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AKADÉMIAI KIADÓ

Evaluation of *Silybum marianum* seed extract and vitamin B6 derivatives on methylglyoxal and sugar-induced oxidative DNA damage

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ABSTRACT

Reducing sugars are known to generate reactive oxygen species (ROS), mainly by means of the glycation reaction. The hydroxyl radical, a prominent entity of ROS, is known to alter cellular DNA and induces damage to DNA, and plays a role in diseases such as diabetes mellitus. In this study, the oxidative damage of DNA induced by the lysine/Fe^{3+}/MG reaction was investigated. *Silybum marianum* seeds extract (*Sly*E), standard silymarin (Sly), and vitamin B6 derivatives, pyridoxal-5-phosphate (PLP), pyridoxamine (PM), and pyridoxine (P) in reversing glycation-induced damage in DNA were evaluated. In addition, different sugars and sugar phosphates were incubated with plasmid pBR 322 DNA to control and compare their harmful effects. Our results revealed that *Sly*E protected lysine/Fe^{3+}/MG induced oxidative DNA damage more effectively than Sly. Vitamins, on the other hand, prevented this DNA damage in the order of PLP>P>PM. The DNA altering and damaging intensity of sugars and sugar phosphates tested increased considerably in the following order: Ribose-5-phosphate > fructose-6-phosphate > ribose > fructose > fructose-1,6 biphosphate > glucose-6 phosphate > glucose. The results show that the lysine/Fe^{3+}/MG glycation reaction can cause oxidative damage of DNA through a mechanism involving hydroxyl radicals. It also provides evidence that ribose-5-phosphate and fructose and its phosphate metabolites can alter DNA more rapidly *in vitro* than glucose and its phosphate metabolites.

KEYWORDS

Silybum marianum, methylglyoxal, hydroxyl radical, DNA cleavage, oxidative DNA damage

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1. INTRODUCTION

The heterogeneous group of chemical products proceeding from the non-enzymatic reaction of reducing sugars and proteins, lipids, and nucleic acids are called AGEs. AGEs induce damage to DNA, which is affiliated with significant factors for mutagenesis, carcinogenesis, and diabetes mellitus (Negre-Salvayre et al., 2009). The amino groups of DNA bases can react non-enzymatically with reducing sugars, and studies have shown that *in vivo* deoxyguanosine is the most reactive nucleotide under physiological conditions (Schneider et al., 1988; Papoulis et al., 1995). The major DNA-AGEs formed upon reaction of DNA with various sugars *in vitro* are N^2-carboxyethylguanosine ($CEG_{A,B}$) and N^2-carboxyethylguanine (CEguanine), the two diastereomers of N^2-carboxyethyl-2′-deoxyguanosine ($CEdG_{A,B}$), are similar analogous formed during the glycation of guanosine or guanine, respectively (Schneider et al., 1988).

The most abundant carbohydrate in body fluids is glucose, but fructose has more potent reducing capacity than glucose, and the glycation reaction is readily triggered by fructose. As a result, fructose is supposed to play a considerable role in the deterioration caused by diabetes in various organ systems (Suarez et al., 1989). However, there are numerous other sugars in blood plasma that could potentially also play roles. Fructose-6-phosphate, fructose-1,6-bisphosphate, glucose-6-phosphate, ribose, ribose-5-phosphate, which are carbohydrate derivatives, are more reactive glycation agents and cause glycation more effectively than glucose. Hemiacetal or hemiketal sugars, since they do not enter into the amadori reaction, with the free carbonyl group in the linear structure are more effective glycation agents. Phosphorylation of sugar also increases reactivity (Gemayel et al., 2007). Reducing sugars are known to generate reactive oxygen species (ROS) essentially via glycation reaction. Among ROS, hydroxyl radical ($^{\cdot}OH$) is the strongest and can react with DNA, lipids, and proteins. DNA is the most sensitive to damage (Ortwerth et al., 1998). It has been reported that DNA damage and mutations are induced by ROS, and also affect cytoplasmic and nuclear signal transduction pathways (Suarez et al., 1989).

Another mechanism in the formation of AGE is the formation of low molecular weight dicarbonyl compounds, such as 3-deoxyglucosone (3-DG), glyoxal (GO) and methylglyoxal (MG), with high reactivity as glycolysis intermediates generally due to oxidative stress, as a consequence of degradation of glycated proteins and oxidation of lipids. These are potent glycation agents in the physiological system, and are more reactive than parent sugars in their ability to react with amino groups of proteins to form crosslinks (Thornalley et al., 1999). Different situations are known to disrupt the balance between ROS production and cellular defense and give rise to cellular destruction and dysfunction. An imbalance between pro and antioxidant factors plays a considerable role in many disease processes, including diabetes mellitus (Dypbukt et al., 1994).

