substrate according to the method of Clairborne, (1995). Protein concentration was determined according to Lowry *et al*. (1951).

Statistical Analysis

Statistical analysis were carried with one-way analysis of variance (ANOVA) to compare the experimental groups followed by Bonferroni's test, to identify significantly different groups (SPSS for Windows, version 17). Values at $P<0.05$ was considered statistical significance.

RESULTS

LIVER

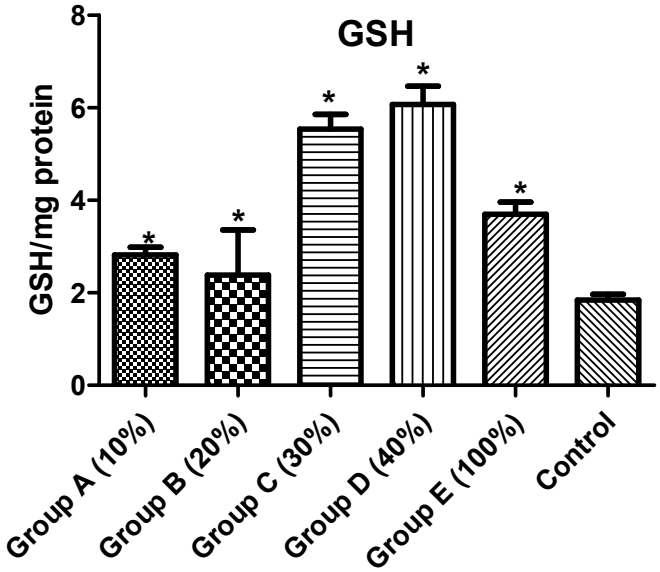

Figure 1a: Levels of GSH in the liver of rats following consumption of unprocessed pigeon pea for 21 days. Values are expressed as mean ± S.D of five rats. *P < 0.05 versus Control.

KIDNEY

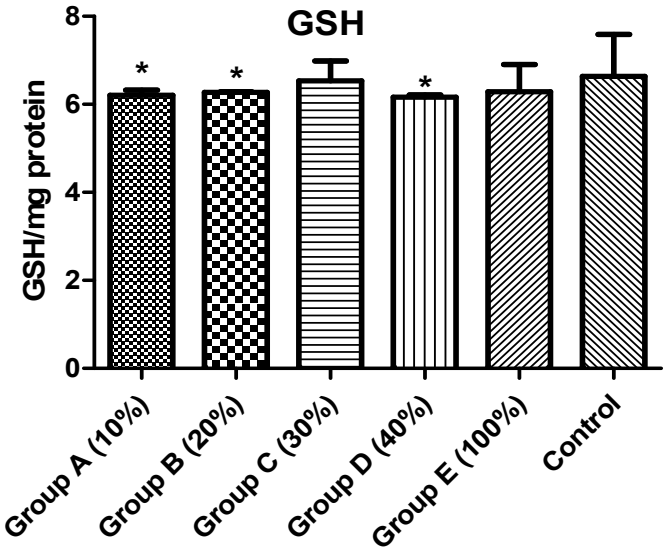

Figure 1b: Levels of GSH in the kidney of rats following consumption of unprocessed pigeon pea for 21 days. Values are expressed as mean ± S.D of five rats. *P < 0.05 versus Control.

