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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

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Antioxidant Potential of the Young Leave Methanolic Extract of *Magnifera Indica* in Alloxan Induced Diabetic Rat

M. Ugbenyen Anthony[1]*, A. Odetola Adebimpe[2] and K.E. Ekpo[1]
[1]Department of Biochemistry, Faculty of Natural Sciences, Ambrose Alli University, Ekpoma, Nigeria
[2]Department of Biochemistry, Faculty of Basic Medical Sciences, University of Ibadan, Ibadan, Nigeria

Abstract: The study was designed to investigate the antioxidant potential of *Magnifera indica* young leave methanolic extract in alloxan induced diabetic rat. Albino rats each weighing 100-200g were given a peritoneal injection of 120mg of Alloxan per kg body weight. After 7 days the blood glucose level of the animals were checked to ascertain a diabetic state. Those that were diabetic were selected for the study. Significant increase ($p<0.05$) in lipid peroxidation and reduction in reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) was observed in the serum, liver, kidney and heart of diabetic rats as compared to the normal control. Administering *Magnifera indica* young leave extract significantly reduced ($p<0.05$) lipid peroxidation and elevated the level of GSH, SOD, and CAT in serum, liver, kidney and heart of diabetic rats compared to untreated diabetic rats. These findings indicate that *Magnifera indica* young leave extract can reduce free radical mediated oxidative stress to cells in experimental diabetes mellitus.

Key words: Antioxidant, *Magnifera indica*, diabetes, alloxan, induced

INTRODUCTION

Reactive oxygen species(ROS) have been implicated in more than 100 diseases (Ali *et al*., 2001), including diabetes. A number of plants have been shown to possess hepatoprotective properties by improving the antioxidant status e.g *caesalpinia bonducella* (Gupta *et al*., 2005). All aerobic organism including humans have antioxidant defense mechanisms that protect against oxidative damage. However, the natural antioxidant defense mechanisms can be insufficient and hence dietary intake of antioxidant component is important and recommended (Duh, 1998). Medicinal plants have become so important in this present generation in the treatment of numerous types of diseases, (Fola, 1993). Therefore, this work is aimed at investigating the antioxidant potential of *Magnifera indica* young leave methanolic extract by studying its role on lipid peroxidation, enzymic and non-enzymic antioxidant in experimental diabetes mellitus.

MATERIALS AND METHODS

Experimental animals: Female Albino rats (Wistar strain) each weighing 100-200g were obtained from the primate, Biochemistry department, University of Ibadan. They were all fed *ad-libitum* on rat feed manufactured by Ladokun feeds Ibadan. All animals were fasted before the start of the experiment.

Each animal for diabetic assay was given a peritoneal injection of 120mg of Alloxan per kg body weight. The blood glucose level of the animals were checked using a glucometer (a one touch test strips) after alloxan injection.

The blood glucose level of the animals were again checked after 7 days to ascertain a diabetic state, and rats with moderate diabetes were used for the experiment.

Collections and extraction of plants sample: Fresh young leaves of *M. indica* were collected around the environs of the University of Ibadan.

The leaves were air dried under laboratory conditions and grinded to powdery form. 560g of the fine powder was packed into the compartment of the soxhlet apparatus/extractor. The solvent(methanol) was then poured into the compartment containing the leaves until it reaches the maximum point. Heat is applied to the apparatus by using the steam bath principle. The solvent vapourises from the round bottom flask back into soxhlet extractor. The solution in the round bottom flask is then distilled with steam bath and the extract concentrated while the solvent is recovered.

The extract was then cooled and poured into a collecting bottle and refrigerated at -20^{0}C till the time of use.

Experimental design: In this experiment a total of 30 rats (20 diabetic surviving rats, 10 normal rats) were used. Diabetes was induced in rats a week before the start of the experiment. The rats were divided into six group (n=5) after the induction of diabetes.

Group 1 : Normal untreated rats.
Group 2 : Diabetic untreated rats
Group 3 : Diabetic rats given extract (1000mg/kg body weight) In aqueous solution daily using a canular for 14 days
Group 4 : Diabetic rats given extract (500mg/kg body weight) in aqueous solution daily using a canular for 14 days.