Many natural compounds are known to prevent glycation and glycation-mediated ROS formation (İnceören et al., 2022). Silymarin extracted from seeds of *Silybum marianum* L. contains multiple active ingredients, including antioxidant flavonoids, which increase cellular glutathione levels and cellular membrane stabilising properties, and is a powerful antioxidant that scavenges reactive compounds that damage DNA and cellular structures (Von Schonfeld et al., 1997). DNA damage may lead to diseases such as neurological disorders, aging cancer, and inflammation. Recently, a group of B vitamins has been shown to exert an inhibitory effect on AGE formation. Especially, pyridoxamine (PM), an active form of vitamin B6, has attracted attention for its therapeutic effects on diabetic complications (Degenhardt et al., 2002).

Therefore, the aim of this study was to investigate the *in vitro* glycation of DNA by various sugars and sugar metabolites and the oxidative damage in pBR 322 plasmid DNA with a glycation model system consisting of lysine/Fe^{3+}/MG. In addition, to determine the protective effects of *S. marianum* seed extract and silymarin on DNA glycation with the lysine/Fe^{3+}/MG glycation model system.

2. MATERIALS AND METHODS

2.1. Plant materials and chemicals

The seed of cultivated *S. marianum* L. Gaertner (Milk thistle) harvested in 2015 was obtained from Dicle University, Field Crops Department, Agriculture Faculty Diyarbakır, Turkey. The plant seed was identified as *S. marianum* (Ref No. 2012-108 (DUF)) by Dr. Selçuk Ertekin, Dicle University, Department of Biology, Faculty of Science, Diyarbakır, Turkey.

All chemicals and reagents used in the experiments were of analytical-grade. Silymarin, pBR 322 plasmid DNA, HindIII DNA, EcoR1 digested DNA, pyridoxine (P), pyridoxal 5-phosphate (PLP), pyridoxamine (PM), glucose, glucose- 6-phosphate, ribose, ribose-5-phosphate, fructose, fructose-6-phosphate, fructose 1,6-bisphosphate, lysine, and methylglyoxal (MG) were bought from Sigma-Aldrich (USA), and sodium azide was obtained from Merck (USA).

2.2. *S. marianum* seed extract preparation

S. marianum seed (40 g) was ground in an electronic laboratory type blender and transferred to soxhlet cartridges. The seeds were defatted with 400 mL of petroleum ether for 6 h at 60 °C using soxhlet apparatus to remove lipids. Then, the defatted seeds were extracted with 400 mL of ethanol: water (1:1) solvent system for 6 h using soxhlet apparatus. Ethanol was evaporated with the rotary evaporator. The remaining water extract was frozen in liquid nitrogen and freeze-dried in a Christ Alpha 1-2 LD plus lyophiliser under −86 °C at 0.0021 mbar pressure for 24 h (Elwekeel et al., 2013). *S. marianum* seed extract (2.45 g) was obtained with a yield of 6.12%. The lyophilised solids were stored at −20 °C until use.

2.3. Analysis of total silymarin in *S. marianum* seed extract by using HPLC

2.3.1. Preparation of standard and sample solutions. The milk thistle sample was first dissolved with methanol, and the main stock solution was prepared. Intermediate stock solutions were prepared by diluting this master stock solution with mobile phases A and B mixed at a 1:1 ratio. 20 µL was injected into the device at a certain rate (for example, 50 ppm). Silymarin stock solution was prepared in methanol. This standard stock solution, which was used to create the calibration curve, was diluted with methanol to certain concentrations.

2.3.2. HPLC-DAD assay. The HPLC system consisted of an LC system from Agilent equipped with a diode array detector (DAD), and chromatographic separation was achieved using a reverse phase Eclipse C18 column (150 × 4.6 mm, 3.5 µm). The mobile phase consisted of [A] water with 0.1% formic acid; [B] methanol (1:1) and the separation was performed at 35 °C. It was made at a flow rate of 0.6 mL min^{-1} and UV detection was carried out at 254 nm (Tayoub et al., 2018).

