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Hepatoprotective effect of *Cajanus cajan* on tissue defense system in D-galactosamine-induced hepatitis in rats

[*Cajanus cajan*'ın D-galaktozamin ile indükte hepatitli sıçanların doku savunma sistemlerine hepatik koruyucu etkisi*]

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ABSTRACT

Purpose: The aim of the study to investigate the hepatoprotective effect of the *Cajanus cajan* ethanolic extract on hepatic antioxidant status in D-galactosamine-induced hepatitis rats by measuring the extent of oxidative damage as well as the state of the antioxidant defense system.

Material and Methods: Ethanolic extract of *Cajanus cajan* was administered orally (100mg/kg body weight) and the effect of the extract on the activities of Alanine Aminotransferase, Aspartate Aminotransferase, Catalase, Superoxide Dismutase, Glutathione Peroxidase, Glutathione-*S*-transferase as well as the levels of malondialdehyde and reduced glutathione were estimated in D-galactosamine induced hepatitis rats.

Results: A significant ($p<0.05$) increase in the activities of liver marker enzymes (Alanine Aminotransferase, Aspartate Aminotransferase) levels, and decrease in antiperoxidative enzymes (Catalase, Superoxide Dismutase, Glutathione Peroxidase, Glutathione-*S*-Transferase) activities were observed in the tissues of D-galactosamine-induced hepatitis rats. Prior administration of *Cajanus cajan* extract (100 mg/kg body weight for 10 days) significantly ($p<0.05$) reversed the D-galactosamine-induced increases in the levels of Alanine Aminotransferase and Aspartate Aminotransferase with corresponding increases in antiperoxidative enzymes activities. The extract also reduced the extent of lipid peroxidation as evidenced by decreased level of malondialdehyde concentrations.

Conclusion: Since the study of induction of the antioxidant enzymes is considered to be a reliable marker for evaluating antiperoxidative efficacy of medicinal plant, these findings suggest a possible/potential antiperoxidative role for *Cajanus cajan* plant extract in hepatic system.

Keywords : *Cajanus cajan*, hepatoprotective, D-galactosamine, hepatitis, rats.

ÖZET

Amaç: Çalışmanın amacı, *Cajanus cajan*'ın etanolik ekstraktının D-galaktozamin ile hepatit oluşturulmuş sıçanların hepatik antioksidant seviyeleri üzerine etkisini, antioksidant savunma sistemi üzerinde oluşan oksidatif hasarı ölçerek belirlemektir.

Gereç ve Yöntem: *Cajanus cajan*'ın etanolik ekstraktı, 100mg/kg vücut ağırlığı olmak üzere D-galaktozamin ile indükte hepatitli sıçanlara oral olarak verilmiştir ve sıçanlarda ekstraktın, Alanin Aminotransferaz, Aspartat Aminotrasferaz, Katalaz, Süperoksit Dismutaz, Glutatyon Peroksidaz, Glutatyon *S*-transferaz aktiviteleri ve malondialdehit, glutatyon düzeyleri üzerine etkisi belirlenmiştir.

Bulgular: D-galaktozamin ile indükte hepatitli sıçanlardan alınan dokularda, karaciğer marker enzimleri Alanin Aminotransferaz ve Aspartat Aminotrasferaz aktivitelerinde önemli artış görülmekle beraber ($p<0.05$), Katalaz, Süperoksit Dismutaz, Glutatyon Peroksidaz ve Glutatyon *S*-transferaz aktivitelerinde düşüş gözlenmiştir. *Cajanus cajan* ekstraktı uygulamasından sonra (10 gün boyunca 100 mg/kg vücut ağırlığı) Alanin ve Aspartat Aminotransferazların D-galaktozamin ile indükte düzeylerinde, antiperoksidatif enzim aktiviteleri ile birlikte önemli bir geri artış gözlenmiştir ($p<0.05$). Ekstraktın aynı zamanda lipit peroksidasyonunu azaltığı, malondialdehit konsantrayon düzeylerindeki düşüşle gösterilmiştir.

Sonuç: Tıbbi bitki *Cajanus cajan* ektraktının antioksidant enzimleri düzeylerine indüksiyonu ile gösterilen antiperoksidatif etkisi, bu bitkinin hepatik sistem üzerine olası antiperoksidatif rolü olduğunu göstermektedir.

Anahtar kelimeler: *Cajanus cajan*, hepatik koruyucu, D-galaktozamin, hepatit, sıçan.

