Revista Brasileira de Farmacognosia
Brazilian Journal of Pharmacognosy
23(3): 481-487, May/Jun. 2013

Comparative effects of mature coconut water (*Cocos nucifera*) and glibenclamide on some biochemical parameters in alloxan induced diabetic rats

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Abstract: In the present study, comparative effects of mature coconut water (*Cocos nucifera* L., Arecaceae) and glibenclamide in alloxan induced diabetic rats were evaluated. Diabetes mellitus was induced in Sprague-Dawly rats using alloxan monohydrate (150 mg kg^{-1} body weight). Treatment with lyophilized form of mature coconut water and glibenclamide in diabetic rats reduced the blood glucose and glycated hemoglobin along with improvement in plasma insulin level. Elevated levels of liver function enzymes markers like alkaline phosphatase, serum glutamate oxaloacetate transaminase and serum glutamate pyruvate transaminase in diabetic rats were significantly reduced on treatment with mature coconut water. In addition to this, diabetic rats showed altered levels of blood urea, serum creatinine, albumin, albumin/globulin ratio which were significantly improved by treatment with mature coconut water and glibenclamide. Activities of nitric oxide synthase in liver and plasma L-arginine were reduced significantly in alloxan induced diabetic rats while treatment with mature coconut water reversed these changes. The overall results show that mature coconut water has significant beneficial effects in diabetic rats and its effects were comparable to that of glibenclamide, a well known antidiabetic drug.

Article

Received 20 Dec 2012
Accepted 14 Feb 2013
Available online 26 Mar 2013

Keywords:
albumin
alloxan
L-arginine
insulin
glycated hemoglobin
serum nitrite

ISSN 0102-695X
DOI: 10.1590/S0102-695X2013005000027

Introduction

Diabetes mellitus is a metabolic syndrome, initially characterized by a loss of glucose homeostasis resulting from defects in insulin secretion, insulin action both resulting in impaired glucose metabolism and other energy-yielding fuels such as lipids and protein (El-Soud et al., 2007). As per WHO, 346 million people worldwide have diabetes and it is also projected that death due to this will be the double between 2005 and 2030 (Rai et al., 2012). The beneficial effect of synthetic drugs provide good glycemic control but long term use have side effects and thus searching for a new class of compounds is essential to overcome diabetic problems (Prasad et al., 2009). There has been increasing demand for the use of plant products with antidiabetic activity due to low cost, easy availability and lesser side effects (Sharma et al., 2010). There are several published reports that plants like *Aegle marmelos, Ficus exasperata, Annona muricata, Syzygium cumini, Gymnema sylvestre* etc. exhibit significant antidiabetic potential (Anandharajan et al., 2006; Adewole et al., 2012; Adeyemi et al., 2010; Kang et al., 2012). So many people often combine the herbal remedies with oral hypoglycemic agent (Rai et al., 2012). Functional foods with preventive and therapeutic effects on metabolic disorders are very helpful for the improvement of lifestyle-related diseases (Muraki et al., 2011). The bioactive phytochemicals have become a very significant source for nutraceutical ingredients (Espín et al., 2007).

Coconut water is a natural nutritious beverage can be considered as a functional food/nutraceutical as it contains several biologically active components and possess cardioprotective, hepatoprotective, hypolipidemic and antihypertensive properties in experimental animals (Anurag & Rajamohan 2003; Loki & Rajamohan 2003; Sandhya & Rajamohan 2008; Bhagya et al., 2010; Prathapan & Rajamohan 2011). Results of our previous studies have shown that mature coconut water has hypoglycemic and antioxidant activities in rats induced diabetes (Preetha et al., 2012).

Among the various synthetic drugs, glibenclamide has been widely used in the management of non-insulin dependent diabetes mellitus (Figueroa-Valverde et al., 2012). The aim of the present study was to investigate the effects of lyophilized mature coconut water (LMCW) in comparison with glibenclamide in alloxan induced diabetic rats.

Materials and Methods

Collection of mature coconut water and the preparation of lyophilized mature coconut water (LMCW)

Coconut water from mature coconuts (*Cocos nucifera* L., Arecaceae) of 10-12 months of age, (West Coast Tall variety) grown in the University campus were used for this study. The coconuts were dehusked and liquid endosperm was collected filtered and pooled. The pooled mature coconut water was then lyophilized at -4 ^{o}C in a freeze drying chamber (Savanth Instruments, USA). The lyophilized mature coconut water was stored at 0 ^{o}C and used for the experiment. It was freshly reconstituted with distilled water prior to administration to rats.