2.4. Analysis of DNA damage

pBR 322 plasmid DNA (0.5 μg), lysine (20 mM), methylglyoxal (20 mM), Fe^{3+} (100 μM) in 100 mM potassium phosphate buffer (pH 7.4) were incubated at 37 °C for 2 h in the presence and absence of *Sly*E and Sly (100–1,000 μg mL^{-1}). Sodium azide (250 mM), pyridoxine (5 mM), pyridoxal 5-phosphate (5 mM) and pyridoxamine (5 mM) were used as positive controls. After the incubation period, the mixture was frozen at −20 °C for 60 min, and the reaction was stopped. 3 μL loading dye was added to the samples, 1% agarose gel was prepared in 100 mL Tris acetate buffer, and the samples were analysed by electrophoresis. The gel was scanned on Gel documentation system (Suji and Sivakami, 2007).

2.5. Incubation of DNA with sugar and sugar phosphates

The pBR 322 plasmid DNA (0.5 μg) was incubated with sugar and sugar phosphates at 37 °C for 2 h under sterile conditions in the dark, with either lysine (20 mM), methylglyoxal (20 mM), Fe^{3+} (100 μM) and glucose (250 mM), glucose-6-phosphate (250 mM), ribose (250 mM), ribose-5-phosphate (250 mM), fructose (250 mM), fructose-6-phosphate (250 mM), and fructose 1,6-bisphosphate (250 mM) in 100 mM potassium phosphate buffer (pH 7.4). After the incubation period, the mixture was frozen at −20 °C for 60 min and the reaction was stopped. Samples were mixed with 3 μL loading dye and loaded on 1% gel. Gels were run at 60 V in tris-acetate-EDTA buffer until the dye came out of the gel. The gel was scanned on Gel documentation system (Ali et al., 2014).

2.6. Continued incubation of pBR 322 DNA with fructose

The pBR 322 DNA was incubated in the dark at 37 °C with 0.8 M fructose in Tris-HCl buffer (50 mM; pH: 8.0). After 0, 3, 5, and 7 days, samples were withdrawn from this mixture and mixed with 3 μL of loading dye and loaded on 1% agarose gel. Gels were run at 60 V in tris-acetate-EDTA buffer until the dye came out of the gel. The gel was scanned on Gel documentation system (Waris et al., 2010).

2.7. Statistical analysis

Values are expressed as mean ± SD. These analyses were performed using SPSS software 25 (IBM Corp, USA). The DNA strand break was analysed by one-way ANOVA. Differences were analysed by Tukey's post hoc test. Difference at $P < 0.05$ was considered to be statistically significant. All figures were constituted using GraphPad Prism version 4.0 (GraphPad Software Inc., USA).

3. RESULTS AND DISCUSSION

3.1. Analysis of total silymarin in *S. marianum* seed extract by using HPLC

Figure 1 shows the chromatogram of *Sly*E compared to the Sly. As can be seen in the chromatograms, eight major peaks were observed, with each peak identified as one of the flavolignan components of the silymarin. The peaks were defined as: (1) taxifolin, (2) silychristin, (3) apigenin-7-glucoside, (4) silydianin, (5) silybin A, (6) silybin B, (7) isosilybin A, and (8) isosilybin B, respectively. The total amount of silymarin in *Sly*E was found to be 2.59 mg g DW^{-1} by HPLC.

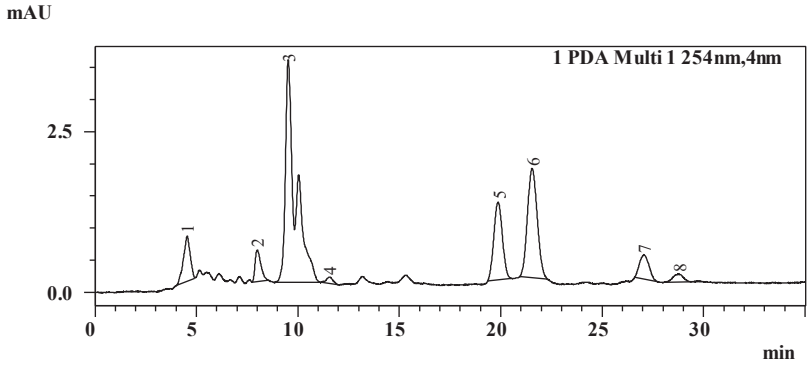


Fig. 1. HPLC chromatogram of silymarin in *S. marianum* seed extract(conditions: column Eclipse C18 (150 × 4.6 mm, 3.5 µm), mobile phase: [A] water with 0.1% formic acid; [B] methanol (1:1 v/v), flow rate: 0.6 mL min^{-1}, UV detection at 254 nm). (Retention time: 4.5 min peak 1: Taxifolin; 8.0 min peak 2: Silychristin; 9.5 min peak 3: Apigenin 7-glucoside; 11.5 min peak 4: Silydianin; 19.8 min peak 5: Silybin A; 25.5 min peak 6: Silybin B; 27.0 min peak 7: Isosilybin A; 28.7 min peak 8: Isosilybin B)

