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Full Length Research Paper

Effect of *Gongronema latifolium* crude leaf extract on some cardiac enzymes of alloxan-induced diabetic rats

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The effect of *Gongronema latifolium* on serum cardiac enzymes in alloxan induced diabetic rat models and normal control rats were determined using graded doses of 80% ethanol extract of *G. latifolium* leaf. The serum activities of aspartate aminotransferase (AST), creatine kinase (CK), creatine kinase isoenzyme (CKMB) and lactate dehydrogenase (LD) were determined in diabetic Wistar albino rats; diabetic control and normal Wistar albino rats after 2 weeks administration of *G. latifolium* leaf extract. Serum CKMB and LD activities of all the experimental groups significantly ($p \leq 0.001$) increased in diabetic rats when compared to non-diabetic rats. CK and LD decreased significantly in diabetic and non-diabetic rats treated with *G. latifolium* leaf extract when compared with the control. Equally, CKMB and LD of diabetic rats treated with *G. latifolium* leaf extract decreased significantly except CMB of group 4 and LD of group 3 animals. There were significant ($p \leq 0.001$) decreased activity of serum AST in diabetic rats when compared to the non-diabetic rats. However, AST did not change significantly in non-diabetic rats while in diabetic rats AST increased significantly ($p \leq 0.001$) when compared with their respective controls. These data suggest that the effects of *G. latifolium* leaf extract at our concentrations are not dose dependent and hepatotoxic. The extract of this plant is likely to be of biological significance in cardiovascular complication of diabetic and non-diabetic users.

Key words: Alloxan-induced diabetes, *Gongronema latifolium,* Cardiac enzymes, Myocardial infarction.

INTRODUCTION

Gongronema latifolium Benth Hook, (Asclepiadaceae) is an herbaceous shrub, with yellow flowers and the stem that yields characteristic milky exudates when cut. It is commonly grown in gardens in Calabar, Cross River State, Nigeria. It is locally called “utasi” by the Efiks, Ibibios and Quas; “utazi” by the Igbos and “arokeke” by the Yorubas in Nigeria. The Efiks and Quas in Calabar use *G. latifolium* crude leaf extract in the treatment of malaria, diabetes, hypertension, and as laxative. Also it is used as a spice and vegetable (Morebise, 2002). The use of crude leaf extract of this shrub in maintaining healthy blood glucose levels have been reported (Okafor, 1981, 1987, 1996). Scientific studies have established the hypoglycaemic, hypolipidaemic and antioxidative effects of aqueous and ethanol extracts of *G. latifolium* leaf (Ugochukwu et al., 2003; Ogundipe et al., 2003). Morebise et al. (2002) showed that the leaf extract has anti-inflammatory properties while its potential nutritional and food processing and Eleyinmi (2007) investigated preservation values of food.

Some phytochemicals such as B-sistosterol, lupenyl esters, pregnane ester, glycosides, essential oils and saponins are associated with parts of this herb (Ekundayo, 1980; Schneider et al., 1993; Morebise et al., 1998; Morebise et al., 2002). It is plausible that one or more of these phytochemicals that are found in *G. latifolium* is likely to influence cellular proteins with enzymatic activity. Some plants extract have influenced markers of myocardial infarction and clinical diagnosis of myocardial infarction is most often establish by the measurement of marker enzymes (Adam et al., 1993; Chatterjea and Shinde, 2002). Aspartate aminotransferase

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(AST), creatine phosphokinase (CK), creatine kinase isoenezyme (CKMB) and lactate dehydrogenase (LD) are often use as markers of myocardial infarction These enzymes are tightly bound to the contractile apparatus of the cardiac muscle tissue and any serious insult to the heart muscle will evoke the release of these enzymes into the serum.

A serum creatine kinase and creatine kinase isoenzyme activities have been reported in hypertension (Lott et al., 1978; Daniel et al., 1983). The present study was therefore undertaken to determine the extent to which *G. latifolium* leaf extract would influence some cardiac enzymes such as aspartate aminotransferase (AST), creatine phosphokinase (CK), creatine kinase isoenzyme (CKMB) and lactate dehydrogenase (LD) in diabetic and non-diabetic rats. Our findings may provide some useful information to support the age-long practice of using *G. latifolium* as an anti-diabetic and anti-hypertensive herb.

MATERIALS AND METHODS

Chemicals and reagents

All reagents and chemicals used in this work were of analytical grade. Alloxan monohydrate (sodium salt) was purchased from Alpha Aesar (Johnson Matthey Shore Road, Heysham, Lance, Canada). The quantitative *in-vitro* determination of serum creatine kinase isoenzyme and creatine kinase activities were carried using TECO diagnostics kits (Szasz, 1976; Gerhardt et al., 1977). Serum aspartate aminotransterase (AST) and lactate dehydrogenase (LD) activities were determined by the use Randox kit reagents (Randox Laboratories, UK). The absorbances were read using Optima Spectrophotometer SP-300 (Optima Inc. Chicago, USA).

