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Syringic acid, a novel natural phenolic acid, normalizes hyperglycemia with special reference to glycoprotein components in experimental diabetic rats

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ABSTRACT

Objective: To evaluate the antidiabetic effect of syringic acid, a natural phenolic compound on the levels of glycoprotein components in plasma and tissues of alloxan induced diabetic rats. **Methods:** Diabetes was induced in male Wistar rats by a single intraperitoneal injection of alloxan (150 mg/kg b.w). Syringic acid (50 mg/kg b.w) was administered orally for 30 d. The effects of syringic acid on plasma glucose, insulin, C-peptide, plasma and tissue glycoproteins were studied. **Results:** Oral administration of syringic acid (50 mg/kg b.w) for 30 d positively modulates the glycemic status in alloxan induced diabetic rats. The levels of plasma glucose were decreased with significant increase of plasma insulin and C-peptide level. The altered levels of plasma and tissue glycoprotein components were restored to near normal. No significant changes were noticed in normal rats treated with syringic acid. **Conclusions:** The present findings suggest that syringic acid can potentially ameliorate glycoprotein components abnormalities in addition to its antidiabetic effect in experimental diabetes, further clinical studies are required to evaluate the use of syringic acid as an effective therapeutic agent for the treatment of diabetes mellitus.

1. Introduction

Diabetes is a prototypical, growing, costly chronic non-communicable disease (NCD) causing increasing morbidity and mortality worldwide, often disproportionately hurting the poor and young sub-populations in developing countries[1]. Globally there are 366 million people currently known to have diabetes which is estimated to grow to 552 million by 2030 with 80% of all people with diabetes living in the developing world[2]. An estimated 61.3 million people in India have diabetes and the number is projected to reach 101.2 million by 2030. The pathophysiology of diabetes involves a very complex cascade of several interrelated mechanism. Elevated blood glucose induced microvascular and macrovascular changes, such as atherosclerosis, retinopathy, nephropathy, coronary artery disease, cerebral vascular disease, and peripheral artery disease[3], the reasons for injury related to hyperglycemia is increased polyol pathway flux, increased glycation of proteins (enzymatic or non enzymatic) and increased hexosamine pathway flux[4]. Among the above stated possibilities, glycosylation of proteins has been the prime subject of much interest. In the diabetic state, glucose is used by the insulin independent pathways, leading to the synthesis of oligosaccharide moieties of glycoprotein.

Glycoproteins can be simply defined as proteins that have carbohydrate moiety covalently attached to their peptide portion. They have multiple and complex function and are found as enzymes, hormones, blood group substances and as constituents of extracellular membranes[5]. The commonest glycoproteins are those in which the carbohydrate is linked to the protein by glycosyl linkages, usually hexose, hexosamine, fucose and sialic acid, joined together covalently linked to polypeptide chain. Hyperglycemia in

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experimental diabetic rats leads to a decreased utilization of glucose by insulin dependent pathways, thereby enhancing the formation of glycoproteins[6]. At the cell surface or inside the cells, the glycoprotein components such as fucose and sialic acid form specific structures, called glycanic chains covalently linked to lipids or proteins. An increase in the biosynthesis or a decrease in the metabolism of glycoproteins could be related to the deposition of these materials in the basal membrane of pancreatic cells. Alterations in glycoproteins level leads to the pathogenesis of diabetes mellitus. Many studies confirm the involvement of glycoproteins in diabetic complications[7].

Plants constitute an important source of active natural products which differ widely in terms of structure and biological properties[8]. They have a remarkable role in the traditional medicine in different countries. Recently, plants and their active components have been widely used in the treatment of diabetes. Plant derived phenolic compounds have a high potential for lowering blood glucose levels. Syringic acid (4-hydroxy-3,5-dimethoxybenzoic acid; Fig. 1) is an active phenolic compound isolated from *Alpinia calcarata Roscoe*[9]. The leaves of *Alpinia calcarata Roscoe* are a well-known traditional indian ayurveda medicine and have been used to treat for diabetes. Since, no systematic studies exist in the literature on the effect of syringic acid on glycoprotein components in diabetes, in this view, the aim of the present study was to establish the antidiabetic effect of syringic acid and also mediated through its salubrious effect on plasma and tissue glycoproteins in alloxan induced diabetic rats. The outcome, we believe, might provide important information about its usefulness as a therapeutic agent for the treatment of diabetes and related complications.

