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PROTECTIVE EFFECTS OF LEAF EXTRACTS OF *Hydrocotyl bonariensis* (ARALIACEAE) AGAINST LIVER AND KIDNEY DAMAGE IN RATS FOLLOWING THE CONSUMPTION OF GALACTOSE RICH DIET

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Abstract

The study investigated the efficacy of Hydrocotyl bonariensis leave extract in providing tissue protection against dietary galactose-induced tissue oxidative stress. Fifty six (56) weanling rats were employed in the study. The control group was placed on AIN-93 diet while the diet of the test groups was supplemented with 30% galactose. Mechanisms of action of the extract were determined by measuring the malondialdehyde level. The level of reduced glutathione (GSH), the activities of catalase and superoxide dismutase (SOD) were also determined. Malondialdehyde (MDA) levels of tissues of animals fed galactose diet significantly increased ($P < 0.05$) when compared with the control. The liver had the highest level of malondialdehyde compared with the kidney. The level of GSH and the activities of catalase and superoxide dismutase were significantly reduced in the tissues of rats fed galactose diet. The extract reduced oxidative stress significantly in rats fed galactose diet. It also reduced the degree of tissues peroxidation, increased the level of GSH, tissue catalase and SOD activity. It therefore suggests that Hydrocotyl bonariensis protects against galactose.

1.0 Introduction

Galactose is a simple monosaccharide that serves as an energy source and as an essential component of glycolipids and glycoprotein. Galactose contributes to energy metabolism via its conversion to glucose by the enzymes that constitute the Leloir pathway. Defects in the genes encoding these proteins lead to the metabolic disorder galactosemia [1]. The defect in galactose metabolism due to galactose enzyme (galactokinase) deficiency or disorder results in inability to utilize galactose which results in galactosemia, galactosuria and cataract. It occurs when one of the enzymes converting galactose to glucose is mutated. As a result, galactose levels in the blood and the tissues accumulate leading to serious complications such as an enlarged liver, kidney failure, cataract and brain damage [2].

Free radicals can be defined as molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbital [3]. Free radicals are highly unstable molecules that interact quickly and aggressively with the molecules in the body causing damage. They have been implicated in the pathogenesis of many diseases such as cataract, cancer, diabetes, hypertension, etc. [4, 5]. The rise in free radicals associated with antioxidant deficiency is said to result in tissue damage.

During consumption of galactose rich diet, free radicals are generated. Studies have indicated that free radicals production and subsequent lipid peroxidation are normal sequel to the rise in oxygen consumption with exercise. Consequently, antioxidant supplementation may detoxify the peroxide produced during exercise and diminish muscle damage and soreness [6]. *Hydrocotyl bonariensis* (large leave pennywort), once a member of the family Apiaceae, now in the family Araliaceae and of the genus *Hydrocotyl* is an herbaceous, hairless, creepy, perennial plant, prostrate, with creeping lateral stems [7]. They are mostly found in Africa and America. It's common name in West Africa, Nigeria is Karo. Its habitats are beach dunes, moist, open sandy areas, wet

ditches and edges of ponds ([8, 9]. Its medicinal use as emetics, diuretics and laxatives has been reported [9].

A study by Ajani et al (2005) identified alkaloids, flavonoids, tannins, phenolic compounds and saponins as the bioactive components of the leave of *Hydrocotyl bonariensis* which may be responsible for its antioxidant properties. The aim of the present study therefore is to evaluate the efficacy of the leave extract of *Hydrocotyl bonariensis* in delaying or preventing the generation of free radicals in the liver and kidney of rats fed galactose rich diet.

2.0 Materials and methods

2.1 Plant authentication and extraction

Mature fresh leaves of *Hydrocotyl bonariensis* were collected from a local garden and were authenticated at the herbarium of the Botany Department, University of Lagos, Nigeria. The herbarium voucher number is 13478. The leaves were then oven dried at 40 ^{0}C for 24 hrs. They were blended using a local kitchen blender, and 240 g of the blended leaves were soaked in 2500 ml of water at room temperature for 48 hrs. The extract was then sieved into a clean container and further concentrated using a rotary evaporator at 40 ^{0}C. The concentrated product was then lyophilized. The yield of the extract was 5.8 %.