Introduction

Cajanus cajan (L.) Millsp (*Leguminosae*) otherwise known as pigeon pea, Congo pea, Gungo pea, Gunga pea or no-eye pea, is a perennial medicinal plant widely used for the treatments of wounds, aphtha, bedsore and malaria [1, 2]. Protective effects of extract from pigeon pea leaf against hypoxic-ischemic brain damage and alcohol-induced liver damage have been reported [3, 4]. Antioxidant activities and chemical constituents' investigations have indicated that *Cajanus cajan* (C.c) leaves are rich in flavonoids and stibenes [5, 6, 7]. Also, hepatoprotective activity of the methanolic extract of C.c against CCl_4 hepatotoxicity in rats was reported by Ashan *et. al* [8].

Hepatitis is one of the major public health problems worldwide and is known to be responsible for considerable morbidity and mortality from liver disease [9]. Part of the urgent need for the clinical development of safe and relatively non-toxic cytoprotective agent for the adequate management of hepatitis is the use of local herbs of proven safety property. For instance, antihepatotoxic/preventive effects of *Sargassum polycystum* ethanol extract and whey protein on hepatic antioxidant defense system in galactosamine-induced hepatitis in rats was reported by Meena *et. al.,* [10] and Kume *et. al* [11], respectively.

Hepatitis induced by D-galactosamine (D-galn) have been reported to show many metabolic and morphological aberration in the liver of experimental animals and its mode of action have been attributed to peroxidation of endogenous lipid and loss of plasma lipid membrane integrity [12, 13]. This study was carried out to assess the hepatoprotective effect of *C. cajan* methanol extract on tissue defense systems in D-galactosamine-induced hepatitis in rats.

Material and Methods

Drug and Chemicals

D-Galn was obtained from Sigma Chemical Company St. Louis, MO, USA. All other chemicals were of analytical grade. *Cajanus cajan* authenticated by Dr Aworinde of the Biological Sciences Department, (Botany unit), University of Agriculture, Abeokuta, Nigeria, prepared from the dried leaves (yield 12.5%). The alcoholic extract of C.c contains cajaninstilbene acid (3-hydroxy-4-phenylmmethoxylstilbene-2-carboxylic acid), pinostrobin, vitexin and orientin [5, 6, 7].

Animals

Twenty four (24) wistar rats (weighing 180-200g) of both sexes used in this study were purchased from the Department of Veterinary anatomy, University of Ibadan, Nigeria. They were housed under standard condition and allowed water and standard pellet diet (Ladokun and sons feeds, Nig. Ltd.) *ad libitum*. The experiment was carried out as per the guidelines of committee for the purpose of control and supervision of experiment on animal care and handling. The protocol conforms to the guidelines of the National Institute of Health (NIH).

Hepatoprotective activity

The experimental animals were divided into four groups of six animals each. Group 1 served as control, group II were normal animals orally treated with C.c (100mg/kg per day) for 10 days. Group III animals were intraperitoneally injected with D-galn (500mg/kg, dissolved in saline for 2 days) for induction of hepatitis as described by Deaciue *et. al*., (1993). Group IV animals were orally pretreated with C.c (100mg/kg per day, for 10 days, dissolved in distilled water) and then intraperitoneally injected with D-galn (500mg/kg per day) for 2 days.

At the end of the experiment, animals were killed by decapitation. Blood was collected without anticoagulant and serum was separated for biochemical assays of alanine transaminases (ALT) and aspartate transaminases (AST) using commercial Randox diagnostic kits. The liver was excised immediately and homogenized in ice-cold 0.1M Tris-HCl buffer, using Potter-Elvehjem homogenizer. The homogenate was used for estimation superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), glutathione-*S*-transferase (GST) and glutathione peroxidase (GPx) according to the methods of Misra and Fridovich, [15], Aebi [16], Moran [17], Habig *et. al*., [18] and Pagila and Valentine [19], respectively.

Statistical analysis

Data generated were expressed as mean ± S.D, the level of significance was tested using Kruskal-Wallis test while the groups were compared with Wilcoxon rank sum test. The level of significance was tested at $p<0.01$.

Results

Use of indigenous plants have received considerable attention for the treatment of hepatitis in the recent times not only because currently available drugs for the treatment of hepatitis have a number of limitations but also due to adverse and high rate of secondary failures associated with them. Previous reports have shown that C.c possess significant free radical scavenging property. The focus of this study was to evaluate the effects of ethanolic leaves extract of C.c for its antioxidant and membrane-stabilizing properties during galactosamine-induced hepatitis in rats.

