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Protective effect of *Gymnema sylvestre* L. against advanced glycation end-product, sorbitol accumulation and aldose reductase activity in Homoeopathic Formulation

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ABSTRACT

Background: Oxidative stress ensues due to the imbalance between the production and elimination of reactive oxygen species. Chronic hyperglycemia along with oxidative stress plays major role in aggravation of chronic disorders such as diabetes and its related complications.

Objective: This study was designed to evaluate the protective effect of *Gymnema sylvestre* L. against oxidative stress.

Materials and Methods: Potencies of *G. Sylvestre* were procured from Dr. Willmar Schwabe India Pvt. Ltd. *In vitro* antioxidative potential of *G. sylvestre* was evaluated by employing various *in vitro* antioxidant methods.

Results: The total phenol content was found to be 2124, 998 and 546 mg/g Gallic Acid Equivalents in Mother tincture, 6C and 30C of *G. sylvestre* and total antioxidant capacity was found to be 2940,802 and 559 µM/g ascorbic acid equivalents respectively. Mother tincture, 6C and 30C of *G. sylvestre* were found to have strong reducing power, 2,2-diphenyl-1-picrylhydrazyl radical, hydrogen peroxide, nitric oxide and superoxide radical scavenging activity. Percentage inhibition of advanced glycation end-products formation by Mother tincture, 6C and 30C of *G. sylvestre* (10-50 µl) was found to be 38.66 to 95.80%, 30.93 to 81.48% and 31.34 to 60.92% respectively. Mother tincture, 6C and 30C of *G. sylvestre* showed an inhibitory effect against sorbitol accumulation with inhibitory concentration (IC_{50}) value 27.55 µl, 197.96 µl and 1.009 ml respectively whereas in aldose reductase inhibition assay, the IC_{50} value of 28.10 µl, 159.71 µl and 0.82 ml respectively.

Conclusion: These results suggested that Homoeopathic preparations of *G. Sylvestre* had potent antioxidant and antiglycation activity.

Keywords: Advanced glycation end-products, Aldose reductase inhibition, Diabetic complications, *G. sylvestre,* Homoeopathic preparations, Oxidative stress, Sorbitol accumulation

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INTRODUCTION

Overproduction of reactive oxygen species (ROS) have been entailed as the causative factor for a variety of pathological effects *viz.,* DNA damage, cellular degeneration inducing various diseases such as cancer, diabetes and its complications, atherosclerosis and aging.[1] Various synthetic and natural antioxidants are being used for attenuating the production of ROS. Toxicity and carcinogenesis caused by synthetic antioxidants are the leading cause for their declining use. Therefore, it is necessary to evolve natural antioxidants to produce effective and non-toxic amelioration of ROS.[2] *G. sylvestre* commonly known as Gurmar, Gurmabooti, is a traditional medicinal plant, with reported use as a remedy for diabetes mellitus, stomachic and diuretic problems. The plant extracts are also used in folk, Ayurvedic and Homoeopathic systems of medicine.[3] *G. sylvestre* has been proved to treat diabetes as it showed tendency to reduce serum glucose concentrations and also improves glucose tolerance.[4,5] Besides antidiabetic potential, *G. sylvestre* has the ability to lower triglyceride and total cholesterol in serum and its anti-atherosclerotic potential were almost similar to that of a standard lipid lowering agent-clofibrate.[6] In Homoeopathy, *G. sylvestre* in different potencies is used to abolish taste of bitter things, sense of altered taste and snake bite.[7]

In this study *in vitro* antioxidative potential of Mother tincture, 6C and 30C potencies of *G. sylvestre* were evaluated by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging assay, nitric oxide (NO) scavenging assay, hydrogen peroxide (H_2O_2) scavenging assay, reducing power, superoxide radical scavenging activity, total phenolic content by Folin-Ciocalteau method and total antioxidant activity by phosphomolybdenum method.

MATERIALS AND METHODS

Materials and Reagents

Potencies (Mother tincture, 6C and 30C) of *G. sylvestre* L. were procured from Dr. Willmar Schwabe India Pvt. Ltd. 2,2-diphenyl-1-picrylhydrazyl (DPPH) and nicotinamide adenine dinucleotide phosphate (NADPH) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Bovine serum albumin (BSA), Nitroblue tetrazolium (NBT), Folin-Ciocalteu reagent and *N*-(1-Naphthyl) ethylenediamide dihydrochloride were purchased from Molychem Pvt. Ltd., India. All other chemicals and reagents used were of analytical grade.