3.0 Experimental design and dietary regimen

Fifty six (56) male wistar albino rats (21 days old) having an average body weight of 30 g were used in the study. The rats were randomized into groups and treated as follow; Group A: received normal stock diet based on the AIN-93 (Reeves, 1997) + 30 % galactose and were administered with 500 mgKg^{-1} extract (7 rats). Group B: received group A diet + 30 % galactose and were administered with 1000 mgKg^{-1}extract (7 rats). Group C: received AIN-93 (galactose free diet) and were administered with 1000 mgKg^{-1} extract (7 rats). Group D: received group A diet with 30 % galactose + 0.5 ml of normal saline (21 rats). Group E: received galactose free diet + 0.5 ml of normal saline (14 rats).

Animals were housed in individual cage in a temperature and humidity controlled room, having a 12 hour light and dark cycle. All the animals had free access to their respective feed and clean drinking water. The treatment was carried out for four (4) weeks after which food was withdrawn from the animals overnight. They were then sacrificed.

The remaining rats in Group D and E were reassigned into groups labeled as follow: D1 (7 rats); D2 (7 rats) and E2 (7 rats). All the rats were maintained on normal diet for two (2) weeks. Group D1 and E2 rats were placed on 0.5 ml of normal saline while Group D2 rats were treated with 500 mgkg^{-1} dose of the extract. All administration was carried out orally as a single dose with the aid of an oral incubator.

4.1 Animal care

The care of the animals was in accordance with the U.S. Public Service Guidelines [17].

4.2 Tissue preparations

At the end of the treatment period, rats were sacrificed by cervical dislocation after diethyl ether anaesthesia. The tissues of interest (liver and kidney) were removed, (kidneys were decapsulated), washed free of blood, weighed, rinsed in ice-cold saline and then homogenized in 10 volumes of ice cold phosphate buffer solution (pH 6.2). The homogenates were kept frozen overnight before enzyme assay to allow unbroken cells to lyse.

4.3 Biochemical analysis

Tissues MDA were estimated as thiobarbituric acid reactive substances (TBARS) as described by Bhuyan et al (1981). Reduced glutathione (GSH) was assayed for by the method of Beutler et al (1981); Catalase activity was assayed for by the method of Sinha (1971). The method of Del-[8] was used to determine superoxide dismutase activity. Protein content was determined by the method of Lowry et al (1951) using BSA as standard.

4.0 Statistics

All data were expressed as mean ± SEM. One-way analysis of variance (ANOVA) was used to analyze the data. Comparisons between the groups were made at a two- sided alpha level of 0.05 $p < 0.05$ was considered statistically significant.

5.0 Results

Data obtained from the study indicates that high dietary galactose elicits significant increase in both liver and kidney peroxidation (Table 1 and 2) as the thiobarbituric acid reactive substances (TBARS) observed for both the liver and kidney of rats placed on dietary galactose were significantly higher than that obtained for the normal control value. Maintenance of rats on dietary galactose along with simultaneous administration of the extract at all the two tested doses prevented the increase in TBARS significantly. The values obtained at 500 mgKg^{-1} and 1000 mgKg^{-1} were however different from each other in liver but not different from each other in kidney. The values were also found to be significantly different in liver but not significantly different in the kidney compared with the observed values in the normal control. Withdrawal of dietary galactose after the fourth week further reduced the liver and kidney TBAR significantly as determined at the end of the sixth week.

When dietary galactose was withdrawn and the animals were simultaneously treated with the extract (D2), the observed TBARS was not significantly different in kidney but significantly different in liver when compared with the normal control at the end of the sixth week.

The result of the GSH status after treatment (Table 1 and 2) indicates that rats placed on dietary galactose showed a significant reduction in their liver and kidney reduced glutathione (GSH) level when compared with the normal control. The value observed for rats placed on dietary galactose was also significantly lowered compared with the control that was simultaneously administered with the extract. Combined administration of the extract with the dietary galactose prevented reduction in GSH levels of both the liver and kidney. Withdrawal of dietary galactose and subsequent treatment with the extract significantly raised both the liver and kidney GSH levels above that of the group that was not treated. The observed GSH was not significantly different from that of the normal control and the control group that was not administered with the extract.