3.2. Analysis of DNA damage

Various evidences indicate that the reaction between lysine and MG can produce superoxide and hydroxyl radicals. Lysine, an important component of proteins, is part of enzyme active sites and plays critical roles in catalysis. At the same time, it is the group of choice for non-enzymatic sugar attachment followed by the formation of AGEs. Under aerobic conditions, oxidized metal ions such as Fe^{2+}, Fe^{3+}, Cu^{+} and Cu^{2+} can catalyse the oxidation of sugars, proteins, nucleic acids, and other substances involved in AGEs formation and promote the production of free radicals and carbonyl compounds. Previous studies have demonstrated that in the presence of iron, MG triggers DNA cleavage by reaction with lysine. The cellular precaution against the oxidative damage in DNA is realised by enzymatic and non-enzymatic antioxidants (Kang, 2003). In this study, the effects of *Sly*E and Sly on lysine/Fe^{3+}/MG mediated DNA cleavage were investigated.

Silymarin, derived from milk thistle (*S. marianum* L.), is a complex mixture with antioxidant and anti-inflammatory properties. Iron is a necessary component of the respiratory chain. The total amount of silymarin in *SlyE* was found to be 2.59 mg g DW^{-1} by HPLC. Generally, the concentration of extract used is much higher than the pure bioactives. In this study, however, the same concentration of both *SlyE* and Sly, 100–1,000 µg mL^{-1} have been used. However, free iron has the capability to promote ROS formation through Fenton reaction (Halliwell and Gutteridge, 2007). As shown in Figs 2 and 3, the amount of supercoiled (SC) DNA was slightly reduced by lysine/MG. SC-DNA loss induced by lysine/MG was clearly enhanced by the addition of ferritin. Consequently, DNA breakage of the MG-mediated reaction may be caused by traces of transition metals. Silymarin has an important effect on increasing gene expression of antioxidant enzymes (Karimi et al., 2011). Both compounds showed a dose-independent inhibition of DNA cleavage induced by lysine/Fe^{3+}/MG (Figs 2 and 3). *Sly*E inhibited DNA cleavage more effectively than Sly (Fig. 2). Thus, it is obvious that there is a strong relationship between inhibition of superoxide and hydroxyl radical production and protection of DNA.