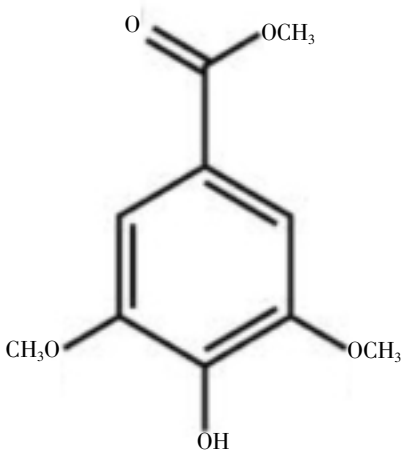

Figure 1. Structure of syringic acid.

2. Materials and methods

2.1. Animals

Male Albino Wistar rats weighing around 180-200 g were procured from the Adhiparasakthi College of Arts and Science, Kalavai, Tamilnadu and were housed under standard vivarium conditions (12 h light and dark cycle, relative humidity 55%). The rats were given normal pellet diets (Hindustan Lever Ltd, Bangalore, India) and water ad libitum. The experimental design was performed in accordance with the current ethical norms approved by the Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines (No.1282/ac/09/CPCSEA) for the investigation of experimental pain in conscious animals.

2.2. Chemicals

All the chemicals used in this experiment were obtained from Sigma Chemical Company (St Louis, MO, USA), Hi Media (Mumbai, India), and SD-Fine Chemicals (Mumbai, India). All chemicals used were of analytical grade.

2.3. Induction of diabetes

Preparation of alloxan-induced diabetic rats Diabetes was induced in rats that had been fasted for 12 h by intraperitoneal injection of 150 mg/kg body weight (bw) of alloxan, freshly dissolved in sterile normal saline immediately before use to give a concentration of 30 g/L. The diabetic state was assessed by measuring non-fasting serum glucose concentration 72 h after alloxan treatment. The rats with a serum glucose level above 300 mg/dL, as well as with polyuria, polydipsia, and polyphagia were selected for the experiment.

2.4. Experimental design

The animals were randomly divided into four groups of six animals in each group (12 diabetic surviving and 12 normal). Syringic acid was dissolved in vehicle solution of of 0.9% saline and syringic acid (50 mg/kg b.w) were administered orally using an intragastric tube for a period of 30 d.

Group I: Normal control (vehicle treated; saline: 1 mL/kg b.w)

Group II: Normal + syringic acid (50 mg/kg b.w)

Group III: Diabetic control

Group IV: Diabetic + syringic acid (50 mg/kg b.w)

After 30 d of treatment, the animals were deprived of food overnight, anaesthetized and sacrificed by cervical decapitation. Blood sample was collected in a tube containing potassium oxalate and sodium fluoride (3:1) for the estimation of glucose, insulin and glycoproteins. Liver and kidney were dissected out, washed in ice-cold saline, patted dry and weighed.

2.5. Biochemical assays

2.5.1. Determination of plasma glucose, insulin and C-peptide

Measurements of plasma glucose and insulin were estimated colorimetrically using commercial diagnostic kits (Sigma Diagnostics (I) Pvt Ltd, Baroda, India). Plasma insulin was assayed by ELISA using a Boehringer-Mannheim kit with an ES300 Boehringer analyzer (Mannheim, Germany). Both the analyses were done according to the

manufacturer's instructions. Connecting peptide (C-peptide) was determined using Rat C-Peptide RIA Kit from LINCO Research, Missouri, USA. The Millipore Rat C-Peptide assay utilized 125I-labelled Rat C-Peptide antiserum to determine the level of C-peptide in plasma by the double antibody technique.