In Vitro Antioxidant Activity

Determination of total phenol content

Total phenolic content in the Mother tincture, 6C and 30C of *G. sylvestre* were determined with Folin-Ciocalteu reagent using gallic acid as a standard phenolic compound. The sample was diluted appropriately to obtain absorbance in the range of calibration curve. An aliquot of 1 ml of sample solution was mixed with 1 ml of Folin–Ciocalteu reagent. Three min later 3.0 ml of 2% sodium carbonate was added and the mixture was allowed to stand for 3 h with intermittent shaking. The absorbance of the blue color that developed was measured at 760 nm. The concentration of total phenolic compounds in the sample was obtained as mg of gallic acid equivalent (GAE) per gram dry weight.[8]

Total Antioxidant Capacity

An aliquot of 0.3 ml of Mother tincture, 6C and 30C of *G. sylvestre* were mixed with 3 ml of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). In case of blank 0.3 ml of water was used instead of sample. The tubes were capped with aluminium foil and incubated in boiling water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance was measured at 695 nm against a blank. Ascorbic acid was used as a standard. Total antioxidant capacity (TAOC) was expressed as equivalents of ascorbic acid (μmol/g).[9]

2,2-Diphenyl-1-Picrylhydrazyl Scavenging Activity

The DPPH radical scavenging ability of Mother tincture, 6C and 30C of *G. sylvestre* was evaluated according to the method given in literature with slight modification.[10] The different concentrations in each reaction set, were mixed with 1.0 ml of 0.1 mM of DPPH in ethanol. The mixture was incubated in the dark for 30 min at room temperature. Degree of inhibition of DPPH by monitoring the decrease in absorbance measured at 517 nm. Ascorbic acid was used as positive control. Radical scavenging activity was expressed as inhibition percentage of free radical by the sample and was calculated using the following formula:

$$\%\,\text{Inhibition} = \frac{A_0 - A_t}{A_0} \times 100$$

Where A_0 was the absorbance of control (blank without sample) and A_t was the absorbance in the presence of sample. All the tests were performed in triplicate and the graph was plotted with mean values.

Hydrogen Peroxide Scavenging Activity

H_2O_2 scavenging activity was evaluated as described previously.[11] An aliquot of 40 mM H_2O_2 solution (0.6 ml) was mixed with various concentrations of Mother tincture, 6C and 30C of *G. sylvestre*. To the mixture 2.4 ml of phosphate buffer (0.1 M, pH 7.4) was added and the mixture was shaken vigorously and incubated at room temperature for 10 min. Then, the absorbance of the reaction mixture was determined at 230 nm. Ascorbic acid was used as positive control. The H_2O_2 scavenging activity was calculated as follows:

$$\%\,\text{Inhibition} = 1 - \left(\frac{A_1 - A_2}{A_0} \right) \times 100$$

Where A_0 is the absorbance of the control (water instead of sample), A_1 is the absorbance of the sample and A_2 is the absorbance of the sample only (phosphate buffer instead of H_2O_2 solution). The inhibitory concentration 50% (IC_{50}) value represented the concentration of the compounds that caused 50% inhibition of H_2O_2.

Reducing Power Assay

The Fe^{3+}-reducing power of Mother tincture, 6C and 30C of *G. sylvestre* was determined according to the method described in literature.[12] Different concentrations of samples (2.5 ml) were mixed with 2.5 ml of 0.2M sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide and incubated at 50°C for 20 min. After incubation, 2.5 ml of 10% trichloroacetic acid (*w/v*) was added and the mixture centrifuged at 1000 rpm for 8 min. The supernatant (5 ml) was mixed with 5 ml of distilled water and 1 ml of 0.1% of ferric chloride, and the absorbance was measured spectrophotometrically at 700 nm. The assay was carried out in triplicate and the results expressed as mean values ± standard deviations. Ascorbic acid was used as positive control. The sample concentration providing 0.5 of absorbance (EC_{50}) was calculated from the graph plotted between absorbance at 700 nm against sample concentration.

