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Research Article

Antioxidant Properties of Some Local Herbs Extract On STZ-Induced diabetics Rats

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Abstract: To determine the effect of antioxidant properties of two African herb extract on Streptozotocin induced diabetic Wistar albino rats by evaluation of the reduced glutathione (GSH) level pre and post treatment with the extract compared with a known antidiabetic drug Daonil. Induction of diabetes was actualized through the intraperitoneal injection of 70mg/kg body weight of Streptozotocin dissolved in 1m citrate buffer, p^H 4.5 twice daily for 2 days. A total of 102 rats were used among those that have attained and exceeded glucose threshold (>10.0 mmol/l) 2 weeks after Streptozotocin induction. Reduced GSH was determined spectrophotometrically. Data was analysed by one way and two analysis of variance. In vivo effect of the herb extract on GSH levels show there is a corresponding relationship between the degree of diabetes and GSH. Levels of GSH decreased with increase in the severity of diabetes. The extracts improved GSH levels in the rats and were concentration and time dependent with Tapinanthus bengwensis showing greater antioxidant properties. Reduced GSH is a reductant involved in maintaining the redox status of erythrocytes and other cells. Reduced concentration of GSH would mean an exposure of the cells to free radicals/antioxidants and thus to oxidant damage with accompanying complications such as haemolysis. The marked elevation of GSH shown with rats treated with Tapinanthus bengwensis indicates possible capacity of this extract as possessing high antioxidant property which could be very useful for management of patient with diabetes mellitus.
Keywords: Antioxidant, herbs, Streptozotocin, diabetic rats

INTRODUCTION

There is now evidence to show that oxidative injury which causes cellular damage can be attributed to free radicals or reactive oxygen species (ROS). The formation of free radicals now appear to give credence as a supporting basis and fundamental mechanism underlying a number of human disorders including diabetes mellitus. Free radicals are known to be generated by zenobiotics, such as metabolism of drugs, and environmental chemicals as well as stress hormones (adrenalin and nor-adrenalin). It has been shown that ROS can be scavenged through chemotherapy utilizing natural antioxidants compounds which are readily available in herbs [1, 2]. The production of free radicals in living organisms are modulated as part of the body's normal metabolic process. This chain reaction normally occur in the liver mixed function oxidase, mitochondrial respiratory chain, by bacterial through xanthine oxidase activity and from transistional metal catalyst.

It has been established that diabetes mellitus is known to increase oxidative stress which co-exist with reduction in anti-oxidant status. Oxygen free radicals can potentiate peroxidation of lipids which can excite the formation of glycated end proteins which plays a major role in complications of diabetes mellitus [3, 4]. There is enough evidence to demonstrate the chemopreventive capabilities of ethnobotanicals. The medicinal properties of herbs are predominantly attributed to the presence of flavonoids and other organic compounds such as coumarins, phenolic acid and antioxidant micronutrients, Cu, Mn and Zn. As shown by [5] aqueous and ethanolic extract of *Gongonema latifolium* leaves obtained from eastern part of Nigeria significantly increased the activity of superoxide dismutase and glucose-6-phosphate while decreasing lipid peroxidation. Didem in [6] has evaluated the hypoglycemic and antioxidant activity of three viscum album subspecies, European Mistletoe in Streptozotocin diabetic rats. Africa is inundated with several flora which can be harnessed to cure several ailments.

MATERIALS AND METHOD

Biochemical Assays

With the aid of tissue homogenator ultra turrax T-25 polyton at 4°C homogenate were obtained. The homogenates (1:10 w/v) were prepared with 100mM Kcl Buffer (p^H7) containing 0.3mM (buffer 1).

