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Extract of Leaves of *Moringa stenopetala* Alleviates Glycation in *In vitro* Model

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Abstract

This study was conducted to characterize antiglycation activity of ethanol extract of *Moringa stenopetala* leaves in a bovine serum albumin (BSA)/fructose system. Antiglycation activity was determined using inhibition of formation of advanced glycation end products, level of N^{ε}-(carboxymethyl) lysine, concentration of fructosamine, and amyloid cross β-structure formation in bovine serum albumin after incubation with fructose. The oxidation protein was evaluated using the concentration of protein carbonyl content formation and thiol group deterioration in the BSA/fructose system. The findings demonstrated the extract of *Moringa stenopetala* leaves significantly inhibited the formation of AGEs by approximately 54.75 ± 0.94% at a concentration of 2 mg/ml. Furthermore, *Moringa stenopetala* leaves reduced the concentration of fructosamine, decreased the formation of N^{ε}-(carboxymethyl) lysine (CML), and the amount of amyloid cross β-structure. Besides, the leaves protected from protein oxidation, including damages on carbonyl formation and thiol oxidation of BSA in fructose induced system. The results suggest importance of the extract of *Moringa stenopetala* leaves for alleviating AGE-mediated complications in diabetic patients.

Keywords: *Moringa stenopetala; Advanced Glycation Products; Diabetic Complications; Fructose Induced Glycation*

Background

Long term rise in blood glucose initiates non-enzymatic reaction of reducing sugars with amino groups of macromolecules through a series of reaction to form advanced glycation end products (AGE); the Maillard reaction, initiating several non-communicable diseases in human beings [1]. Chronic accumulation the Millard products in body tissue is the leading cause of several age-related diseases such as neurodegenerative disorders, atherosclerosis and diabetic complications [2,3]. Glycated albumin comprises about six to fifteen percent of the total albumin in normal individuals and increased more than a double in hyperglycemia. Altered conformation and consequently altered binding of albumin by non-enzymatic reaction results in diabetes mellitus, liver diseases and nephropathy which are just a few disorders among others [3,4]. Currently a lot of efforts focus on investigation of new medicinal products to reverse glycation associated with hyperglycemia [1-4]. However, antiglycation activity of *Moringa stenopetala* leaves extract not yet investigated. Therefore, the aim of this study was to investigate the antiglycation activity of *Moringa stenopetala* in *In vitro* model.

Materials and Methods

Extraction of plant material

The fresh leaves of *Moringa stenopetala* were collected from Arbaminch town, identified and authenticated by taxonomist, dried under shade and grinded to powder for extraction using standard procedures. The leaves of the plant material was deposited in Herbarium for future reference with a voucher number "AL-001". The powdered leaves were extracted by percolation using 70% (v/v) ethanol: water system in Ethiopia Public Health Institute, and the mixture was then filtered using filter paper. Rotary vaporizers under reduced pressure at a temperature of 40 - 45°C was used to dry the extract. The filtrate obtained was dried by steam bath at 40°C and kept in a refrigerator at 8°C for experimentation.

Advanced glycation end products assay in *In vitro* model

Glycated BSA formation in *In vitro* system was done according to a previously reported methods with little modifications [5]. BSA of 10 mg/mL concentration was incubated with fructose of about 0.5M concentration in 0.1M phosphate buffer (PBS), pH 7.4 containing 0.02% sodium azide (NaN_3) at 37°C for fourteen days in the absence or presence of *Moringa stenopetala* leaves extract of different concentrations and aminoguanidine (1 mg/ml) as positive standard. Dimethylsulfoxide (DMSO, 4%) was used as a solvent for this study. Aliquots of the reaction mixtures were then assayed for AGEs formation, fructosamine level, protein carbonyl content, thiol group level, amyloid cross β structure formation, and CML level determination according to standard procedures [6-10].

Data analysis

The results are presented as Mean ± SEM. Significance of the data was determined using one-way ANOVA. A Turkey post-hoc comparison was used to analyze the sources of significant differences. Statistically significant level was considered at a p-value < 0.05.

Results and Discussion

The effect of hydroalcoholic extract of *Moringa stenopetala* leaves at the concentration of 0.5 mg/ml, 1 mg/ml and 2 mg/ml on the formation of total AGEs during 14 days of incubation period was shown in figure 1. Extract of *Moringa stenopetala* leaves showed the strongest anti-glycating inhibition based on *in vitro* in fructose induced BSA glycation in this study. When the glycation occurred in the presence of test material, it was observed that *Moringa stenopetala* significantly reduced the formation of AGEs by 54.75 ± 0.94% at the concentration of 2 mg/ml, as compared to BSA incubated with fructose but less potent than AG (60.25 ± 1.13%), which was used as control.