Nitric Oxide Scavenging Activity

At physiological pH, an aqueous solution of sodium nitroprusside spontaneously generates NO[13] which interacts with oxygen to produce nitric ions that can be estimated using Griess reagent. Scavengers of NO compete with oxygen leading to reduce the production of NO. The reaction mixture of 5mM Sodium nitroprusside in phosphate buffer saline (PBS) and 3.0 ml of different concentrations of the Mother tincture, 6C and 30C of *G. sylvestre* was incubated at 25°C for 150 min. After incubation, the samples were added to Griess reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% napthylethylenediamine dihydrochloride). The pink chromophore generated during the diazotization of nitrite with sulfanilamide and subsequent coupling with napthyl-ethylenediamine was measured at 546 nm. Ascorbic acid was used as positive control. The percentage of inhibition was measured by the following formula:

$$\%\,\text{Inhibition} = \frac{A_0 - A_t}{A_0} \times 100$$

Where A_0 was the absorbance of the control (blank, without sample) and A_t was the absorbance in the presence of the sample. All the tests were performed in triplicate and the graph was plotted with the mean values.

Superoxide Radical Scavenging Activity

The activity was measured by the reduction of NBT (NBT reagent) method as described by Shukla *et al*., 2009.[14] The method is based on the generation of superoxide radical (O^{2-}) by auto-oxidation of hydroxylamine hydrochloride in the presence of NBT, which gets reduced to nitrite. Nitrite in the presence of ethylene diamine tetra acetic acid (EDTA) gives a color that was measured at 560 nm. Different concentrations of Mother tincture, 6C and 30C of *G. sylvestre* were taken in a test tube. To this, a reaction mixture consisting of 1 ml of (50 mM) sodium carbonate, 0.4 ml of (24 mM) NBT and 0.2 ml of 0.1 mM EDTA solutions were added to the test tube and immediate reading were taken at 560 nm. After incubating the reaction mixture at 25°C for 15 min, about 0.4 ml of (1 mM) of hydroxylamine hydrochloride was added to initiate the reaction and reduction of NBT was measured at 560 nm. Ascorbic acid was used as the positive control. Decreased absorbance of the reaction mixture indicates increased superoxide anion scavenging activity. The

percentage of inhibition was calculated according to the following equation:

$$\%\text{Inhibition} = \frac{A_0 - A_t}{A_0} \times 100$$

Where A_0 was the absorbance of the control (blank, without sample) and A_t was the absorbance in the presence of the samples. All the tests were performed in triplicate and the graph was plotted with the mean values.

Anti-glycation Activity

In vitro antiglycation activity of Mother tincture, 6C and 30C of *G. sylvestre* was examined by testing their ability to inhibit the fluorescence of BSA in accordance with a previous method.[15] The reaction mixture of BSA (10 mg/ml), 1.1 M fructose in 0.1 M PBS, pH 7.4 containing 0.02% sodium azide with or without sample (Mother tincture, 6C and 30C of *G. sylvestre* dissolved in PBS) was incubated in darkness at 37°C for 1, 2, 3, and 4 weeks. Advanced glycation end-product (AGE) formation was measured by fluorescent intensity at an excitation wavelength 355 nm and emission wavelength 460 nm using Elico-SLI74 Spectrofluorometer fitted with Xenon Lamp (Elico, India, Pvt Ltd.). Aminoguanidine (AG; 500 μg/ml) was used as a positive control for this study.

Erythrocyte Sorbitol Accumulation Inhibition

Five-mililitre ml of Heparinized human blood was collected from overnight fasted healthy male volunteer and erythrocytes were separated from the plasma by centrifugation at 3000 g for 30 min. The cells were washed 3 times with isotonic saline at 4°C and in the final washing, the cells were centrifuged at 1500 g for 15 min to obtain a consistently packed cell preparation. The packed cells (1 mL) were then incubated in Krebs-Ringer bicarbonate buffer (pH 7.4) (4 mL) containing 55 mM glucose in the presence or absence of samples (Mother tincture, 6C and 30C of *G. sylvestre*) at 37°C for 3 h. The erythrocytes were washed with cold saline by centrifugation at 2000 g for 5 min, precipitated by adding 6% of cold perchloric acid (3 mL), and centrifuged again at 2000 g for 10 min. The supernatant was neutralized with 2.5 M K_2CO_3 at 4°C and used for sorbitol determination.[16] The relative fluorescence due to NADH was measured by a fluorescence spectrometer (Elico-SLI74 Spectrofluorometer fitted with Xenon Lamp, Elico, India) at an excitation wavelength of 366 nm and an emission wavelength of 452 nm. The experiments were performed in triplicates.