GSH Evaluation

GSH was evaluated spectrophotometrically by a slightly modified version of the method of [7]. Into 1ml of the homogenate, 1.5ml of 5% metaphosphoric acid was added and centrifuged at 3000g for 10 minutes at room temperature. Five hundred microlitres of this acidic

supernatant buffer and 0.25ml of 0.04% $5,5^1$-dithiobis (1-2-Nitrobenzoic acid) was mixed. Measurement of the yellow solution was taken after 10 minute at a wavelength of 412nm. A molar extinction coefficient ($13.6Mcm^{-1}$) that describes the formation of the thiolate anions by the reaction of the sulfhydryl groups with 5^1, 5^1-dithiobis-2-nitrobenzenoic acid (DTNB) at 412 nm was used for the quantitation of GSH.

Animals and Extract Administration

Males and female wistar albino rats weighing between 250-300g were used in the experiment. The animals were obtained from the Department of Zoology and Environmental Biology in the University of Port Harcourt, Nigeria. They were housed in an environment ($t=25^oC\pm2^oC$) and air humidity (60%) controlled room with a 12-hour light-dark cycle, and were placed on a standard laboratory chow and drinking water *ad libitum*. The experiments were conducted in accordance with the ethical guidelines for investigation in laboratory animals and were approved by the Ethical Committee for Animal Experimentation of the University of Port Harcourt, Nigeria.

Histological Examination

The pancreas, kidney and liver of control, diabetic control and diabetic test rats were extracted for histological smears and immediately transferred into 10% formalin. The samples were sliced to facilitate complete fixative penetration. They were then processed with paraffin infilteration and embedded in paraffin. The formalin-fixed-parafin embedded tissue blocks were cut with a rotary microtome into 4-6µm thick, and were placed on positively charged glass slide. They were dried and stained with haematoxylin/eosin for histopathological examination. A special stain Gomoris (1941) chrome alum/hematoxylin phyloxine method was used for staining the pancreas.

RESULTS

Values obtained after treatment with different concentration of the herbs extract are shown on the table below. There was sustained decrease in GSH concentration in the diabetic control rats. However, treatment with the extract elucidated marked improvement in the GSH concentration. The effect was more pronounced on treatment with *T.bengwensis.*

Table 1: Reduced Glutathione GSH (µu/mol Hb) Assay value for different groups

Day	Control	Diabetic control rats	DTR 5% T.beng.	DTR 10% T.beng.	DTR 5% O.grat	DTR 10% O. grat.	DTR on Daonil	DTR on 10% T.beng + 10% O. grat
0	0.40 ± 0.03^a	0.32 ± 0.11^c	0.35 ± 0.02^c	0.33 ± 0.00^c	0.36 ± 0.11	0.53 ± 0.01^s	0.34 ± 0.11^c	0.36 ± 0.02
2	0.44 ± 0.00^a	0.30 ± 0.12^n	0.30 ± 0.02^n	0.44 ± 0.12^d	0.35 ± 0.12	0.35 ± 0.02	0.34 ± 0.12	0.4 ± 0.02
4	0.42 ± 0.01^a	0.33 ± 0.01^b	0.30 ± 0.00^b	0.48 ± 0.02	0.33 ± 0.00^k	0.37 ± 0.03	0.4 ± 0.01	0.47 ± 0.03
6	0.36 ± 0.00^e	0.30 ± 0.01^e	0.28 ± 0.00^e	0.52 ± 0.02^f	0.31 ± 0.011	0.39 ± 0.02^t	0.5 ± 0.11^b	0.5 ± 0.04
8	0.37 ± 0.00^w	0.25 ± 0.12^p	0.34 ± 0.03^d	0.55 ± 0.22^g	0.33 ± 0.02^m	0.32 ± 0.02	0.68 ± 0.17^z	0.8 ± 0.04^d
10	0.41 ± 0.01^r	0.20 ± 0.13^y	0.54 ± 0.02^e	0.65 ± 0.02^t	0.31 ± 0.11^n	0.38 ± 0.02	0.93 ± 0.21^y	0.9 ± 0.12
12	0.42 ± 0.02^l	0.15 ± 0.13^v	0.52 ± 0.02^h	0.77 ± 0.02^l	0.33 ± 0.01^p	0.38 ± 0.02	0.93 ± 0.12^x	1.0 ± 0.04
14	0.43 ± 0.0^u	0.09 ± 0.12^t	0.52 ± 0.03^m	0.82 ± 0.12^t	0.32 ± 0.12^q	0.39 ± 0.02	1.3 ± 11^w	1.1 ± 0.11
16	0.44 ± 0.00^k	0.10 ± 0.02^x	0.50 ± 0.02^n	0.91 ± 0.02^x	0.30 ± 014^r	0.39 ± 0.02^u	1.5 ± 0.31^v	1.3 ± 0.12

Values are mean ±SEM of triplicate determination values on the row having the same superscript are not significantly different from each other.