Animals and experimental design

Male Sprague- Dawley rats weighing between 160-190 g were used for the study. The rats were housed individually in polypropylene cages in room maintained at 25±1 ^{o}C with alternate exposure to light and dark for 12 h. The rats were maintained on a standard Chow diet (Sai Feeds, Bangalore, India) and water *ad libitum* prior to dietary manipulation. The protocol was approved by the animal ethics committee of the University of Kerala (BC-TR 1/2004).

Dose response study

For dose response study, rats were divided into seven groups of six rats each. Group A: Control rats; Group B: Diabetic control; Group C: Diabetes+LMCW (100 mg kg^{-1} bw); Group D: Diabetes+LMCW (250 mg kg^{-1} bw); Group E: Diabetes+LMCW (500 mg kg^{-1} bw); Group F: Diabetes+LMCW (750 mg kg^{-1} bw); Group G: Diabetes+LMCW (1000 mg kg^{-1} bw).

After the optimization of the dose of LMCW, further experiments were carried out with 24 rats which were divided into four groups of six rats each.

Group I: Normal control

Group II: Diabetic control

Group III: Diabetes+LMCW (1000 mg kg^{-1} bw)

Group IV: Diabetes+glibenclamide (0.6 mg kg^{-1} bw)

Diabetes was induced in rats of groups II, III and IV by a single intraperitoneal (*i.p*) injection of alloxan monohydrate (150 mg kg^{-1} bw) after fasting the animals for 24 h. The rats were then kept on 5% glucose solution for the next 24 h to prevent hypoglycemia. After 72 h, rats with fasting blood glucose more than 250 mg dL^{-1} were considered diabetic and included in the study. Lyophilized mature coconut water (LMCW) was fed daily using an intragastric tube for 45 days. After the experimental period, animals were fasted overnight and they were sacrificed by sodium pentothal injection. Blood and tissues were collected for various estimations.

Biochemical estimations

Serum glucose was determined (Trinder, 1969) using Agappe diagnostics, Ernakulam, Kerala, India. Serum insulin was measured with an automated immunochemiluminometric (ICL) assay according to the manufacturer's instruction and was provided by Bayer Diagnostics (ADVIA Centaur insulin assay). Estimation of glycated hemoglobin was done using a Micromat2 hemoglobin Acc test, using a micromat II instrument, Catalogue No. 280-00016XI (Biorad). Liver glycogen was estimated by the method of Carroll et al., (1956). Blood urea was estimated by modified Berthelot method (Wheatherburn, 1967). Serum and urinary nitrate concentration, was estimated using the Griess reaction (Green et al., 1982). Serum protein was estimated by the method of Lowry et al, 1951. Albumin was estimated based on bromocresol green method using Agappe Diagnostics Albumin Kit (Doumasa et al., 1971). Serum glutamate oxaloacetate transaminase (SGPT) and Serum glutamate pyruvate transaminase (SGOT) was assayed by DNPH method (Reitman & Frankel, 1957) using the enzyme kit from CML Biotech (P) Ltd, Ernakulam, India. Quantitative determination of alkaline phosphatase was done as described by King & King (1954) using the enzyme kit procured from Dr.Reddy`s laboratories, Hyderabad, India. Creatinine in serum was estimated as per Bowers & Wong (1980). Activity of nitric oxide synthase was estimated by the method of Salter & Knowles (1997). Concentration of plasma L-arginine was estimated as described by Gopalakrishnan & Nagarajan (1979).

Statistical analysis

The results are expressed as the mean values with their standard deviation. Intergroup comparison was performed by one-way ANOVA followed by Duncan's variance. Significance was set at $p<0.05$.

Results

Concentration of blood glucose

Dose dependant response of LMCW in alloxan

induced diabetic rats were also evaluated and found that 1000 mg kg^{-1} of LMCW was effective in reducing blood glucose when compared to other doses in rats (Table 1). Significant increase of blood glucose levels were observed in alloxan induced diabetic rats (275.32±4.25 mg dL^{-1}) when compared to normal control rats (96.42±2.31 mg dL^{-1}). Treatment of diabetic rats with LMCW (1000 mg kg^{-1}) and glibenclamide showed significant reduction of blood glucose (129.23±1.95 and 120±2.3 mg dL^{-1} respectively) when compared to diabetic control.

Table 1. Dose response study of mature coconut water (*Cocos nucifera* L., Arecaceae) in alloxan induced diabetic rats.