Aldose Reductase Enzyme Inhibition

Partial purification of Aldose reductase 1 (ALR1) from rat kidney (IAEC Protocol No.: MMCP/IAEC/13/07) was carried out following the previously described methods.[17] Isolated kidney was homogenized in 3 volumes of 10 mM sodium phosphate buffer, pH 7.2 containing 0.25 M sucrose, 2.0 mM EDTA, and 2.5 mM 2-mercaptoethanol. The homogenate was centrifuged at 10,000 g for 20 min and the supernatant was subjected to ammonium sulfate precipitation. Precipitate obtained between 45% and 75% saturation was dissolved in the above buffer. The supernatant was used as the source of ALR1. The activity of ALR1 was measured spectrophotometrically by monitoring the oxidation of NADPH at 340 nm as a function of time at 37°C using glyceraldehyde as substrate. The assay mixture in 1 ml contained 50 mM sodium phosphate buffer of pH 7.2, 0.2 M ammonium sulfate, 10 mM DL-glyceraldehyde, 5 mM β-mercaptoethanol and 0.1 mM NADPH. Various concentrations of Mother tincture, 6C and 30C of *G. sylvestre* were added to assay mixtures of ALR1 and incubated for 5 min before initiating the reaction by NADPH as described above. The percentage inhibition was calculated considering the activity in the absence of Mother tincture, 6C and 30C of *G. sylvestre* as 100%. The IC_{50} values were determined by linear regression analysis of the plot of percent inhibition versus inhibitor concentration.

RESULTS AND DISCUSSION

Total Phenol Content

Phenols are very important plant constituents because of their scavenging ability owing to their hydroxyl groups.[18] The phenolic compounds may contribute directly to antioxidative action. It is known that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans when ingested up to 1 g daily from a diet rich in fruits and vegetables.[19] Natural antioxidants may be useful in preventing deleterious effects of oxidative stress. Results obtained in this study revealed a considerable level of phenols in the Mother tincture, 6C and 30C of *G. sylvestre*. Total phenol content present in Mother tincture, 6C and 30C of *G. sylvestre* was found to be 2124, 998 and 546 mg/g GAE respectively.

Total Antioxidant Capacity

TAOC reflects the total antioxidant defence system. The phosphomolybdenum method used in this study involves the reduction of molybdenum VI (Mo^{6+})

to a green Mo^{5+} complex in acidic medium. TAOC of Mother tincture, 6C and 30C of *G. sylvestre* was found to be 2940, 802 and 559 μM/g ascorbic acid equivalent respectively.

2,2-Diphenyl-1-Picrylhydrazyl Scavenging Activity

DPPH free radical scavenging method has been widely adopted as a tool for estimating the free radical scavenging activities of lipophilic radicals. A chain of lipophilic radicals is initiated by the lipid auto-oxidation.[20] Radical scavenging activity is very important due to the deleterious role of free radicals in foods and in biological systems. Diverse methods are currently used to assess the antioxidant activity of plant phenolic compounds. Chemical assays are based on their ability to scavenge synthetic free radicals, using a variety of radical-generating systems and methods for detection of the oxidation end-point. DPPH radical-scavenging method is a common Spectrophotometric procedure for determining the antioxidant capacities of components.[21] The reduction capacity of DPPH was determined by the decrease in absorbance at 517 nm. On the DPPH radical, Mother tincture, 6C and 30C of *G. sylvestre* had a significant effect with increasing concentration from 10-50 μl. The IC_{50} value of Mother tincture, 6C and 30C of *G. sylvestre* was 33.88, 43.62 and 46.88 μl respectively [Figure 1] and that of ascorbic acid was found to be 1.43 μg/ml. A higher DPPH radical scavenging activity is associated with lower IC_{50} value.

Hydrogen Peroxide Scavenging Activity

H_2O_2 is generated *in vivo* by several oxidase enzymes. H_2O_2 *via* OH · acts as a messenger molecule in the synthesis and activation of inflammatory mediators.[22] The loss of H_2O_2 can be measured at 230 nm when it is incubated with a scavenger. [Figure 2] show the scavenging ability of Mother tincture, 6C and 30C of *G. sylvestre* on H_2O_2 at different concentrations. The IC_{50} value of Mother tincture, 6C and 30C of *G. sylvestre* was found to be 25.82 μl, 0.2 ml and 0.2 ml respectively. IC_{50} value of ascorbic was found to be 80 μg/ml.