Statistical analysis

Statistical analysis was performed using one way and two way analysis of variance (ANOVA). Differences between treatment groups were determined by the SPSS method. A statistical probability of $P<0.05$ was considered to be significant.

DISCUSSION

In this study an experimental model of Streptozotocin-induced diabetes mellitus in rat was used. The activity of reduced glutathiome (GSH) was evaluated. Our results demonstrated that repeated treatment with 5% and 10% of the extract for 16 days induced biochemical and histological changes. As shown on Table 1 a significant increase in the activity of GSH was observed on treatment with the 10% concentration of the extract with T.bengwensis elucidating greater effect. The results obtained show a clear close negative linear association between GSH levels and the progress of diabetes mellitus. This has been demonstrated by the fasting glucose levels and the GSH concentration. As has been shown earlier, Streptozotocin-induced diabetes is mediated by a depletion of the antioxidant pool and through a lowering of the β-cells nicotinamide adenine dinucleotide. NAD^+ which result in histological alteration of pancreatic islet beta cells. Similar observation have been made in [8,9]. In tandem with our findings, the plausible explanation for this increase in GSH activity could be adduced to improvement in function of the liver. The liver is the main organ involved in storage as well as detoxification of

zenobiotics. Hepatocytes are highly specialized to synthesize GSH from its precursors or to recycle it from oxidized gluthione (GSSH). Considering the result of this study, we can conclude that the administration of the extract has produced beneficial effect by increasing the GSH level with a significant modification in the activity of endogenous antioxidants enzyme in the liver, kidney and the blood[10,11].

The possible mechanism through which the extract brought about a decrease in blood glucose may be a potentiation of insulin effect by increasing either pancreatic secretion of insulin from β-cells of islet of langerhans or its responsiveness. Hyperglycemia results in free radical formation through various biochemical reactions. Free radicals may also be formed via the auto-oxidation of unsaturated lipids in plasma and membrane lipids. The free radicals produced may react with polysaturated fatty acids in all membranes leading to lipid peroxidation. Lipid peroxidation will inturn result in elevated production of free radicals. Lipid peroxidation mediated tissue damage has been observed in the development of both type I and II diabetes. It is known that secretion is closely associated with lipoxygenase-derived peroxides. GSH has multifactorial role in antioxidant defense. It is a direct scavenger of free radicals as well as a co-susbtrate for peroxide detoxification by glutathione peroxidase[12,13]. As earlier suggested, decrease in synthesis or increased degradation of GSH results in oxidative stress in diabetes mellitus.

Increased oxidative stress resulting from significant increase in aldehydic products of lipid peroxidation has probably decreased hepathic GSH content. In the present study, the elevation of GSH was noticed with both extract and the generic drug daonil treated diabetic rat. This indicate that both extract can increase the biosynthesis of GSH and consequently reduce the oxidative stress leading to less degradation. Increase in the activity of the enzyme might have protected β-cells against damage by the ROS. Treatment with antioxidant in diabetic mice was shown to have suppressed cell apoptosis and decreased blood glucose level. Here we conclude that extract used in the present study offered protection of β-cells against ROS mediated damage by enhancing antioxidant properties and thus reduced hyperglycemia in chemically induced diabetes. This finding suggest that the increasing use of the herbs for therapy will delay the complication in diabetes. Furthermore, it elucidate the fact that GSH levels could be used to assess efficacy, enhance patients assessment and monitoring.

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