LIVER

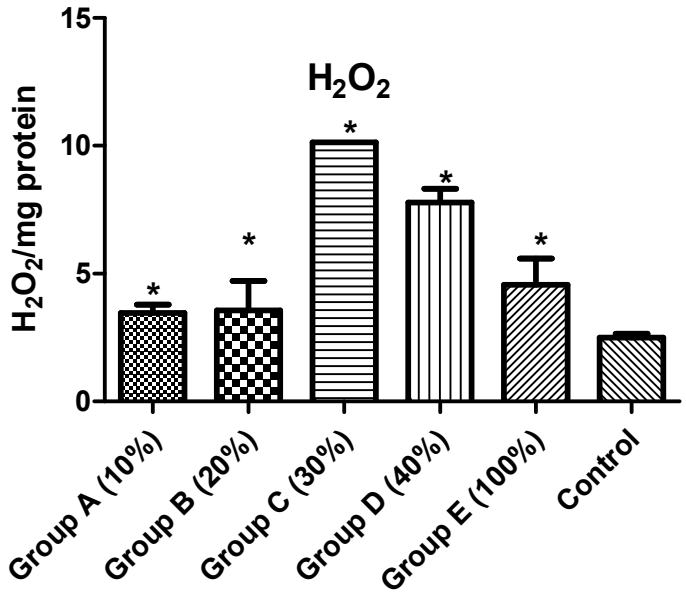

Figure 2a: Levels of H_2O_2 in the liver of rats following consumption of unprocessed pigeon pea for 21 days. Values are expressed as mean ± S.D of five rats. *P < 0.05 versus Control.

KIDNEY

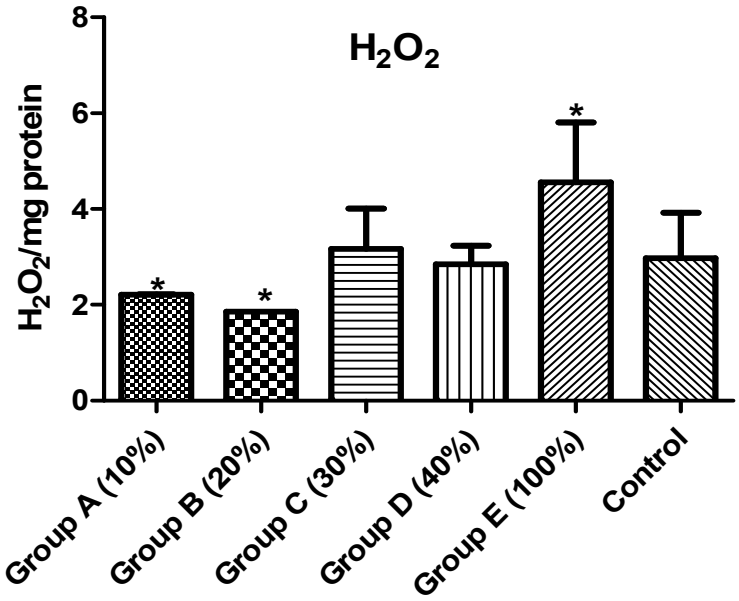

Figure 2b: Levels of H_2O_2 in the kidney of rats following consumption of unprocessed pigeon pea for 21 days. Values are expressed as mean ± S.D of five rats. *P < 0.05 versus Control.

LIVER

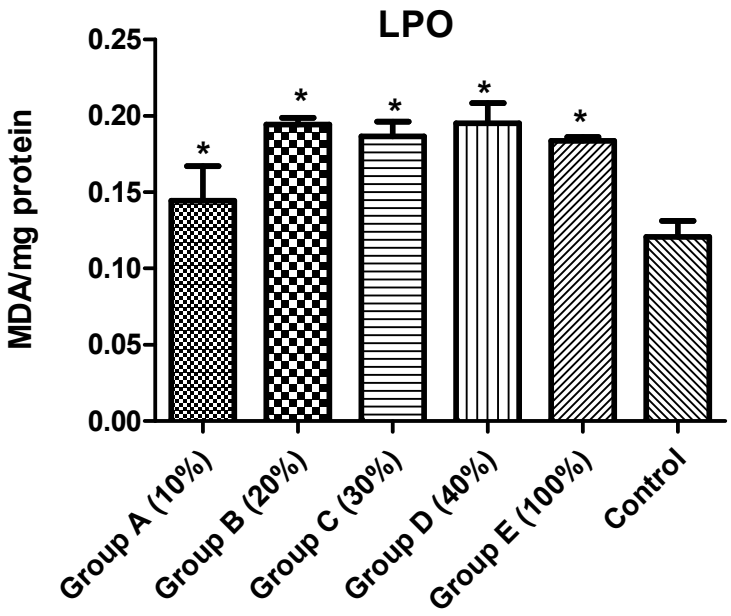

Figure 3a: Levels of LPO (quantified as MDA) in the liver of rats following consumption of unprocessed pigeon pea for 21 days. Values are expressed as mean ± S.D of five rats. *P < 0.05 versus Control.