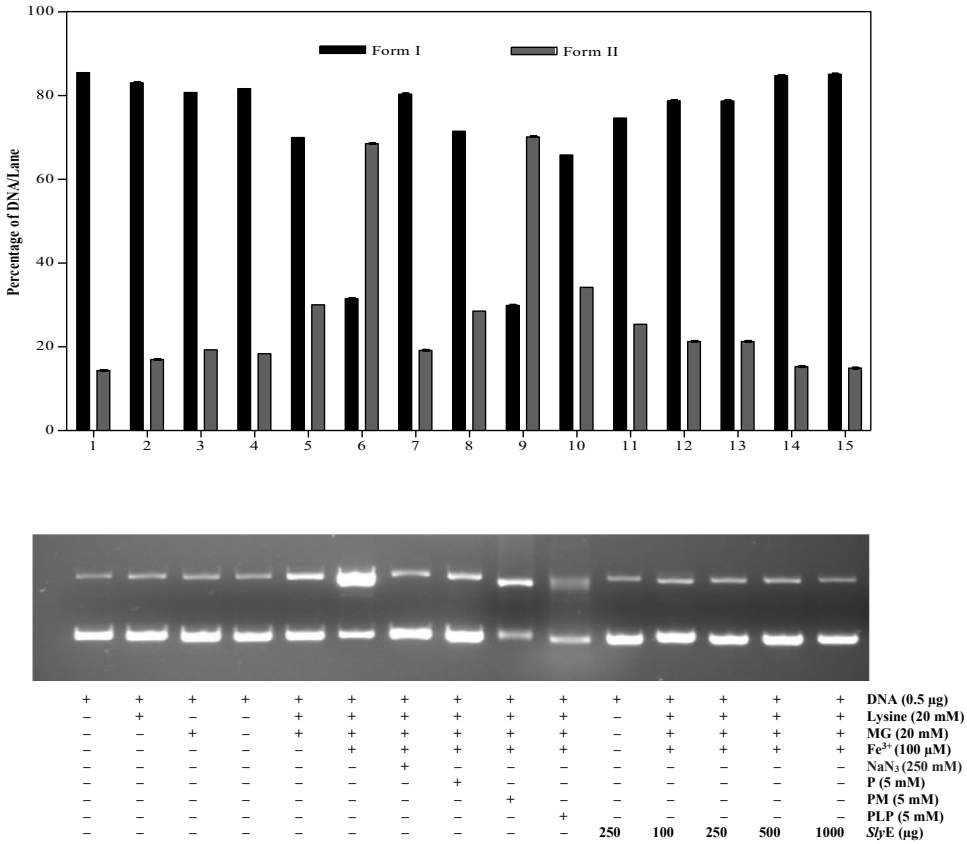
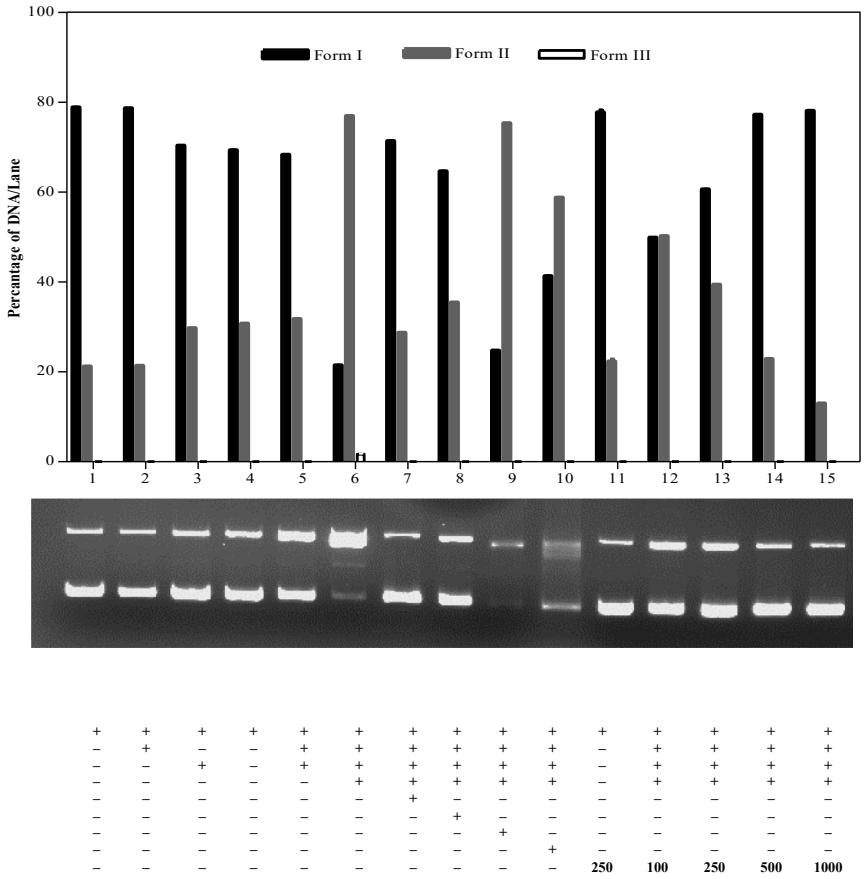


Fig. 2. Effect of SlyE and vitamin B_6 derivatives on DNA cleavage by the lysine/Fe^{3+}/MG system. Each value represents the mean ± SD (n = 3).

pBR 322 plasmid DNA was incubated at 37 °C for 2 h with the above: The reactions were stopped by freezing at −20 °C for 60 min and samples were analysed by 1% agarose gel electrophoresis. I and II indicate the position of the supercoiled and nicked circular DNA plasmid forms, respectively. The relative staining intensity of agarose gel was analysed by densitometric scanning (Quantity One program, version 4.5.2, Bio Rad Co.). Each value represents the mean ± SD (n = 3)

Recently, a group of vitamin B has been shown to exert inhibitory effect on AGE formation. Especially pyridoxamine, an active form of vitamin B6, has raised attention for its therapeutic effects on diabetic complications (Degenhardt et al., 2002). Previous studies show that the glycation reaction of MG/lysine in the presence of Fe^{3+} can give rise to oxidative damage of DNA by means of a mechanism involving hydroxyl radicals. In our study, we aimed to determine the ability of pyridoxine (P), pyridoxamine (PM), and pyridoxal-5-phosphate (PLP), which are derivatives of vitamin B6, to scavenge ROS, which causes oxidative DNA damage in the presence of lysine, transition metals, and MG.