Intraperitoneal administration of D-galn caused a significant ($p<0.01$) increase in the level of marker enzymes ALT and AST in the serum of group III D-galn-injected rats compared to that of group I control rats (table 1). This suggests cellular leakages and loss of functional integrity of the liver cell membrane. This is in agreement with the reports of Kucera *et.al.,* [13], Kume *et. al*., [11], that the amount of diagnostic marker enzymes presents

Table 1. Effects of *C. cajan* Extract of The Activities of Antioxidant and Antiperoxidative Enzymes Status in Rats

GROUPS / PARAMETERS	CONTROL GRP I	EXTRACT GRP II	HEPA GRP III	EXTR + HEPA GRP IV
ALT	72.1± 6.30[a]	74.8± 6.07[a]	288±24.6[b]	109±9.20[c]
AST	79.2± 6.15[a]	76.7±6.10[a]	276±22.8[b]	125±9.96[c]
SOD	7.35± 0.82[a]	7.47±0.92	3.02±0.66[b]	5.99±0.78[c]
CAT	68.52± 5.4[a]	69.30±5.5	30.62±3.8[b]	57.49±5.3[c]
GST	11.53±1.3[a]	12.07±1.4[a]	4.98±0.96[b]	10.42±1.2[a]
GP_x	70.48± 5.5[a]	72.63±6.0[a]	49.45±4.4[b]	68.34±4.8[a]
GSH	6.11± 0.36[a]	5.06±0.34	2.97±0.21[b]	4.51±0.31[a]
MDA	1.24± 0.16[a]	1.41±0.18[a]	3.69±0.27[b]	1.55±0.19[a]

Values are expressed as means ± SD; n=6. Values that have different superscript letter (a,b,c) differ significantly with each other ($p<0.01$; Wilcoxon rank sum test)

Grp II : C.c 200mg/kg, p.o for 10 days
Grp III : D-galn, 500mg/kg per day, i.p for 2 days
ALT = U/L
AST = U/L
SOD = One unit of SOD activity is the amount of protein required to give 50% inhibition of nitrite formation.
CAT = nmol. of H_20_2 decomposed/min/mg protein
GST = µmol. of 1-chloro 2, 4-dinitrobenzene conjugate formed/min/mg protein.
GPx = nmol. glutathione oxidized /min / mg protein
GSH = nmol. /g wet liver.
MDA = nmol. malondialdehyde / mg protein

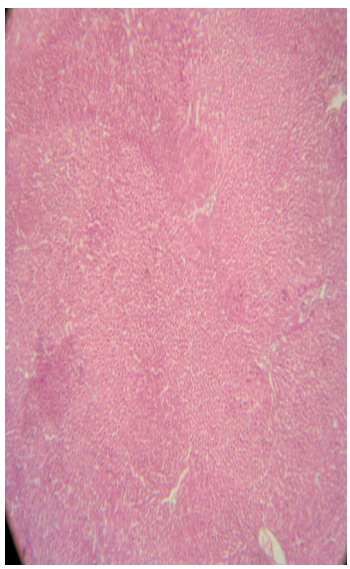

Plate 1. Section of the liver tissue showing nor mahepatocytes (control) x 400

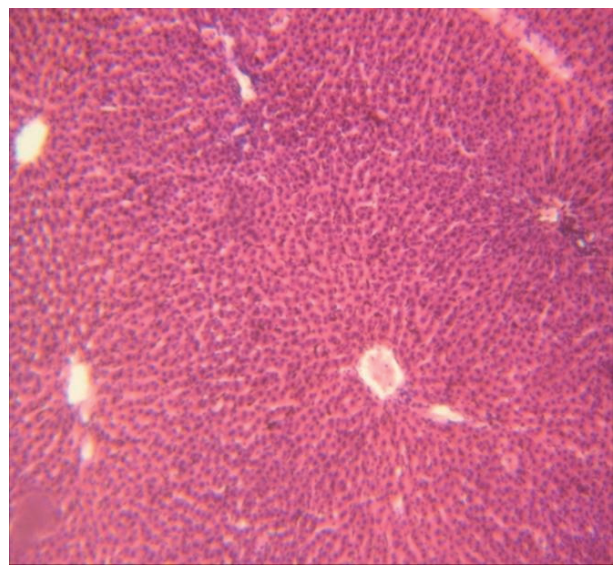

Plate 2. showing liver section of group II rats with no visible leison (400)

in serum is directly proportional to the number of necrotic cells present in the liver tissue. Administration of C.c (pre-treatment model) significantly ($p<0.01$) reduced the D-galn-induced elevation in the levels of these marker enzymes in the serum of group IV rats compared to group III animals. This thereby indicates potential cytoprotective activity of the C.c extract. It further revealed that the ethanol extract of C.c has potential of being able to confer some levels of protection not only on the structural integrity of the hepatocellular membrane but also on architecture of the D-galn-damaged liver cells. This further buttressed the assertion that natural antioxidant molecules impact stabilization to cell membrane depending on their degree or intensity of their free radical sca-