KIDNEY

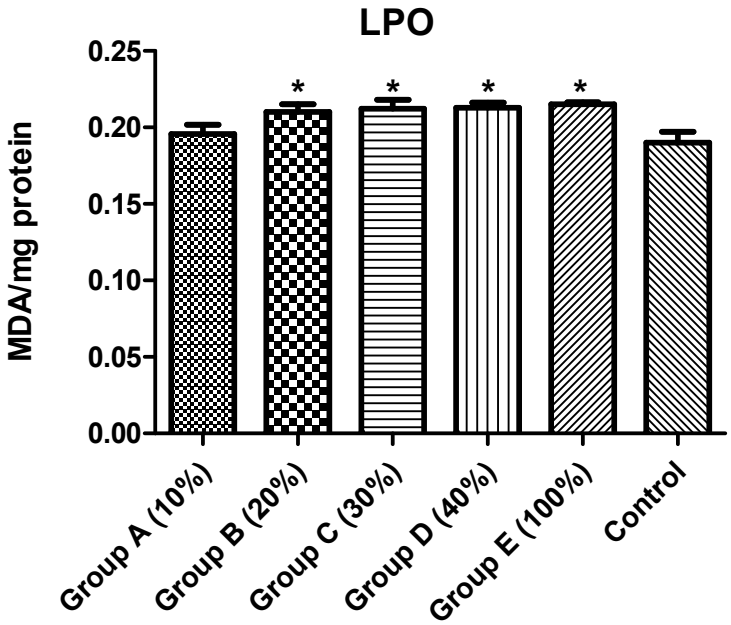

Figure 3b: Levels of LPO (quantified as MDA) in the kidney of rats following consumption of unprocessed pigeon pea for 21 days. Values are expressed as mean ± S.D of five rats. *P < 0.05 versus Control.

LIVER

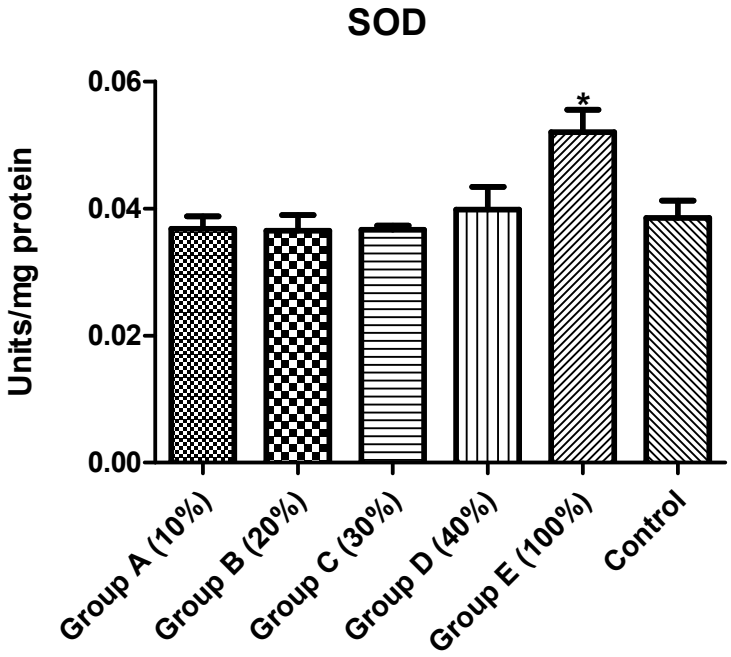

Figure 4a: Activities of SOD in the liver of rats following consumption of unprocessed pigeon pea for 21 days. Values are expressed as mean ± S.D of five rats. *P < 0.05 versus Control.