Groups	Concentration of blood glucose (mg dL^{-1})
A	96.42±2.31
B	275.32±4.25[a]
C	244.32±2.75[b]
D	216.14±1.65[b]
E	182.29±2.17[b]
F	156.75±2.52[b]
G	129.23±1.95[b]

Values expressed as mean±SD of six rats. Significance accepted at $p<0.05$. 'a' indicates values are significantly different from group I. 'b' indicates values are significantly different from group II.

Concentration of plasma insulin, glycosylated hemoglobin (HbA1c) and liver glycogen

Figure 1 shows the concentration of plasma insulin, glycosylated hemoglobin (HbA1c) and liver glycogen in control and experimental rats. Alloxan induced diabetic rats showed significant decrease in plasma insulin and liver glycogen compared to normal control. On the other hand, treatment of diabetic rats with LMCW and glibenclamide increased the insulin level and concentration of liver glycogen along with reduction of HbA1c level.

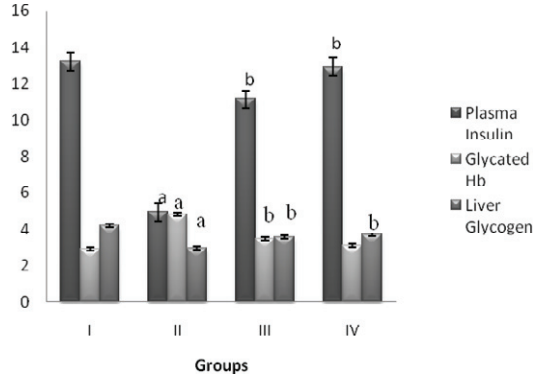

Figure 1. Concentration of plasma insulin (μ $IUmL^{-1}$), glycosylated hemoglobin (%) and liver glycogen (mg 100 g^{-1} tissue). Values expressed as mean±SD of six rats. Significance accepted at $p<0.05$. 'a' indicates values are significantly different from group I. 'b' indicates values are significantly different from group II.

Concentration of blood urea, serum creatinine, serum and urinary nitrite

Concentration of blood urea, serum creatinine and urinary nitrite were significantly increased in alloxan induced diabetic rats when compared to normal control rats. On the other hand, diabetic rats treated with LMCW and glibenclamide reversed these changes when compared to diabetic rats (Table 2, Figure 2).

Table 2. Concentration of blood urea; serum and urinary nitrite.

Groups	Blood urea mg dL^{-1}	Serum nitrite μmol L^{-1}	Urinary Nitrite mg dL^{-1}
I	19.36±3.21	13.23±2.35	32.47±3.62
II	39.99±1.73[a]	9.51±1.98[a]	20.13±2.15[a]
III	22.09±2.95[b]	11.68±2.35[b]	33.15±2.27[b]
IV	21.27±2.52[b]	12.13±3.26[b]	24.68±1.83[b]

Values expressed as mean±SD of six rats. Significance accepted at $p<0.05$. 'a' indicates values are significantly different from group I. 'b' indicates values are significantly different from group II.

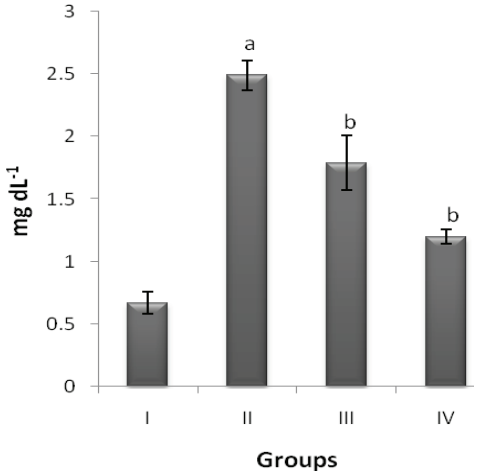

Figure 2. Concentration of creatinine in serum (mg dL^{-1}). Values expressed as mean±SD of six rats. Significance accepted at $p<0.05$. 'a' indicates values are significantly different from group I. 'b' indicates values are significantly different from group II.

Concentration of serum protein, serum albumin and A/G ratio

Table 3 shows the concentration of serum protein, serum albumin and A/G ratio in control and experimental rats. Significant decrease in the concentration of serum protein, serum albumin and A/G ratio were observed in alloxan induced diabetic rats when compared to normal control rats. Treatment of diabetic rats with LMCW and glibenclamide showed an increase in the concentration of serum protein, serum albumin and A/G ratio when compared to diabetic control.

Table 3. Concentration of serum protein, serum albumin and A/G ratio.