2.5.2. Extraction of glycoproteins

To 0.1 mL of plasma, 5.0 mL of methanol was added, mixed well and centrifuged for 10 min at 3 000×g. The supernatant was decanted and the precipitate was again washed with 5.0 mL of 95% ethanol, recentrifuged and the supernatant was decanted to obtain the precipitate of glycoproteins. This was used for the estimation of hexose and hexosamine. For extraction of glycoproteins from the tissues, a known weight of the tissue was homogenized in 7.0 mL of methanol. The contents were filtered and homogenized with 14.0 mL of chloroform. This was filtered and the residue was successively homogenized in chloroform-methanol (2:1 v/v) and each time the extract was filtered. The residue (defatted tissues) was obtained and the filtrate decanted. A weighed amount of defatted tissue was suspended in 3.0 mL of 2 N HCl and heated at 90 ℃ for 4 h. The sample was cooled and neutralized with 3.0 mL of 2 N NaOH. Aliquots from this were used for estimation of fucose, hexose, hexosamine and sialic acid.

2.5.3. Determination of glycoproteins

The plasma and tissue hexose content was estimated by the method of Niebes[10], sialic acid in plasma and tissues were estimated by the method of Warren[11] and hexosamine by the method of Wagner[12]. Fucose was estimated by the method of Dische and Shettles[13], respectively.

2.6. Statistical analysis

Data presented as means± SD and subjected to statistical significance were evaluated by one way analysis of variance (ANOVA) using SPSS Version 13.0 (SPSS, Cary, NC, USA) and the individual comparisons were obtained by Duncan's[14] multiple range test (DMRT). Values were considered statistically significant when $P<0.05$.

3. Results

3.1. Effect of syringic acid on the levels of plasma glucose and insulin

Figure 2a and 2b shows the level of plasma glucose and insulin in control and experimental diabetic animals. There was a significant ($P<0.05$) elevation in plasma glucose level with significant ($P<0.05$) decrease in plasma insulin levels in alloxan diabetic rats, compared with normal rats. Administration of syringic acid tended to bring blood glucose and plasma insulin towards near normal levels. The plasma glucose and insulin levels of normal rats were not altered when administered with syringic acid (50 mg/kg b.w).

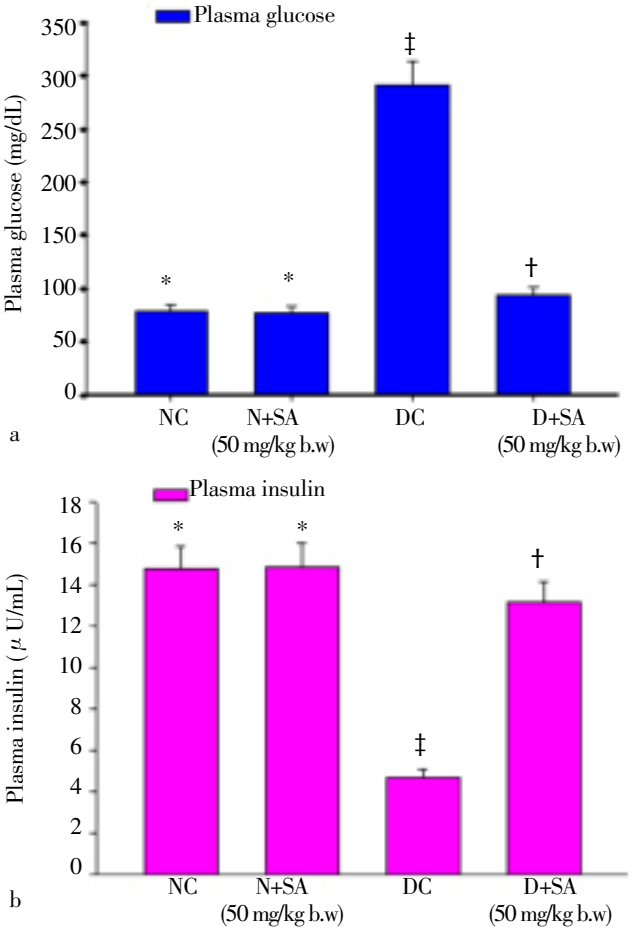

Figure 2. Each value is mean± S.D. for 6 rats in each group. In each bar, means with different superscript symbol (* – †) differ significantly at $P<0.05$ (DMRT). NC: normal control, DC: diabetic control, SA: syringic acid.

3.2. Effect of syringic acid on the levels of plasma glycoproteins and C-peptide

Table 1 shows the changes in the level of plasma glycoproteins and C-peptide of control and experimental

Table 1.
Effect of syringic acid on plasma glycoprotein and C-peptide levels in normal and experimental rats.