KIDNEY

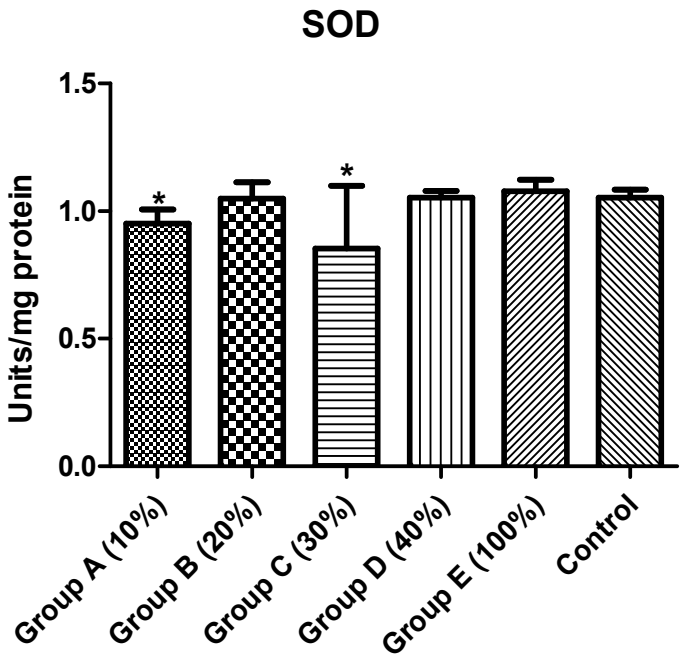

Figure 4b: Activities of SOD in the kidney of rats following consumption of unprocessed pigeon pea for 21 days. Values are expressed as mean ± S.D of five rats. *P < 0.05 versus Control.

LIVER

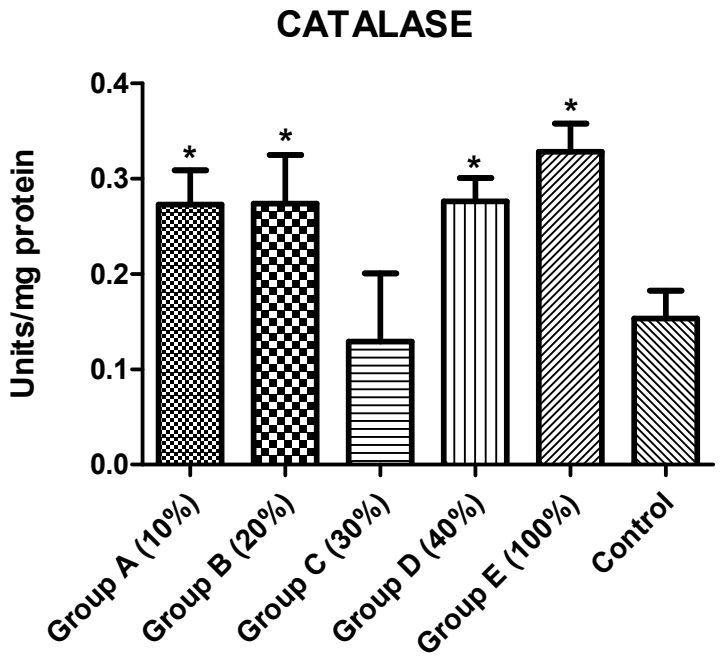

Figure 5a: Activities of catalase in the liver of rats following consumption of unprocessed pigeon pea for 21 days. Values are expressed as mean ± S.D of five rats. *P < 0.05 versus Control.