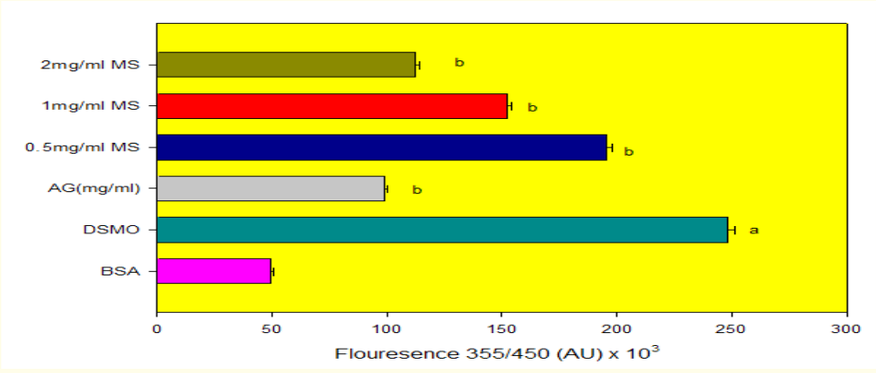

Figure 1: *The effects of hydroalcoholic extract of Moringa stenopetala (MS) at different concentration on fluorescent formation in the BSA/fructose system. Expressed in Mean ± SEM (n = 3). aP < 0.05 versus normal control, bP < 0.05 versus to negative control.*

Figure 2 shows the activity of the extract of *Moringa stenopetala* on macromolecules bound CML formation. The results revealed the formation of non-fluorescent CML in BSA incubated with fructose was 3.64-times higher than normal control group. The supplementation of *Moringa stenopetala* leaves extract at the concentration of 2 mg/ml to the solution reduced CML-derived AGE formation by approximately 19.25 ± 0.18%, whereas positive standard inhibited the formation of non-fluorescent CML by 45.46% when compared to negative control.

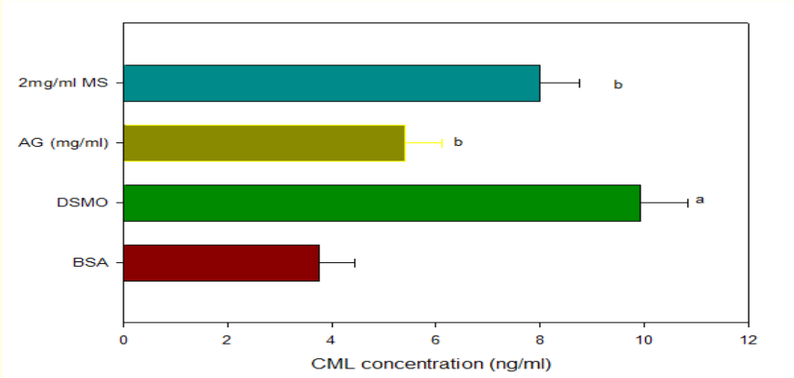

Figure 2: *The effects of hydroalcoholic extract of Moringa stenopetala (MS) at different concentration on on the level of Nε-(carboxymethyl) lysine (CML). Expressed in Mean ± SEM (n = 3). aP < 0.05 versus normal control, bP < 0.05 versus to negative control.*

After a couple of weeks of the study, the concentration of fructosamine in BSA incubated with fructose produced a 3.3-fold elevation when compared to normal control. The extract of *Moringa stenopetala* significantly decreased the level of fructosamine by about 36.1 ± 0.11%, reduced the oxidation of thiol groups by approximately 19.4%, at a concentration of 2 mg/ml whereas positive control prevented the damage of protein thiol groups by 23.6% at a concentration of 1 mg/ml, as compared to BSA incubated with fructose and repressed protein carbonyl creation by nearly 18.7% when compared to negative control (Table 1).