Reducing Power Assay

Reducing power may serve as a significant indicator of potential antioxidant activity. In this method reducing power was determined based on the ability of the sample to reduce ferric ions/ferricyanide complex to ferrous ion complex. The formation of ferrous ion complex was observed by the formation of Perl's Prussian blue color at 700 nm [Figure 3]. EC_{50} (effective concentration at which the absorbance is 0.5) was calculated from the calibration curve and was found to be 39 μl, 0.22 ml and 1.01 ml for Mother tincture, 6C and 30C of *G. sylvestre* respectively and 21.42 μg/ml for ascorbic acid.

Nitric Oxide Scavenging Activity

NO is an essential regulating molecule required for several physiological processes such as vasodilation, nerve transmission and control of blood pressure.[23] Excess generation of NO leads to a chain of reactions *via* formation of peroxynitrite ions that are extremely toxic and can be detrimental to human health.[24] The plant products have the property to counteract the effect of excessive NO generation. The various concentrations of Mother tincture, 6C and 30C of *G. sylvestre* showed significant inhibition against NO radical in a dose dependent manner. The concentration of Mother tincture, 6C and 30C of

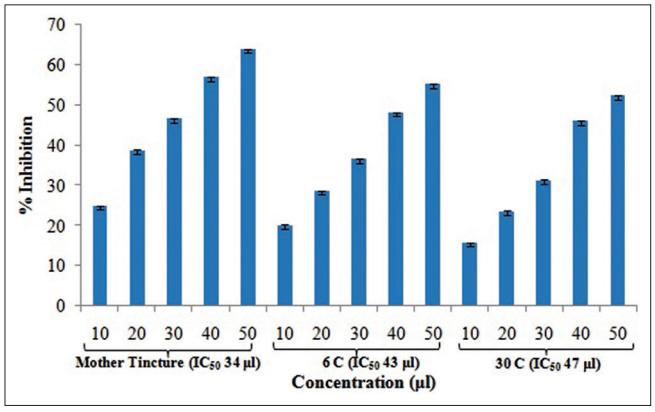

Figure 1: Effect of *Gymnema sylvestre* on 2,2-diphenyl-1-picrylhydrazyl radical-scavenging activities in homoeopathic formulation. Values are mean ± standard deviation for *n* = 3

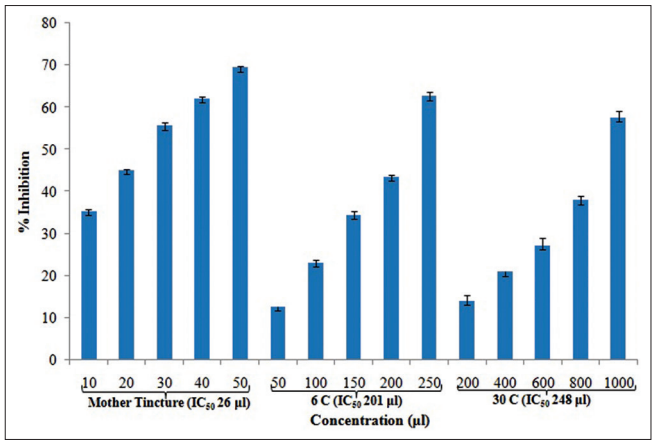

Figure 2: Effect of *Gymnema sylvestre* on hydrogen peroxide radical-scavenging activities in homoeopathic formulation.Values are mean ± standard deviation for *n* = 3

G. sylvestre required for IC_{50} was found to be 24.83 μl, 0.15 ml and 0.82 ml respectively [Figure 4]. IC_{50} of ascorbic acid was found to be 63.55 μg/ml.

Superoxide Scavenging Activity

Superoxide radical is a harmful free radical acting as a precursor of more reactive oxidative species such as oxygen and hydroxyl radicals that contributes to tissue damage and various other pathological conditions.[23] Mother tincture, 6C and 30C of *G. sylvestre* had significant activity against superoxide radicals in a dose dependent manner [Figure 5]. IC_{50} of ascorbic acid was found to be 27.96 μg/ml and that of Mother tincture, 6C and 30C of *G. sylvestre* was found to be 45.50 μl, 0.16 ml and 0.707 ml respectively.