KIDNEY

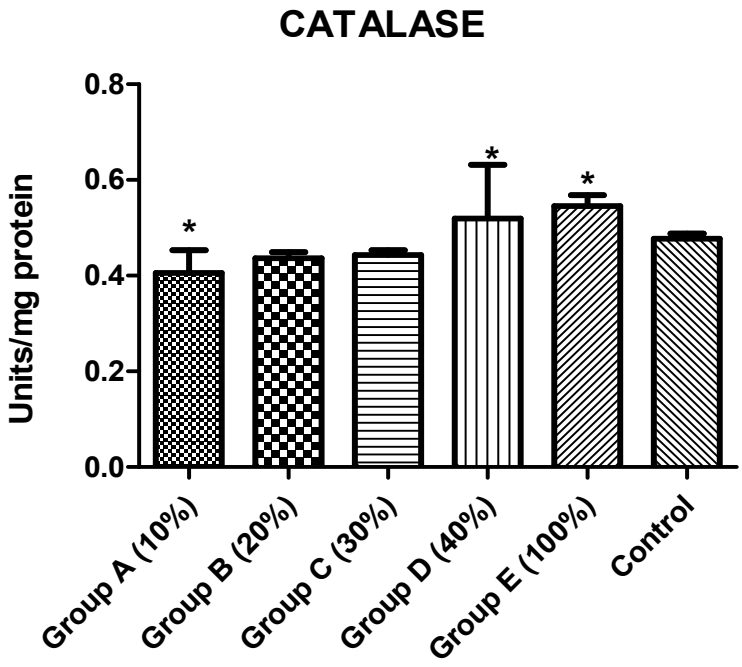

Figure 5b: Activities of catalase in the kidney of rats following consumption of unprocessed pigeon pea for 21 days. Values are expressed as mean ± S.D of five rats. *P < 0.05 versus Control.

LIVER

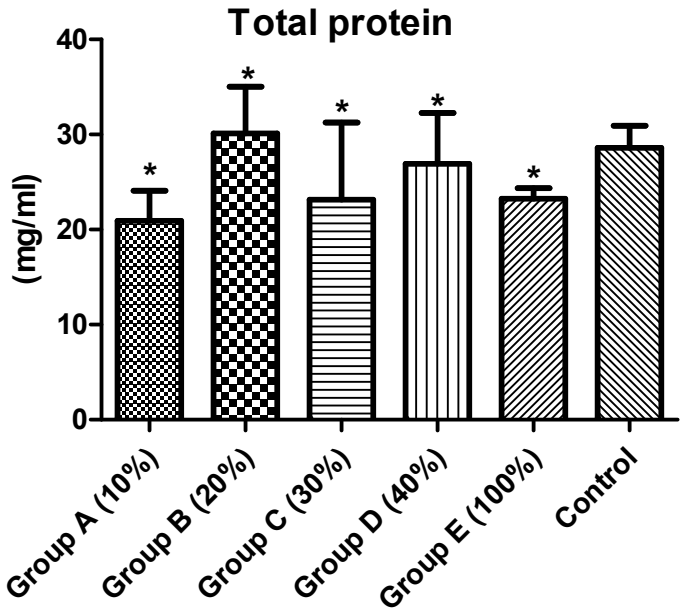

Figure 6a: Total protein concentration in the liver of rats following consumption of unprocessed pigeon pea for 21 days. Values are expressed as mean ± S.D of five rats. *P < 0.05 versus Control.

KIDNEY

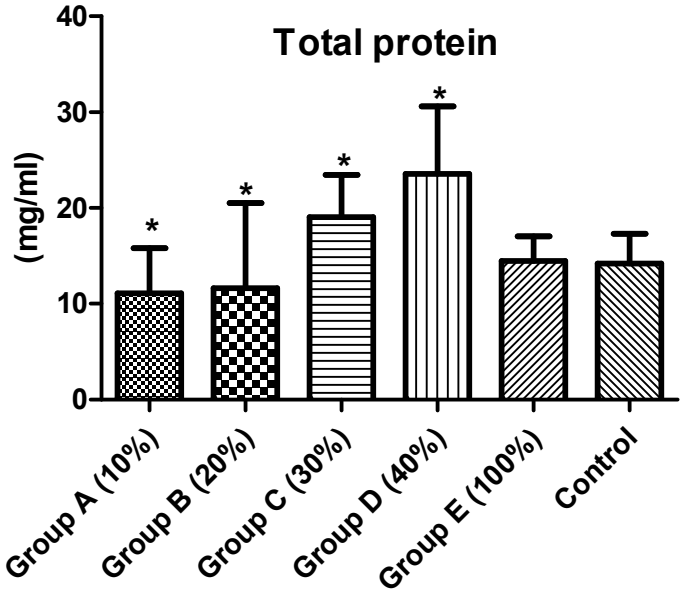

Figure 6b: Total protein concentration in the kidney of rats following consumption of unprocessed pigeon pea for 21 days. Values are expressed as mean ± S.D of five rats. *P < 0.05 versus Control.