Treatment groups	Fructosamine (mMol/l)	Thiol content (mMol/mg)	Carbonyl content (nMol/mg)
BSA	1.67 ± 0.07	0.93 ± 0.04	0.38 ± 0.03
DSMO	5.46 ± 0.21[a]	0.72 ± 0.03[a]	1.98 ± 0.04[a]
AG (1 mg/ml)	3.21 ± 0.10[b]	0.89 ± 0.02[b]	1.42 ± 0.02[b]
MS (0.5 mg/ml)	5.01 ± 0.18	0.76 ± 0.04	1.91 ± 0.03
MS (1 mg/ml)	4.21 ± 0.12[b]	0.81 ± 0.03[b]	1.87 ± 0.02
MS (2 mg/ml)	3.49 ± 0.00[b]	0.86 ± 0.02[b]	1.61 ± 0.01[b]

Table 1: *The effect of Moringa stenopetala leaves extract on advanced end products content in fructose induced glycation system. Expressed in Mean ± SEM (n = 3). aP < 0.05 versus normal control, bP < 0.05 versus to negative control.*

In the present study the results on the formation of the AGEs demonstrated that *Moringa stenopetala* leaves efficiently suppressed fluorescent and non-fluorescent formation of advanced glycation end products. Moreover, the plant material also suppressed the concentration of fructosamine and amyloid cross β-structure development in fructose-induced BSA. There were significant rise of carbonyl content and oxidation of thiols in negative control. After *Moringa stenopetala* extract was added to the same systems, there was significant suppression of carbonyl content and thiols oxidation in *in vitro* system. Polyphenolic compounds block the carbonyl group in reducing sugars and break the cross linking structure in the formed AGEs have been suggested for antiglycation activity of compounds. The falling of free radical generation by antioxidant activity of polyphenols may indicate other mechanisms for the prevention of glycation. Hence, the antiglycation activities demonstrated in this experiment may be associated with the presence of high amount of phytochemicals such as polyphenolic compounds in the plant materials.

Major molecular modifications of structural changes in plasma albumin can be investigated by carbonyl formations and damage of thiol groups which are associated with free radical generations [11,12]. The noticeable increase in carbonyl content formation and the oxidation of thiols in BSA were observed in this experiment after a couple weeks incubation. We found that supplementation of the test material significantly declined the protein carbonyl formation and oxidation of thiols. Hydroalcoholic extract of *Moringa stenopetala* leaves have, therefore, a potential to ameliorate glycation induced damage to thiols and to decrease carbonyl content *in vitro*.

To ratify the thought that the plant material deters beta- amyloid sheet formation of albumin experimentally, Congo red was used as shown in figure 3. With this assay, the negative albumin control exhibited the strongest absorbance as expected and all the test groups with *Moringa stenopetala* leaves showed significant decline in absorbance (P < 0.05).

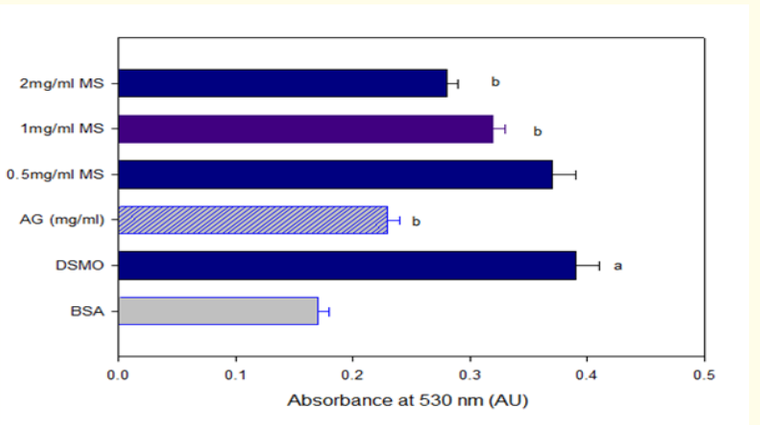

Figure 3: *The effects of hydroalcoholic extract of Moringa stenopetala (MS) at different concentration on absorbance of Congo red in the BSA/fructose system. Expressed in Mean ± SEM (n = 3). aP < 0.05 versus normal control, bP < 0.05 versus to negative control.*

It is well noted that aggregation of advanced glycation end products may result in pancreatic islet amyloidosis that causes damage of β-cell and compromised insulin secretion [9,13]. The plant extract suppressed the formation of amyloid cross β-structure of in fructose induced BSA. This beneficial effect of hydroalcoholic extract of *Moringa stenopetala* may ameliorate a risk degenerative diseases in diabetic patients.

Trapping free radicals as well as reducing reactive carbonyl group formation by antioxidant compounds is one best strategy for inhibition of advanced glycation end products effect in the body [13-15]. The plant material has antioxidant activity [16,17], the antioxidant(s) may contribute to antiglycation activity of the plant extract [18-31].

Conclusions

The results revealed that *Moringa stenopetala* leaves effectively protect BSA from fructose-induced glycation. Therefore, the *Moringa stenopetala* leaves have the potential to be used as supplement in the prevention/treatment of glycation induced diseases. Further characterization of active principle(s) with their mechanism(s) of action should be explored

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