Groups	Hexose (mg/dL)	Hexosamine (mg/dL)	Fucose (mg/dL)	Sialic acid (mg/dL)	C-peptide (pmol/L)
Normal	90.30 ± 6.88*	72.17 ± 5.50*	29.91 ± 2.28*	55.39 ± 4.22*	235.30 ± 17.92*
Normal + syringic acid (50 mg/kg)	87.97 ± 6.73*	71.36 ± 5.46*	27.35 ± 2.09*	54.31 ± 4.16*	239.03 ± 18.30*
Diabetic control	144.34 ± 11.05†	99.08 ± 7.58†	44.71 ± 3.42†	75.33 ± 5.77†	65.29 ± 4.97†
Diabetic + syringic acid (50 mg/kg)	103.33 ± 7.91‡	82.85 ± 6.34‡	34.29 ± 2.62‡	61.45 ± 4.70‡	198.47 ± 15.19‡

Values are given as means± S.D. for six rats in each group. Values in a column not sharing a common superscript symbol (* –‡) differ significantly at $P < 0.05$. Duncan's Multiple Range Test (DMRT).

Table 2.
Effect of syringic acid on liver glycoprotein levels in normal and experimental rats (mg/g defatted tissue).

Groups	Hexose	Hexosamine	Fucose	Sialic acid
Normal	26.18 ± 1.99*	11.25 ± 0.86*	15.36 ± 1.17*	9.47 ± 0.72*
Normal + syringic acid (50 mg/kg)	25.34 ± 1.94*	10.79 ± 0.83*	16.50 ± 1.26*	9.92 ± 0.76*
Diabetic control	46.51 ± 3.56†	20.35 ± 1.56†	27.36 ± 2.85†	4.48 ± 0.34†
Diabetic + syringic acid (50 mg/kg)	32.56 ± 2.49‡	15.23 ± 1.17‡	19.36 ± 1.48‡	7.02 ± 0.54‡

Values are given as means± S.D. for six rats in each group. Values in a column not sharing a common superscript symbol (* $-^{\ddagger}$) differ significantly at $P<0.05$. Duncan′s Multiple Range Test (DMRT).

Table 3.
Effect of syringic acid on kidney glycoprotein levels in normal and experimental rats (mg/g defatted tissue).

Groups	Hexose	Hexosamine	Fucose	Sialic acid
Normal	24.16 ± 1.84*	16.85 ± 1.28*	13.67 ± 1.04*	9.03 ± 0.69*
Normal + syringic acid (50 mg/kg)	22.99 ± 1.76*	15.29 ± 1.17*	14.29 ± 1.09*	9.28 ± 0.71*
Diabetic control	43.71 ± 3.35†	33.28 ± 2.55†	30.60 ± 2.34†	5.26 ± 0.40†
Diabetic + syringic acid (50 mg/kg)	29.32 ± 2.24‡	22.25 ± 2.00‡	18.89 ± 1.45‡	7.02 ± 0.54‡

Values are given as means± S.D. for six rats in each group. Values in a column not sharing a common superscript symbol (* $-^{\ddagger}$) differ significantly at $P<0.05$. Duncan′s Multiple Range Test (DMRT).

rats. There was a significant ($P<0.05$) increase of plasma glycoproteins with significant ($P<0.05$) decrease in plasma C-peptide levels in diabetic rats when compared to normal control rats. Oral administration of syringic acid to diabetic rats resulted in significant ($P<0.05$) reduction of glycoproteins and significant ($P<0.05$) increase of C-peptide in the plasma when compared to diabetic untreated rats.

3.3. Effect of syringic acid on the levels of tissue glycoproteins

The levels of liver and kidney glycoprotein of control and experimental rats were shown in Tables 2 and 3. The level of hexose, hexosamine and fucose were significantly ($P<0.05$) increased whereas the level of sialic acid was significantly ($P<0.05$) decreased and those levels were brought back to near normal by treatment with syringic acid.