Discussion

GSH concentration was decreased in the kidneys whereas it increased significantly above the control in the liver tissues of rats in all the *Cajanus cajan* fed groups. Feeding of unprocessed *Cajanus cajan* caused a significant increase in the H_2O_2 level in the liver of 30% PPID and in 40% PPID group and in the kidney at 100% PPD group. MDA concentration was significantly increased in the liver and kidney tissues of the rats with the highest value in the 40% PPID for the liver of the rats. Renal SOD activity was significantly decreased in 10% PPID and in 30% PPID rats whereas hepatic SOD activity increased in the 40% PPID and in 100% PPD group. Hepatic catalase activity increased significantly in all treatment groups whereas the increase in renal catalase activity was noted only in 40% PPID and in 100% PPD group.

Cajanus cajan induced oxidative stress by disrupting the antioxidant defense mechanisms in the liver and kidney of rats. The observed decrease in the renal GSH level in the present study may indicate an increased demand of GSH to detoxify ROS generation in the tissue. GSH enzymes have very high levels in the liver and also serve in detoxification metabolism especially against hydrogen peroxide (Hayes *et al.,* 2005).

Superoxide radical is converted to H_2O_2 by the SOD whereas CAT is responsible for the detoxification of H_2O_2 (Misra and Fridovich, 1972). The significantly elevated levels of H_2O_2 observed in the liver and kidney shows that increase in the antioxidant defense enzymes in rats fed with the *Cajanus cajan* was insufficient to eliminate this noxious substance from the hepatic and renal tissues. The production of H_2O_2 is used as a bio-indicator of oxidative stress in animals (Yung *et al*., 2006). Hydrogen peroxide is a toxic by-product of many normal metabolism which must be converted to less dangerous substances to prevent damage to cells *(*Hill, 2001).

Lipid peroxidation (LPO) disrupts the integrity of cellular membranes and consequently results in tissue injury. The increase in the levels of malondialdehyde (MDA), a biomarker of LPO, in the liver and kidney of *Cajanus cajan*-fed rats may be attributed to the increased production of reactive oxygen species and altered antioxidant defense system. Previous studies indicated that consumption of unprocessed *Lablab purpureus* seeds increased LPO in the liver and kidney of rats (Soetan *et al*., 2016).

In the present study, consumption of unprocessed *Cajanus cajan* seeds caused a significant decrease in renal SOD but increased hepatic SOD activity along with augmentation in CAT activities of both liver and renal tissues, thus suggesting their induction possibly to combat the generation of reactive oxygen species (ROS) during *Cajanus cajan* metabolism in these tissues.

Superoxide dismutases (SODs) catalyze the breakdown of the superoxide anion into oxygen and hydrogen peroxide and are present in almost all aerobic cells and in extracellular fluids. Hence, SODs are an important antioxidant defense in nearly all cells exposed to oxygen and are present in almost all aerobic cells and in extracellular fluids (Zelko *et al.,* 2002). An increase in SOD is known to be a boost to the immune competence of animals. Catalase is produced in the liver and concentrated in peroxisomes located close to mitochondria which are exposed to oxygen, where it functions to catalyze the breakdown of hydrogen peroxide to water and oxygen. (Chelikani *et al.,* 2004). Catalase is therefore frequently used by cells to rapidly catalyze the decomposition of hydrogen peroxide into less reactive gaseous oxygen and water molecules, although all known animals use catalase in every organ and the concentration is highest in the liver (Goodsell, 2004).

Conclusion

Based on the available data presented, it can be concluded that consumption of unprocessed seeds of pigeon pea (*Cajanus cajan*) induced oxidative stress in the liver and kidney of rats via a mechanism involving disruption of antioxidant defense systems and elevation in H_2O_2 generation and lipid peroxidation in the liver and kidney of fed rats.

Conflict of Interest

There is no conflict of interest in this study.

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