Preparation of plant extract

Fresh leaves of *G. latifolium* were collected in Akpabuyo in Cross River State, Nigeria identified and authenticated by a botanist in the Botany Department, University of Calabar, Nigeria. The leaves were picked, sun-dried initially and finally dried in a PLUS II oven at 50°C. The dry sample was crushed to powder with a KENWOOD blender. The leaf powder was stored in a glass bottle with a plastic screw cap and kept in a refrigerator (4°C). Bulk ethanol extraction of the leaf powder was undertaken by shaking for 12 h. The extract was thereafter concentrated at 55°C using a rotary evaporator (Labo rota 3000 Resona) and a vacuum pump (Edward's). The extract was dried in an oven at 55°C until all the ethanol was removed. The dry extract was stored in a refrigerator until required for use. The concentration of the extract was determined by drying a known volume and measuring the dry weight.

Experimental animals/animal treatment

Male albino rats of the Wistar strain weighing 180 - 330 gm obtained from the animal house of Biochemistry department, Faculty of Basic Medical Sciences, University of Calabar, were used in this investigation. The experimental animals were acclimatized for three days in the experimental section of the animal house and there after were fasted for 24 h (Esmerino et al., 1998) before induction of diabetic by intraperitoneal injecting of a single dose of alloxan (150 mg/kg body weight). Diabetes was confirmed after 7 days in rats that showed fasting blood glucose (FBG) levels of ≥ 300 mg/dl accompanied with loss of body weight, polyuria and polyphagia. The mortality ranged from 5 - 10%. The 56 male albino Wistar rats were randomly selected into 8 groups consisting of diabetic (groups 5 - 8) and 4 non-diabetics (groups 1 - 4) of 7 animals each. The animals in groups I and 5 were designated normal and diabetic controls and received (1.5 ml/body weight of distilled water) as placebo for 2 wks., while the test groups 2, 3 and 4 were gavaged graded doses of 1, 1.5 and 2 ml of the extract, corresponding to 200, 300 and 400 mg/kg body weight of the *G. latifolium* extract. The same treatments were given to groups 6, 7 and 8 respectively. Experimental animals had access to feed and water *ad libitum*.

Animal sacrifice and preparation of sera

All the experimental animals were kept off extract and feed 24 h before they were weighed and sacrificed using chloroform fumes for anaesthesia. Blood samples were collected by cardiac puncture into sterile plain tubes for preparation of sera. Sera were collected from clotted blood samples by centrifugation using a bench top centrifuge (MSE, Minor, England) at 3000 g for 10 min. The separated sera were kept in a refrigerator (4°C) until required for analyses. All the enzymes were determined within 24 h of sample collection. This experiment was conducted in line with the Institution Ethical Committee as approved by the University of Calabar Graduate School.

Statistical analysis

Statistical analysis of data was by standard student's t-test method and $p < 0.05$ was regarded as significant. The group data was regarded as mean ± standard deviation (SD) of seven (7) determinations.

RESULTS

Table 1 indicates the activities of cardiac enzymes of non-diabetic and diabetic Wistar albino rats after 14 days oral administration of *G. latifolium* leaf extract in graded doses of 200, 300 and 400 mg/kg body weight. AST and CK activities were significantly lower when diabetic rats in groups 5, 6 and 8 were compared to corresponding non-diabetic animals in groups 1, 2, 4. However, the decrease in AST activity was significant ($p \leq 0.001$) in groups 6, 7 and 8 of diabetic rats when compared to that of non-diabetic rats in groups. In Table 1, the decreases in CK activity of the *G. latifolium* leaf extract treated groups: 6, 7 and 8 were significant ($p \leq 0.05$) when compared to the non-diabetic control. There was a significant increase ($p \leq 0.05$) in serum CK activity in test group 7 and a significant decrease ($p \leq 0.05$) in test group 8 when compared to the diabetic control. Serum CKMB activities were significantly ($p \leq 0.05$) higher in all diabetic rats than non-diabetic rats. Animals in test group recorded significantly ($p \leq 0.001$) lower CKMB activities while group 2 had significant ($p \leq 0.001$) higher CKMB activities, when compared with the normal control. Serum AST activity in all diabetic test groups increased (though not significantly, $p \geq 0.05$), when compared to the diabetic control. There was a non-dose dependent increase in serum CKMB activity in diabetic test groups and a non-dose dependent decrease in serum AST and CK activities fol-

Table 1. Serum activities of cardiac enzymes of diabetic and non-diabetic Wistar albino rats treated with 80% ethanol extract of *Gongronema latifolium.*