As shown in Figs 2 and 3, DNA damage increased dramatically when Fe^{3+} was added to the DNA/lysine/MG reaction mixture, and SC-DNA loss significantly increased with the addition of ferritin (lane 6, $P < 0.05$). Vitamins PLP>P>PM prevented DNA damage. Although all tested



+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	DNA (0.5 µg)
–	+	–	–	+	+	+	+	+	+	–	+	+	+	+	Lysine (20 mM)
–	–	+	–	+	+	+	+	+	+	–	+	+	+	+	MG (20 mM)
–	–	–	–	–	+	+	+	+	+	–	+	+	+	+	Fe^{3+} (100 µg)
–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	NaN_3 (250mM)
–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	P (5 mM
–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	PM (5 mM)
–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	PLP (5 mM)
–	–	–	–	–	–	–	–	–	–	250	100	250	500	1000	Sly (µg)

Fig. 3. Effect of Sly and vitamin B_6 derivatives on DNA cleavage by the lysine/Fe^{3+}/MG system. pBR 322 plasmid DNA was incubated at 37 °C for 2 h with the above: The reactions were stopped by freezing at −20 °C for 60 min and samples were analysed by 1% agarose gel electrophoresis. I, II, and III indicate the position of the supercoiled, the nicked circular, and the linear DNA plasmid forms, respectively. The relative staining intensity of agarose gel was analysed by densitometric scanning (Quantity One program, version 4.5.2, Bio Rad Co.)

vitamins showed varying degrees of ability to inhibit hydroxyl radical production, P and PLP showed maximum abilities. Regarding the mechanism of action of PM, it has been proposed that it may be influential as a metal chelating agent that can compose stable complexes with Cu^{2+} and Fe^{3+} and as a ROS scavenger (Adrover et al., 2008). It also inhibits the occurrence of the hydroxyl radical through the Fenton reaction or reacts directly with it (Chetykrin et al., 2008). Free radical trapping antioxidants have been known to inhibit the glycationreaction. The hydroxyl radical scavenger sodium azide protects DNA from damage. In the presence of lysine, MG, and $Fe^{3+,}$ sodium azide (250 mM) protected DNA from damage (Figs 2 and 3; lane 7, $P < 0.05$). Consequently, the present study displays that the lysine/Fe^{3+}/MG system may bring about oxidative DNA damage.