Group 5 : Diabetic rats given glibenclamide(600µg/kg body weight) in aqueous solution daily using a canular for 14 days

Group 6 : Normal rats given extract(1000mg/kg body weight) in aqueous solution daily using a canular for 14 days.

After 14 days the rats were killed by decapitation. Blood was collected in a tube and centrifuged, the serum was used for the estimation of lipid peroxidation and organs such as liver, kidney, and heart were removed and homogenized in cold 0.1M Phosphate buffer (pH 7.0) using a homogenizer.

The homogenate was centrifuged at 10000 rpm for 10 minutes and the supernatant decanted and used for enzyme assay.

Biochemical analysis: Lipid peroxidation was estimated by measurement of thiobarbituric acid-reactive substances(TBARS) by the method of Varshney and Kale (Vashney and Kale, 1990). The pink chromogen produced by the reaction of thiobarbituric acid with malondialdehyde, a secondary product of lipid peroxidation was measured at 531.8nm.

Reduced glutathione (GSH) was determined by the method described by Ellman (1959). GSH estimation was based upon the development of relatively stable yellow colour on addition of 5,5'-dithio2-nitrobenzoic acid (DTNB) to compounds containing sulfhydryl group.

The activity of Catalase(CAT, EC. 1.11.1.6) was estimated by the procedure of Sinha (1972), based on the fact that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of hydrogen peroxide with the formation of perchromic acid as an unstable intermediate. The chromic acetate thus produced is measured at 570-610nm.

Superoxide dismutase (SOD,EC.1.15.1.1) activity was estimated by the method of Beyer and Fridovich (Beyer and Fridovich, 1987). It is based on the inhibition of auto-oxidation of adrenaline to adrenochrome by SOD.

Statistical analysis: All the results obtained were expressed as mean ± S.D of 5 rats in each group. Statistical significance of difference of means was analyzed by Students t-test. The results were considered statistically significant at $p < 0.05$.

RESULTS

Effect of administering *M. indica* leave extract and glibenclamide on tissue lipid peroxidation in control and experimental diabetic groups is shown in Table 1. Significant increase in lipid peroxidation in the untreated diabetic group compare to the control group. Administration of *M. indica* and glibenclamide to diabetic group significantly reduced lipid peroxidation, as revealed by the reduction in the level of MDA formed in tissues. The administration of *M. indica* and glibenclamide tends to bring the level of lipid peroxidation in diabetic groups near that of the control group.

Effect of administering *M. indica* leave extract and glibenclamide on reduced glutathione(GSH) in some tissues in control and experimental diabetic groups is shown in Table 2. Significant decrease in the concentration of GSH in the diabetic control compare to the control group. Administration of *M. indica* and glibenclamide to diabetic groups brought about a significant increase in the concentration of GSH compared to untreated diabetic control.

Effect of administering *M. indica* leave extract and glibenclamide on tissues Catalase activity(CAT) in control and experimental diabetic groups is shown in Table 3. Significant decrease in the activity of the enzyme catalase (CAT) in diabetic control group compare to the control group. Administering *M. indica* and glibenclamide to diabetic group resulted in a significant increase in the activity of this enzyme compared to the untreated diabetic control.

Effect of administering M. *indica* leave extract and glibenclamide on tissues superoxide dismutase (SOD) in control and experimental diabetic groups is shown in Table 4. Significant decrease in the activity of the enzyme SOD in diabetic control group compared to the control group. Administration of *M. indica* and glibenclamide to diabetic group caused significant increase in the activity of the enzyme in the diabetic group compared to untreated diabetic control.

DISCUSSION

Reactive oxygen species(ROS) have been implicated in more than 100 diseases (Ali *et al*., 2001), including diabetes. Possible source of oxidative stress in diabetes include alter carbohydrate and lipid metabolism, decrease level of antioxidant defenses such as glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and most importantly, increase generation of reactive oxygen species (ROS) as a result of these metabolic derangements, lipid peroxidation and glaciation.