4. Discussion

Alloxan is the most commonly employed agent for the induction of diabetes in experimental animal model. There is increasing evidence that alloxan causes diabetes by rapid depletion of β-cells by DNA alkylation and accumulation of cytotoxic free radicals that is suggested to result from initial islet inflammation, followed by infiltration of activated macrophages and lymphocyte in the inflammatory focus. It leads to a reduction in plasma insulin concentration leading to a stable hyperglycemia state[15]. In this study also significant hyperglycemia was achieved after alloxan injection. The optimum dose of syringic acid was fixed by administering graded doses of syringic acid to diabetic rats for different time periods and the minimum effective dose for 30 d which caused maximum hypoglycemic activity and non-toxic nature was chosen (50 mg/kg b.w) for the present study (data not shown). The antidiabetic effect of syringic acid may be due to the increased release of insulin from the existing β-cells and/or regenerated β-cells of pancreas, restored insulin sensitivity or inhibition of intestinal absorption of glucose or enhanced the utilization of glucose by peripheral tissues. These results are in agreement with pari and Rajarajeswari[16]who reported that administration of coumarin, a phenolic compound to diabetic rats significantly decreased the glucose level to near normal through enhanced release of insulin from the existing β-cells. Since, protective role of syringic acid on glycoprotein components in diabetes remain to be investigated. In the present work, we demonstrated that syringic acid attenuated the elevation of glycoprotein components in normal and diabetic rats.

C-peptide, a cleavage product of proinsulin, is secreted by pancreatic β-cells in equimolar amounts along with insulin. C-peptide has been considered an inert by-product of insulin synthesis and has also been of great value in the understanding of the pathophysiology of type 1 and type 2 diabetes mellitus[17]. Therefore, we checked the C-peptide levels since it is secreted in equimolar amounts compared to insulin and have a much longer half life than insulin. Moreover, C-peptide may interact with insulin signal transduction and also be suggested as a new therapeutic implications for long-term complications of diabetes[18]. In the present study, the significant decrease in the plasma glucose levels and significant increase in plasma insulin and more importantly the C-peptide levels of syringic acid treated rats indicate that this compound augmented the conversion of pro-insulin to insulin in addition to the augmented glucose transport and insulin secretion. These results suggested that syringic acid may increase insulin secretion of pancreatic β-cells and normalize the plasma glucose level.

Diabetes mellitus is often associated with the development of vascular degenerative complications affecting both large vessels and the microvasculature. Several studies have emphasized the multiplicity of disturbances affecting the metabolism of carbohydrates, proteins and lipids in diabetes[19]. Glycation is a nonenzymatic reaction of glucose and other saccharide derivatives with proteins, nucleotides and lipids. Glycation is considered to be one of the key mechanisms responsible for the long-term consequences of diabetes. Glycation occurs inside and outside of cells;

Glycation of cellular proteins produces changes in structure and loss of enzymatic activity. The elevated levels of plasma glycoproteins in diabetic rats could be a consequence of abnormal carbohydrate metabolism. Insulin deficiency and high levels of plasma glucose in diabetic condition may result in an increased synthesis of glycoproteins[20]. The increase in plasma glycoprotein components has been associated with the severity of diabetes. The elevation in the levels of plasma glycoprotein components might be due to secretion from cell membrane glycoconjugates into the circulation[21]. In this study, we have observed the increased levels of hexose, hexosamine, fucose and sialic acid in the plasma of alloxan induced diabetic rats. Administration of syringic acid ameliorates the levels of plasma glycoproteins near to normal. Our results are in harmony with Sundaram et al[22]who reported that iridoid glucoside, improved plasma glycoprotein components in experimantal diabetic rats.

Liver is one of the chief storage organs for glucose reserve in the body and plays a crucial role in maintenance of blood glucose homeostasis, because it consents to amass the superfluous blood glucose and to demobilize it in hypoglycemic states. Chronic hyperglycemia impedes normal inhibition of hepatic glucose production persuaded by an acute escalation of blood glucose level[23]. Liver is primarily responsible for producing a large amount of glycoproteins present in blood. Diabetic nephropathy is one of the major microvascular complications and is a leading cause of end-stage renal disease, is characterized by the protuberance of the glomerular mesangium due to amassing of extracellular matrix proteins synthesized by the mesangial cells with basement membrane thickening, glomerular and tubular hypertrophy, glomerulosclerosis and tubulointerstitial fibrosis[24]. The pathogenesis of diabetic nephropathy is multifactorial in which chronic hyperglycemia plays a crucial role[25]. The excess availability of glucose in the hyperglycemic state accelerates the synthesis of basement membrane components, (glycoproteins) and this leads to the thickening of capillary basement membrane[26]. The luminal surface of epithelial cells in kidney tubules is also lined with a thick carbohydrate rich glycoprotein layer. Stimulation of kidney protein synthesis may contribute to explain the increase in the synthesis of glycoproteins as well as the renal hypertrophy that occurs early in diabetes[27]. The biochemical markers, hexose, hexosamine, fucose and sialic acid have been measured in the liver and kidney because liver is responsible for synthesis of all major proteins, which are then secreted into the blood.