3.3. Incubation of DNA with sugar and sugar phosphates

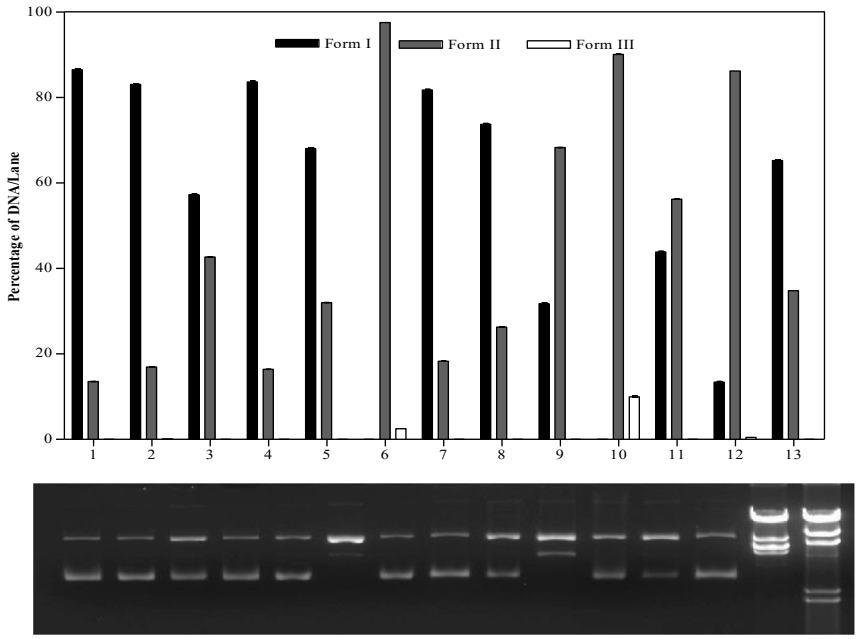
Reducing sugars are known to produce reactive ROS essentially through glycation reaction. Among ROS, ·OH radical is the strongest and can react with DNA, lipids, and proteins, DNA is the most susceptible to damage (Halliwell and Gutteridge, 2007). It has been reported that DNA damage and mutations are caused by ROS and also affect cytoplasmic and nuclear signal transduction pathways (Levi and Werman, 2003). DNA was incubated for 2 h at 37 °C in the presence of various sugars and sugar phosphates (250 mM) and lysine (20 mM), FeCl, (100 μM) and MG (20 mM). Then, the reaction was frozen at −20 °C for 60 min, and the damage to the DNA was analysed. Sugar and sugar phosphates damaged DNA in the following order: Ribose-5-phosphate > fructose-6-phosphate > ribose > fructose > fructose-1,6-biphosphate > glucose-6-phosphate > glucose (Fig. 4). Only two sugar phosphates, ribose-5-phosphate and fructose-6-phosphate, resulted in the formation of both a linear and open circular form of DNA (Fig. 4; lane 10 and 12). However, damage to DNA by lysine/Fe^{3+}/MG was much more extensive than for sugars and sugar phosphates (Fig. 4; lane 6, $P < 0.05$). This is probably due to MG having two carbonyl groups, which makes it highly reactive and more damaging than sugars (Thornalley et al., 1999). To estimate the amount of total DNA left at the end of each incubating period, DNA was digested with Hind III and EcoR1, a restriction enzyme that cleaves pBR 322 DNA at a single site and converts the super-coiled form or open circular form to the linear form that migrates on an agarose gel as a function of its molecular weight. In electrophoresis following digestion with Hind III and EcoR1, the sugars Ribose-5-phosphate and Fructose-6-phosphate seem to cause the most DNA damage (Fig. 4; lane 10 and 12). It was observed for DNA glycation that several sugar phosphates reacted more readily than their non-phosphate analogues, like fructose-6-phosphate compared to fructose or glucose-6-phosphate compared to glucose (Fig. 4; lane 11 and 12; lane 7 and 8, $P < 0.05$). This study provides us with evidence that ribose-5-phosphate and fructose and its phosphate metabolites can alter DNA more rapidly *in vitro* than glucose and its phosphate metabolites.

3.4. Continued incubation of pBR 322 DNA with fructose

More recently, it has been shown that sugars other than glucose can contribute significantly to the glycation reactions *in vivo*, because of their far higher reactivity (Levi and Werman, 2003). As can be seen from Fig. 5, the conversion of the supercoiled form of pBR 322 DNA to the nicked open circular form was detected and progressively increased after 3 days of incubation with fructose. The loss of the supercoiled form was 50%, 52%, and 60% at the end of 3, 5, and 7 days of incubation. Fructose is a stronger and faster glycation agent than glucose *in vitro*. The ration of the open chain form, which is the reactive species, is about 10 times higher in fructose than in glucose. Fructose adducts observed by Kawasaki et al. (1998) in diabetic rat lens suggest a role for fructose in glycation.

DNA reacting non-enzymatically with sugars forms AGEs of DNA (DNA-AGEs) (Papoulis et al., 1995). Fructose can generate free radicals under *in vitro* conditions and cause pBR 322 plasmid DNA modifications and damage. Incubation of pBR 322 plasmid with fructose for 3, 5, and 7 days caused the supercoiled plasmid to transform into open circular and linear forms (Fig. 5, $P < 0.05$).

Fructose-AGE levels have been detected in more than 100 commercial products and have been shown to be highest in yogurt drinks. Fructose in high fructose corn syrup is a modified



+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	DNA (0.5 µg)
–	+	–	–	+	+	+	+	+	+	+	+	+	–	–	Lysine (20 mM)
–	–	–	+	–	+	–	–	–	–	–	–	–	–	–	MG (20 mM)
–	–	+	–	+	+	+	+	+	+	+	+	+	–	–	Fe^{3+}(100 µg)
–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	Glucose (250 µg)
–	–	–	–	–	–	–	+		–	–	–	–	–	–	Glucose-6-P (250 µg)
–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	Ribose (250 µg)
–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	Ribose-5-P (250 µg)
–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	Fructose (250 µg)
–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	Fructose-6-P (250 µg)
–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	Fructose–1,6-P (250 µg)
–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	EcoR1 dig DNA
–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	Hind III dig DNA