Lipid peroxide mediated tissue damage has been observed both in type I and II diabetes mellitus(Halliwell and Gutteridge, 1999; Esterbauer, 1990).

Hyperglycemic generates abnormally high level of free radicals by a mechanism involving autoxidation of glucose, followed by oxidative deneration and protein glaciation (Hunt *et al*., 1988).

Increased level of lipid peroxidation have been reported in the liver and kidney of alloxan diabetes rats (Chattopadhyay *et al*., 1997).

In this study , it was also observed that there was significant increase in lipid peroxidation as revealed by the high level of MDA formed in the tissues of alloxan induced diabetic rats compared to the control rat. This

Table 1: Effect of administering *M.indica* leave extract and glibenclamide on tissue lipid peroxidation in control and diabetic rats

	nM of MDA formed / g protein			
Group	Serum	Liver	Kidney	Heart
Control	5.00± 2.00	4.00 ± 0.97	6.90± 1.13	3.50± 0.69
Control + 1000mg extract	2.92± 1.67	3.48± 0.15	5.68±1.76	3.00± 0.56
Diabetic	79.00± 6.00 *	123.00±12.30*	120.00±20.20*	142.00±18.00*
Diabetic+1000mg extract	7.77±1.89 #,ns	6.07± 1.53 #,ns	8.28± 1.00 #,ns	4.32±0.70 #,ns
Diabetic+500mg extract	12.80± 4.11 #	7.44± 2.81 #	8.23± 2.50 #,ns	4.40±1.92 #,ns
Diabetic+ glibenclamide	18.20 ± 1.39 #	6.10± 0.64 #	8.50± 2.60 #,ns	3.12±0.79 #,ns

Result are expressed as mean ± SEM (n=5). *: Significantly different from the control group (p<0.05). #: Significantly different from the diabetic group (p<0.05). ns: Not significantly different from the control group (p<0.05)

Table 2: Effect of administering *M.indica* leave extract and glibenclamide on reduced glutathione (GSH) in some tissue in control and diabetic rats

	GSH (mg / g tissue)		
Group	Liver	Kidney	Heart
Control	1.80±0.10	13.68±4.37	1.86±0.30
Control+ 1000mg extract	2.20±0.17	15.52±2.20	0.93±0.31
Diabetic	0.20±0.01*	0.14±0.01*	0.14±0.06*
Diabetic + 1000mg extract	3.60±0.22 #	11.80±3.00 #,ns	1.24±0.35 #,ns
Diabetic + 500mg extract	1.70±0.12 #	11.50±3.10 #,ns	1.00±0.44 #
Diabetic + glibenclamide	2.40±0.19 #	10.24±4.00 #,ns	1.35±0.66 #

Result are expressed as mean ± SEM (n=5). *: Significantly different from the control group (p<0.05). #: Significantly different from the diabetic group (p<0.05). ns: Not significantly different from the control group (p<0.05)

Table 3: Effect of administering *M.indica* leave extract and glibenclamide on tissue catalase (CAT) in control and diabetic rats

	CAT(μm H_2O_2 Consumed min / mg / protein)		
Group	Liver	Kidney	Heart
Control	151.00±11.03	328.00±32.21	298.5± 21.23
Control + 1000mg extract	66.60± .00	116.90±8.04	216.00±12.20
Diabetic	24.5±0.40*	18.90±1.00*	31.60±1.00*
Diabetic extract + 1000mg	69.80±1.39 #	121.80±13.61 #	239.00±24.72 #
Diabetic + 500mg extract	57.10±1.82 #	90.90±7.06 #	272.00±24.55 #
Diabetic + glibenclamide	54.80±1.20 #	94.00±5.51 #	174.00±9.59 #

Result are expressed as mean ± SEM (n=5). * Significantly different from the control group (p<0.05). # Significantly different from the diabetic group (p<0.05). ns Not significantly different from the control group (p<0.05)

suggest that the natural antioxidant defense mechanism to scavenge excessive free radical has been compromised in rat induced with diabetes (Pratibha *et al*., 2004)

Administration of *M. indica* leave extract to diabetic group significantly reduced lipid peroxidation, as revealed by the reduction in the level of MDA formed in the tissues. It is therefore suspected that *M. indica* leave contain an active component, which lower lipid peroxidation.