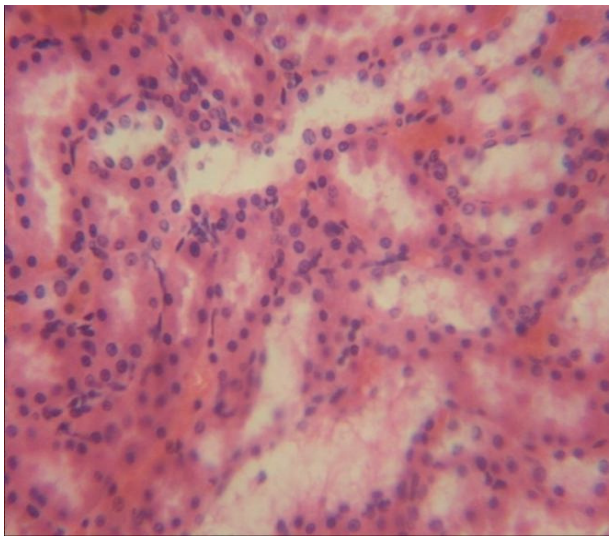

Plate 3. showing liver section of group III rats (induced) with severe vascular degeneration and periportal oedema. (x 400)

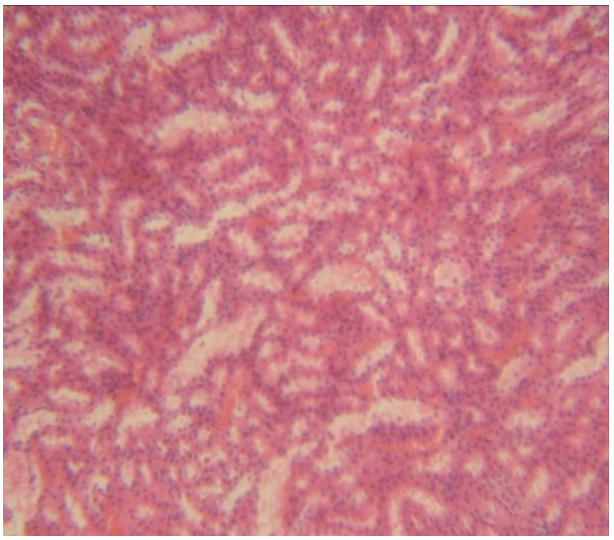

Plate 4. showing liver section of the *Cajanus cajan* treated group with mild hepatic regeneration with no visible pathology (x 400).

venging capability. We therefore hypothesized possible prolonged viability of the liver cell membrane against D-galn-induced necrotic damage in C.c leaves extract administered rats.

A significant ($p<0.01$) increase in lipid peroxidation in the D-galn-administered rats was not surprising, since one of the basic deteriorative reaction in cellular mechanism of D-galn-induced hepatitis have been linked to *in vivo* lipid peroxidation [9, 10, 11]. The results further suggested that rats administered C.c extract showed significant ($p<0.01$) decrease in the level of peroxidation (as measured by malondialdehyde concentration) in the liver tissue of C.c treated group IV rats as compared to group III (D-galn-induced) rats, thereby indicating the antioxidant property of C.c against D-galn-induced lipid peroxidation.

Moreso, significant ($p<0.01$) decline observed in the level of GSH in group III rats compared to others is in agreement with previous reports Adaramoye and Adeyemi [20], indicating that the tissue antioxidant status was been compromised by D-galn, probably as a result of decrease synthesis or increased degradation or inhibition of GSH synthesis. The observed increase GSH levels in C.c administered group of rats suggested potential antioxidant protective properties of this plant extract.

The activities of GSH-dependent enzymes (GPx and GST) was also found to decline in group-III rats, thereby buttressing the observed susceptibility of the liver cells of the rats in this group to oxidative damage. However, the administration of C.c maintained the activities of these enzymes near that of control group. Reduction in activities of antiperoxidative enzymes (CAT and SOD) in group-III rats might be due to enhanced reactive oxygen radicals (superoxide and hydrogen peroxide) generation, which in turn overwhelmed the activities of these enzymes. The animals administered with C.c prior to D-galn administration showed a significant ($p<0.01$) reduction in the level of lipid peroxidation with concomitant enhanced activities of catalase and superoxide dismutase, thereby indicating or reflecting antioxidant properties of C. c in experimentally induced hepatitis condition.

It could therefore be concluded from this study that prior administration of C. c extract at 100mg/g body weight for 10 days could confer some levels of protection against D-galn induced hepatitis by modulating the level of non-enzymic and activities of enzymic anti-oxidants near the non-induced control group. However, further work is needed to compare the effect of this extract with standard anti-hepatitis drug.

There is no conflict of interest in respect of this manuscript.

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