Groups	Serum protein (g dL^{-1})	Serum albumin (g dL^{-1})	A/G ratio
I	8.19±2.12	4.84±1.80	1.44±0.52
II	4.70±1.78[a]	2.21±0.72[a]	0.89±0.41[a]
III	5.69±1.59[b]	2.88±0.92[b]	1.02±0.50[b]
IV	6.12±1.82[b]	3.14±1.05[b]	1.05±0.51[b]

Values expressed as mean±SD of six rats. Significance accepted at $p<0.05$. 'a' indicates values are significantly different from group I. 'b' indicates values are significantly different from group II.

Activities of alkaline phosphatase (ALP), glutamate oxalo acetate transaminase (SGOT) and glutamate pyruvate transaminase in serum (SGPT)

Activities of ALP, SGOT and SGPT in serum were increased in diabetic rats when compared to normal rats. LMCW and glibenclamide treated rats resulted in significant decrease in the activities of these enzymes when compared to diabetic control (Table 4).

Table 4. Activities of alkaline phosphatase (ALP), glutamate oxaloacetate transaminase (SGOT) and glutamate pyruvate transaminase in serum (SGPT).

Groups	ALP (kA Units L^{-1})	SGOT (IU L^{-1})	SGPT (IU L^{-1})
I	10.51±1.45	19.61±0.97	22.08±1.22
II	21.38±2.54[a]	39.37±1.67[a]	45.27±0.92[a]
III	14.95±1.42[b]	25.41±1.48[b]	28.16±1.69[b]
IV	15.54±1.28[b]	28.76±1.21[b]	30.38±1.74[b]

Values expressed as mean±SD of six rats. Significance accepted at $p<0.05$. 'a' indicates values are significantly different from group I. 'b' indicates values are significantly different from group II.

Activity of nitric oxide synthase in liver and concentration of plasma L-arginine

The activity of nitric oxide synthase in liver and concentration of plasma L-arginine were significantly lowered in alloxan induced diabetic rats when compared to normal control rats. Treatment of diabetic rats with LMCW and glibenclamide showed significant increase in the activities of nitric oxide synthase in liver and concentration of plasma L-arginine when compared to diabetic rats (Figure 3).

Discussion

In the preset study, the antidiabetic effects of mature coconut water (MCW) were compared with that of standard drug, glibenclamide in alloxan induced diabetic rats. Concentrations of blood glucose and glycosylated hemoglobin (HbA1c) levels were found to have reduced in diabetic rats treated with LMCW. The non-enzymatic, irreversible covalent bonding of glucose with hemoglobin in the circulation results in the formation of HbA1c and the concentration of HbA1c reflects the average blood glucose levels over a period of time (Venkatesan & Sorimuthu, 2012). LMCW treated diabetic rats exhibited reduced level of HbA1c. This may be due to the restoration of blood glucose levels, thereby reducing the intensity of hemoglobin glycosylation during the experimental period. The effects were comparable to that of standard drug glibenclamide. In addition to this, serum insulin level was increased by the treatment with LMCW in diabetic rats. The reduction of blood glucose and HbA1c in LMCW treated diabetic rats may be due to the increased level of insulin.

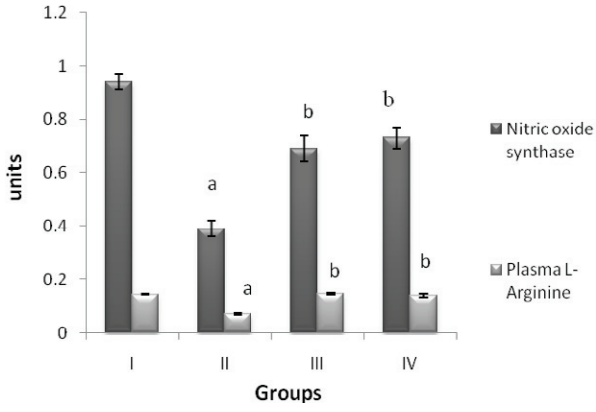

Figure 3. Activity of nitric oxide synthase (Ug^{-1} wet weight) and concentration of plasma L-arginine (µmol mL^{-1}). Values expressed as mean±SD of six rats. Significance accepted at $p<0.05$. 'a' indicates values are significantly different from group I. 'b' indicates values are significantly different from group II.