Groups/Enzyme†	Treatment	AST (U/L)	CK (U/L)	CKMB (U/L)	LD (U/L)
Group 1	Normal control	98 ± 7.60	474.19 ± 73.35	225.23 ± 55.29	1,459.80 ± 26.36
Group 2	200 mg/kgbd/wt	112.45 ± 4.84	387.98 ± 32.54 *	282.14 ± 42.30*	1164.14 ± 61.08 *
Group 3	300 mg/kgbd/wt	96.50 ± 7.01	211.68 ± 59.93 **	177.48 ± 53.73*	734.46 ± 93.21 **
Group 4	400 mg/kgbd/wt	97.07 ± 12.50	251.91 ± 28.73 **	226.67 ± 29.84	858.07 ± 37.42**
Group 5	Diabetic control	52.64 ± 15.48	340.43 ± 72.38	698.35 ± 66.93	1,603.59 ± 83.35
Group 6	200 mg/kgbd/wt	75.46 ± 20.95 *	333.37 ± 39.13	433.57 ± 32.65***	1371.51 ± 81.21*
Group 7	300 mg/kgbd/wt	77.49 ± 16.03*	495.34 ± 75.92 **	445.33 ± 28.45 ***	1581.95 ± 70.32
Group 8	400 mg/kgbd/wt	70.56 ± 20.00 *	214.38 ± 70.43 **	780.82 ± 42.78*	1459.08 ± 73.52 **

†Mean ± SD of 7 determinations. * = p ≤ 0.05; ** = p ≤ 0.01; *** = p ≤ 0.001

lowing oral gavaging with graded doses of *G. latifolium* crude leaf extract for two weeks. In Table 1, serum LDH activity decreased significantly (p ≤ 0.001) when compared to the non-diabetic control rats, while it decreased significantly in groups 6 and 8 but insignificantly different (p ≤ 0.05) for test group 7. The significant change in LDH activities of diabetic rats when compared with the non-diabetic rats is an indication that cellular integrity of diabetic rats is more susceptible to drug metabolism.

DISCUSSION

Herbal medicinal researches have attained an incredible global level in the recent past as well as the continued application of some plant constituents in pharmaceutical industries thereby elevating the growing status of traditional herbal medicine in Africa in general and Nigeria in particular. In Calabar, Cross River State of Nigeria, the culinary and medicinal use of herbs is phenomenal as there is hardly any local meal without herbs. The use of *G. latifolium* in our local community as an anti-malarial, anti-inflammatory, laxative, spice and vegetable is common but its use in the treatment of diabetes and hypertension is one major reason for giving serious attention to research in prognosis of cardiovascular risk factors. The knowledge of toxicity or efficacy of xenobiotic(s) is function of the knowledge of drug metabolizing enzymes and marker enzyme activity. The local use of *G. latifolium* crude leaf extract in folk medicine as an anti-diabetic and anti-hypertensive remedy is one reason that has given the impetus to study its possible involvement in myocardial infarction. Experimental evidence (Kamble and Vaidya, 2002) has shown that antihypertensive drugs such as atenolol and enalapril elicit the release of cellular enzymes from injured organs such as the liver. We also believe that through a similar mechanism, enzymes may leak profusely from infarct myocardial cells.

It is well known that diagnosis of cardiac enzymes is important. Serum CK activity is a more sensitive indicator in early stage of myocardial ischemia, while peak rises in LD is roughly proportional to the extent of injury to the myocardial tissue (Chatterjea and Shinde, 2002). Also, the integrity of the cardiac apparatus in drug biotransformation and metabolism could be assessed by evaluating the levels of AST, CK, CKMB and LD in serum. The results in diabetic animals in this experiment shows a protective effect of *G. latifolium* crude leaf extract on the heart of experimental animals at a dose level of 200 mg/kg body weight. Moreover, the significantly lowered activities of CK, CKMB and LD at 200 mg/kg body weight and CK and LD at 400 mg/kg body weight scientifically suggest that the leaf extract of *G. latifolium* may have the potential of reducing the factors that produce infarction in the myocardium. This is so because the metabolism of alloxan-induced infarct myocardium may be studied by assessing the level of marker enzyme proteins in the serum. It is interesting to know that as myocardial diseases are rich sources of CKMB, so are skeletal muscular diseases good sources of creatine kinase isoenzyme (Hamm et al., 1992). Pathological value has been estimated in injured skeletal muscle. Therefore the significant reduction in CK enzyme at the dose of 400 mg/kg body weight of *G. latifolium* extract may be due to some physiological effects on muscular activity. This fact may be associated with the efficacy of *G. latifolium* crude leaf extract in the treatment of muscular pains, arthritis and inflammation (Morebise et al., 2002).

Conclusion

The findings of this investigation have revealed that the application of *G. latifolium* crude leaf extract in the treatment of hypertension may have significant effects in moderating incident of myocardial infarction.

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