Liver and kidney Catalase activities were observed to be significantly reduced from that of the normal control when rats were placed on dietary galactose (Table 1and 2). Simultaneous treatment with the extract at 500 $mgKg^{-1}$ doses of the extract prevented reduction in catalase activity in the liver only but treatment with both the 500 $mgKg^{-1}$ and 1000 $mgKg^{-1}$ dose of the extract prevented reduction in catalase activity in the kidney.

Treatment with the extract following withdrawal of dietary galactose significantly increased both the liver and kidney Catalase activity above the group that was not treated (D1).

There was a significant reduction in both the liver and kidney superoxide dismutase activity when rats were placed on dietary galactose compared with the normal control and the control rats administered with the extract (C). Treatment with the extract simultaneously with dietary galactose prevented reduction in superoxide dismutase activity in the two tissues from that of the control. The observed activity at both 500 $mgkg^{-1}$ and 1000 $mgkg^{-1}$ dosage was higher in both the liver and kidney when compared with that of the group of rats that was not treated (D1) but was previously placed on dietary galactose. The SOD activity at the end of two weeks following withdrawal of dietary galactose (D1) was not different in both the liver and kidney from the value observed prior to withdrawal of the diet (D). After withdrawal of dietary galactose and subsequent treatment with the extract (D2), the superoxide dismutase activity was significantly higher than the group that was not treated (D1). However, the activity was also not significantly different when compared with that of the normal control.

Table 1: Level of liver peroxidation, Catalase and Superoxide dismutase (SOD) activities and GSH status of rats with the extract

Group/treatment	TBARS ($x10^5$ mmol MDA/g tissue)	GSH ($x10^3$ µg/g tissue)	Catalase ($x10^5$ µg/mg protein)	SOD (10^3ng/mg protein)
A (AIN-93 diet + 30% galactose + 500 $mgKg^{-1}$ extract)	$2.74 \pm 0.10^{c, \#}$	$7.10 \pm 0.05^{\#, c}$	$0.86 \pm 0.01^{c,\#}$	2.29 ± 0.02^{c}
B (AIN-93 diet + 30% galactose + 1000 $mgKg^{-1}$ extract)	2.56 ± 0.02^{d}	$7.07 \pm 0.18^{\#, c}$	0.78 ± 0.03^{c}	$1.95 \pm 0.12^{c, d}$
C (AIN-93 diet + 1000 $mgKg^{-1}$ extract)	2.21 ± 0.02	7.13 ± 0.18	1.10 ± 0.10	1.98 ± 0.61^{d}
D (AIN-93 diet + 30% galactose)	3.00 ± 0.06*	6.75 ± 0.08*	0.73 ± 0.03*	1.07 ± 0.01*
E (AIN-93 diet + normal saline)	2.07 ± 0.04	7.06 ± 0.21	1.03 ± 0.02	1.38 ± 0.08
D1 (AIN-93 diet + normal saline)	2.17 ± 0.13	5.77 ± 0.03^{d}	0.81 ± 0.05^{d}	1.05 ± 0.01*
D2 (AIN-93 diet + 500 $mgKg^{-1}$ extract)	2.37 ± 0.11	7.00 ± 0.10	0.86 ± 0.01	1.38 ± 0.33
E2 (AIN-93 diet + normal saline)	1.97 ± 0.04	$5.80 \pm 0.05^{e, d}$	0.96 ± 0.01	1.41 ± 0.07

Note: [a] Values are mean of 5 determinations ± Standard error of mean (SEM). [b]The *, #, [c] denote that value is significantly different ($p < 0.05$) from others in the same column but not different from value with a similar superscript.

Table 2:Level of Kidney peroxidation, Catalase and Superoxide dismutase (SOD) activities and GSH status of rats after treatment