Hexosamine a nitrogenous sugar in which an amino group replaces a hydroxyl group. It is an excellent precursor for use in the investigation of synthesis of plasma and tissue glycoproteins. The level of hexosamine, increased significantly in the plasma and tissues of diabetic rats, which may be due to insulin deficiency, this leads to depressed utilization of glucose by insulin-dependent pathway, there by enhancing the formation of hexose and hexosamine[28]. In diabetic rats treated with syringic acid significantly lowered hexosamine, which might be due to increased secretion of insulin. Our results are find in line with the study of reduced hexosamine by improved secretion of insulin in tetrahydrocurcumin and chlorogenic acid treated diabetic rats[29].

Fucose is member of a group of essential sugars that the body requires for functioning of cell to cell communication and its metabolism appear to be altered in various disease conditions such as diabetes mellitus[30]. A rise in fucose levels could be due to increased glycosylation in the diabetic state. In diabetes, three proteins (haptoglobin, a1 acid glycoprotein and a1-antitrypsin) synthesized in the liver are mainly responsible for the increase in fucose levels. The metabolism and synthesis of these proteins may be altered in diabetes leading to changes in plasma in the hyperglycemic state accelerates the synthesis of glycoproteins. Due to increased glycosylation in the diabetic state the fucose levels could be increased. Treatment with syringic acid had restored fucose level to near normal, which could be due to improved glycemic status in plasma and tissues. These results are agreement with pari and Rajarajeswari[7] who reported that coumarin a phenolic compound improves fucose level in diabetic rats.

Sialic acid is an acute phase protein derived from neuraminic acid which forms terminal sugar of carbohydrates taking part in the glycoprotein structure[31]. Sialic acids play a role in cell-cell recognition, protein targeting, protease resistance, conformational stabilization, adhesion, and intracellular signaling events in biological systems. In diabetes mellitus, the tissue concentration of sialic acid was found to decrease significantly. The decrease in the content of sialic acid in tissues may be due to the utilization for the synthesis of fibronectin, which contains sialic acid residues in the core structure. The synthesis of fibronectin was also reported to increase significantly in various tissues of diabetic patients and rats. In our study, a significant increase in total sialic acid levels in the plasma was observed when compared with the control group. Various factors might cause an elevation in the concentration of plasma sialic acid. Among these factors, the first is an increase in the synthesis of sialic acid in insulin-independent tissues, such as the liver and the brain, and the second is an increase in the activity of sialytransferase, which transfers the sialic acid residues to the glycolipids and glycoproteins[32]. In our study, administration of syringic acid increased the content of sialic acid in the tissues and decreased the sialic acid level in the plasma by enhancing the secretion insulin level. Insulin has been found to inhibit cytokine-induced acute phase protein synthesis in rat hepatic cell. This may, consequently, account for the decreased plasma sialic acid levels in the alloxan induced diabetic rats. Previous studies show that diosmin, produce the same effect in experimental diabetic rats[21].

From the above findings, we conclude that syringic acid decreased hyperglycemic state in alloxan induced diabetic rats might have been responsible for the decrease of glycoproteins in plasma, liver and kidney. Syringic acid may have beneficial effects in diabetes mellitus by the enhancement of insulin action and C-peptide, as evident by the decreased level of plasma glucose in diabetic rats. The observed effect of syringic acid on reversing the adverse effects of hyperglycemia provides an insight into the pathogenesis of diabetic complications and may be used to advantage in therapeutic approaches.

Conflict of interest statement

The authors of this article do not have any conflict of interest to disclose. No part of the manuscript has been submitted or is under consideration in any other publication.

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