Fig. 4. Investigation of damage by lysine/Fe^{3+}/MG in pBR 322 plasmid DNA and comparison with glycation-induced DNA cleavage caused by lysine/Fe^{3+}/sugar/sugar phosphates by agarose gel electrophoresis. Plasmid DNA was incubated at 37 °C for 2 h with the above: The reactions were stopped by freezing at −20 °C for 60 min and samples were analysed by 1% agarose gel electrophoresis. I, II, and III indicate the position of the supercoiled, the nicked circular, and the linear DNA plasmid forms, respectively. The relative staining intensity of agarose gel was analysed by densitometric scanning (Quantity One program, version 4.5.2, Bio Rad Co.). Each value represents the mean ± SD (n = 3)

sugar, and fructose is not a naturally occurring sugar in the starch from which it is obtained. The hormone leptin is a crucial regulator of energy intake through its interaction with the hypothalamic centers, rising satiety and energy expenditure (Ahima and Flier, 2000). Both fructose consumption and obesity cause a change in leptin function called leptin resistance in which the

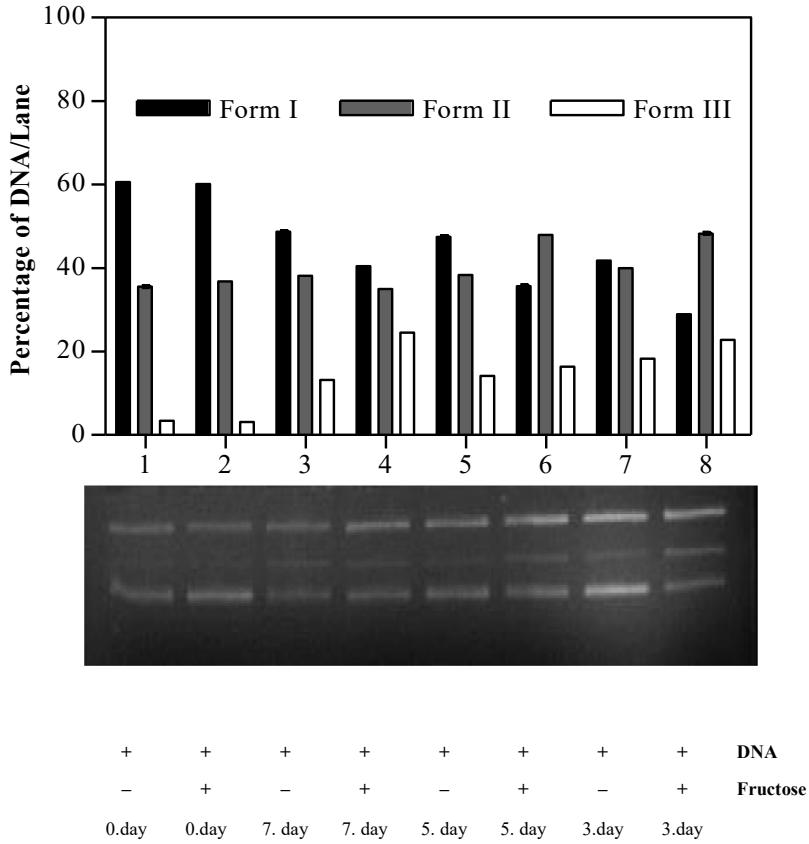


Fig. 5. Agarose gel electrophoresis of pBR 322 plasmid DNA after incubation with fructose was subjected to continuous or interrupted incubation for 3, 5 and, 7 days with 0.8 M fructose. The samples were analysed by 1% agarose gel electrophoresis. I, II, and III indicate the position of the supercoiled, the nicked circular, and the linear DNA plasmid forms, respectively. The relative staining intensity of agarose gel was analysed by densitometric scanning (Quantity One program, version 4.5.2, Bio Rad Co.). Each value represents the mean ± SD ($n = 3$)

hypothalamic center becomes resistant. As a result, the satiety response that must be produced is inhibited, resulting in greater food consumption (Johnson et al., 2017). Western diet is sourced from high fructose corn syrup and this may be inducing biochemical changes that support metabolic syndrome, type 2 diabetes, and nonalcoholic fatty liver disease (Malik and Hu, 2015). In this context, our study provides evidence for DNA damage from fructose.

4. CONCLUSIONS

In conclusion, our study shows that various sugars and sugar metabolites can cause DNA damage. Moreover, the protective effects of *S. marianum* seed extract and silymarin were also

evaluated in inhibiting DNA glycation against the lysine/Fe^{3+}/MG glycation model system. Findings from this study describe the protective effects of *S. marianum* seed extract and silymarin on DNA integrity against MGO-induced oxidative stress. Increase in the formation of DNA AGEs adduct and change in the structure can cause enhancement of diabetes mediated complications associated with hyperglycemia.

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