Group/treatment	TBARS ($x10^5$ mmol MDA/g tissue)	GSH ($x10^3$ µg/g tissue)	Catalase ($x10^5$ µg/mg protein)	SOD (10^3ng/mg protein)
A (AIN-93 diet + 30% galactose + 500 $mgKg^{-1}$ extract)	2.24 ± 0.04^{d}	6.96 ± 0.14	$0.83 \pm 0.08^{d, \#}$	1.92 ± 0.31^{d}
B (AIN-93 diet + 30% galactose + 1000 $mgKg^{-1}$ extract)	2.17 ± 0.05^{c}	7.09 ± 0.17	$0.75 \pm 0.05^{d, \#}$	$1.84 \pm 0.19^{c, d}$
C (AIN-93 diet + 1000 $mgKg^{-1}$ extract)	$1.87 \pm 0.20^{\#, e}$	6.98 ± 0.09	0.93 ± 0.03	1.85 ± 0.03^{c}
D (AIN-93 diet + 30% galactose)	2.81 ± 0.06*	6.67 ± 0.07*	0.65 ± 0.05*	1.33 ± 0.10*
E (AIN-93 diet + normal saline)	2.18 ± 0.01	7.07 ± 0.09	0.85 ± 0.05	1.59 ± 0.05
D1 (AIN-93 diet + normal saline)	1.96 ± 0.06^{e}	7.93 ± 0.07^{c}	0.68 ± 0.02^{c}	1.34 ± 0.01*
D2 (AIN-93 diet + 500 $mgKg^{-1}$ extract)	2.19 ± 0.00^{d}	7.99 ± 0.06	0.85 ± 0.00	1.52 ± 0.02
E2 (AIN-93 diet + normal saline)	1.83 ± 0.05	7.95 ± 0.15	0.88 ± 0.03	1.61 ± 0.04

Note: [a] Values are mean of 5 determinations ± Standard error of mean (SEM). [b]The *, #, [c] denote that value is significantly different ($p < 0.05$) from others in the same column but not different from value with a similar superscript.

6.0 Discussion

Accumulation of galactose is a poison to the body and can cause serious complications such as an enlarged liver, kidney failure, cataract and brain damage [2].

Oxidative stress occurs in a cell or tissue when the concentration of Reactive Oxygen Species (ROS) generated exceeds the antioxidant capacity of the cell [8]. Oxidative stress can also occur when there is a decrease in antioxidant capacity of a cell [15]. Exposure to free radicals from a variety of sources has led organisms to develop a series of defence mechanisms against free radical-induced oxidative stress which includes preventive and repair mechanism, physical and antioxidant defences [1]. The enzymatic antioxidant defense mechanism against "oxidative stress" includes Superoxide dismutase (SOD), Glutathione Peroxidase (GPx) and Catalase and non-enzymatic antioxidants defense mechanism includes Vita. C, Vit. E, and GSH [1]. *Hydrocotyl bonariensis* has been used in traditional medicine for various therapeutic purposes [16] and several antioxidant studies have been carried out on the components of the leaf [10]. The present study evaluated the protective potential of *H. bonariensis* in tissues (liver and kidney) of rats placed on galactose rich diet. The significant increase ($p < 0.05$) in the concentration of malondialdehyde along with a concomitant decrease in the measured antioxidants status observed in the tissues of animals fed with galactose- rich diet is an indication of increased lipid peroxidation in those tissues.

Lipid peroxidation is assessed by determination of thiobarbituric acid reactive substances (TBARS) particularly malondialdehyde (MDA). MDA is formed by cleavage of each side of the end peroxide ring. MDA introduces cross-link in proteins which may induce profound alteration in their biochemical properties [17]. Our result agrees with that of [18] who also reported increased oxidative stress in tissues of animals fed with galactose diet. The deposition of malondialdehyde in the tissues has various significance. MDA levels in the liver may be used to investigate the oxidative damage of proteins and lipoproteins which is a possible pathogenic mechanism for liver injury [19].

Reductions in the concentration of GSH and the activities of superoxide dismutase and catalase in the tissues of rats placed on galactose diet may in part be due to reduction in the rate of the synthesis of these antioxidants. Reduced antioxidant status and increased oxidative stress occurs in a damaged tissue. The alteration in the activity/concentration of the antioxidants as well as enhanced lipid peroxidation could be early markers of toxicity. A consequent increase in GSH level, Catalase and SOD activities after withdrawal of galactose and treatment with the extract as reported in this study, suggest the antioxidant efficacy of the extract in delaying or preventing oxidative stress caused by free radicals. This may support its usage as protective agent by halting chain reaction of lipid peroxidation initiated by free radicals and thereby acting as membrane stabilizer and that the efficiency may not be significantly different between the dosage of the extract at 500 and 1000 $mgKg^{-1}$.

7.0 Conclusion

The study has demonstrated that *Hydrocotyl bonariensis* leave extract offers protection to the liver and the kidney from galactose- induced oxidative

stress by reducing the degree of tissue peroxidation and increasing the endogenous antioxidants status.

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