Liver glycogen level is considered as the best marker for assessing antihyperglycemic activity of any drug (Ahmed et al., 2012). The increase in liver glycogen of diabetic treated with natural products and glibenclamide is due to the increased insulin response which in turn promotes conversion of inactive form of glycogen synthase to the active form and enhances conversion of blood glucose into glycogen (Rawi et al., 2011). The prevention of depletion of glycogen in the liver is possibly caused by stimulation of insulin release from existing pancreatic β-cells, which enhances glycolysis (Ramkumar et al., 2011). Increased liver glycogen content in MCW treated diabetic rats suggests the stimulation of insulin release by LMCW from pancreatic β-cells, which enhances upregulation of glycolysis.

Hyperglycemia induces elevation of the blood urea and creatinine in serum which are considered as significant markers of renal dysfunction. Degradation of

protein and nucleic acid results in the formation of non-protein nitrogenous compound such as urea and creatinine. The elevated levels of serum urea and creatinine in diabetic rats are due to catabolism of the protein and nucleic acids (Wilson et al., 2011). Treatment of diabetic rats with LMCW showed significant decrease in the concentration of blood urea and creatinine in serum which could be due to the prevention of protein and nucleic acid degradation by LMCW. The results were similar to that of glibenclamide treated rats.

In addition to this, the present study showed a decline in total protein, sharp fall in serum albumin, globulin and A/G ratio in diabetic rats. Hypoalbuminemia observed in diabetes is generally attributed in the presence of nephropathy and/ or may be due to increased protein catabolism (Prakasam, 2004; Sivajothi et al., 2008). Significant decrease in the concentration of serum protein, serum albumin and A/G ratio were observed in alloxan induced diabetic rats when compared to normal control rats. Treatment of diabetic rats with LMCW and glibenclamide showed significant increase in the concentration of serum protein, serum albumin and A/G ratio when compared to diabetic rats. These findings suggest that LMCW treatment ameliorates alloxan induced nephrotoxicity.

Serum concentrations of liver function marker enzymes, SGPT, SGOT and ALP in alloxan induced diabetic rats were elevated. This may be due to leaking out of enzymes from the tissues and migrating into the circulation by the adverse effect of alloxan and alloxan can induce the liver injury by free radical mechanism (Kala et al., 2012). LMCW treatment regulated the activity of SGPT and SGOT in liver of rats intoxicated with alloxan. SGPT and SGOT act as indicators of liver function and restoration of normal levels of these parameters indicate normal functioning of liver. The measurement of enzymatic activity of alkaline phosphatase (ALP) is of clinical and toxicological importance as changes in the activity is indicative of tissue damage by toxicants. Elevated levels of ALP in diabetes may be due to extensive damage to liver in the alloxan induced diabetic rats (Ravikumar et al., 2010). Treatment with LMCW in alloxan induced diabetic rats caused a decline in ALP level.

Diabetic rats showed reduced activity of nitric oxide synthase, an enzyme required for the production of nitric oxide. Nitric oxide (NO) represents one of the signalling molecules involved in the modulation of the intracellular redox environment. Previous studies reported that NO acts as a physiological modulator of islet hormone release (Jimenez-Feltstrom et al., 2004). L-arginine, the precursor of NO can enhance insulin secretion and reduce hyperglycaemia, and these beneficial actions are associated with increased NO formation in patients with type 2 diabetes (Das et al., 1993). There are reports that L-arginine and NO can prevent β-cell damage in alloxan induced diabetic rats. In addition to potential action on insulin release, arginine administration may provide multiple benefits to ameliorate diabetes-induced endothelial dysfunction (Pieper 1998). Chemical analysis of LMCW showed that it contains L-arginine (5.85%), ascorbic acid (0.45%), magnesium (0.42%), potassium (7.71%), calcium (1.32), manganese (0.084%), total proteins (13.6%) etc. Among these, L-arginine is the major bioactive component which is reported to possess many beneficial effects against diabetes (Preetha et al., 2012). LMCW treatment enhanced the activities of nitric oxide synthase and increased the concentration of L-arginine in diabetic rats suggests that L-arginine is a major factor responsible for the beneficial effects.

In diabetic rats, mature coconut water treatment showed significant beneficial effects along with antinephrotoxicity and antihepatotoxicity. In conclusion, the present study clearly revealed that the mature coconut water has beneficial effect against diabetes induced complications and its effects were comparable to that of standard drug, glibenclamide.

Acknowledgement

First author thank Govt. of Kerala, India for financial support in the form of Senior Research Fellowship (B3/9272).

Authors' contributions

PPP contributed in carrying out the laboratory experiments systematically, analysis of the data, interpreting the results scientifically and drafted the manuscript. VGD involved in drafting the manuscript. TR designed the study, supervised the laboratory work and contributed to critical reading and finalizing the manuscript. All the authors have read the final manuscript and approved the submission.

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