Glutathione (γ - glutamylcysteinyglycine, GSH) is a sulfhydryl (-SH) antioxidant, antitoxin, and enzyme cofactor. Glutathione is ubiquitous in animals, plants, and microorganisms (Kosower and Kosower, 1978; Meister, 1976).

The reducing power of GSH is a measure of its free radical scanvenging, electron-donating and sulfhydryl-donating capacity. Reducing power is also the key to the multiple actions of GSH at the molecular, cellular and tissues level and to its effectiveness as a systemic protestant against oxidative and free radical damage which is the case in diabetes mellitus (Meister, 1994).

Depletion of hepatic GSH and increased lipid peroxidation are characteristic of diabetes (Mukherjee *et al*., 1994) and the activities of glutathione (GSH) dependent enzymes are altered in various tissues of diabetic rat (Maritime *et al*., 1999; Saxena *et al*., 1993; Kakkar *et al*., 1995).

In this study, low level of GSH was also observed in the tissues of alloxan diabetic rats as compare to the control group. Administering *M. indica* extract elevated the level of GSH in the tissues. There are significant increases in the level of GSH in the diabetic groups compare to the untreated diabetic group.

Effect of *M. indica* on GSH may be considered as a generalized electrophilic counter attack response evoked by alloxan.

It can be speculated that the major mechanisms by which *M.indica* exerts its chemopreventive effect may be through modulation of lipid peroxidation and enhanced elevation of alloxan detoxification systems.

Superoxide and hydroxyl radicals are important mediators of oxidative stress, they induce various injuries to the surrounding organs and play a vital role in some clinical disorders (Slater, 1984). Any compound natural or synthetic with antioxidant activities might contribute towards the total/ partial alleviation of this

Table 4: Effect of administering *M.indica* leave extract and glibenclamide on tissue superoxide dismutase (SOD) in control and diabetic rats

	SOD (unit / min / mg protein)		
Group	Liver	Kidney	Heart
Control	2.47± 0.15	4.50±0.03	7.00±2.61
Control + 1000mg extract	1.94±0.34	4.01±0.08	6.41±2.11
Diabetic	0.23±0.05*	0.36±0.15*	1.11±0.02*
Diabetic + 1000mg extract	1.74±0.36 #,ns	3.87±0.06 #	6.30±2.43 #,ns
Diabetic + 500mg extract	1.62±0.20 #,ns	3.18±0.12 #	6.47±1.93 #.ns
Diabetic + glibenclamide	1.34±0.29 #	2.99±0.14 #	4.59±0.75 #,ns

Result are expressed as mean ± SEM (n=5). *: Significantly different from the control group ($p<0.05$). #: Significantly different from the diabetic group ($p<0.05$). ns: Not significantly different from the control group ($p<0.05$)

damage. Therefore removing superoxide and hydroxyl radicals is the most effective defense of living body against diseases (Jeyekumar *et al*., 1999).

Superoxide dismutase (SOD) convert superoxide to hydrogen peroxides and subsequently catalase(CAT) act on hydrogen peroxide and converts it to water.

In the present study , reduced activities of SOD and CAT were observed in tissues of diabetic untreated rats compared to the control group.

Other workers also reported a decrease in the activities of SOD and CAT in alloxan induced diabetic rat tissues such as liver, kidney, pancreas and brain (Krishnakantha and Lokesh, 1993).

Administering *M.indica* and glibenclamide increased the activities of SOD and CAT in tissues of diabetic rats compared with the diabetic untreated rats.

This result shows that *M.indica* possesses an antioxidant activity , which could exert a beneficial action against pathological alteration caused by the presence of superoxide and hydroxyl radical in alloxan diabetes.

The study shows that *M.indica* extract has the ability to induced antioxidant enzymes such as superoxide dismutase (SOD) and catalase(CAT) and also increase the concentration of reduced glutathione (GSH) in alloxan induced diabetic rats. It can therefore be used to protect the body cells against attacks from free radical.

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