Advanced Glycation End-Products Inhibition Activity

Accumulation of AGEs in the tissues is a significant contributor to diabetic complications such as nephropathy, neuropathy and retinopathy.[25] Scientific studies reveal that the inhibition of AGEs reflects the attenuation of diabetic complications. AGEs by acting on their receptors AGE modify the plasma protein leading to the excessive formation of free radicals.[26] Aminoguanidine inhibits the formation of AGEs by trapping the intermediate Amadori products. In this study, the formation of AGEs was monitored weekly by measuring the fluorescence intensity of the BSA-fructose solutions for 4 weeks. A significant inhibition of AGEs formation (93.37%) was observed in fructose-induced glycated BSA plus aminoguanidine (500 μg/ml). At 4th week of incubation, the percentage inhibitions of AGEs formation by *G. sylvestre* Mother tincture (10-50 μl) was 38.66 to 95.80%, respectively; 6C (10-50 μl) was 30.93 to 81.48% and for 30C (10-50 μl) was found to be 31.34 to 60.92% respectively [Figures 6-8].

Erythrocyte Sorbitol Accumulation Inhibition

In diabetes, activation of sorbitol pathway leads

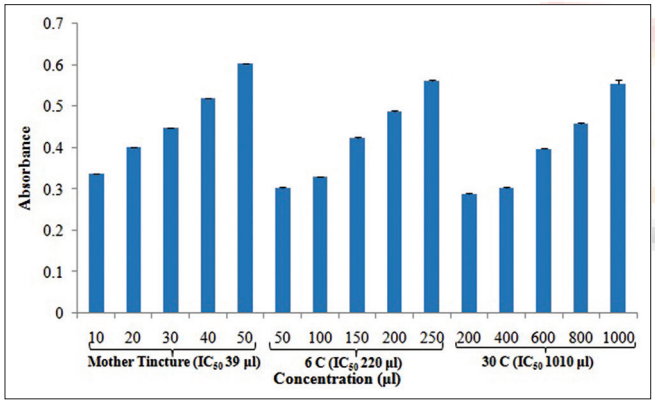

Figure 3: Effect of *Gymnema sylvestre* on reducing power in homoeopathic formulation. Values are mean ± standard deviation for *n* = 3

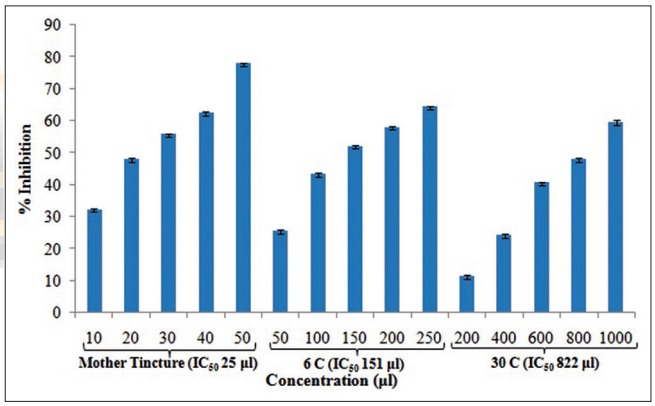

Figure 4: Effect of *Gymnema sylvestre* on nitric oxide radical-scavenging activities in homoeopathic formulation. Values are mean ± standard deviation for *n* = 3

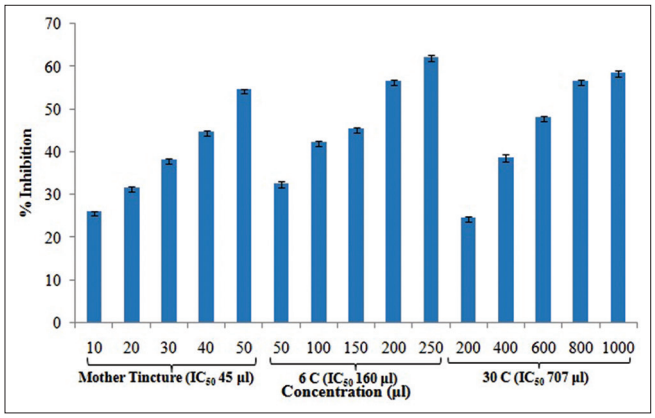

Figure 5: Effect of *Gymnema sylvestre* on superoxide dismutase radical-scavenging activities in homoeopathic formulation. Values are mean ± standard deviation for *n* = 3

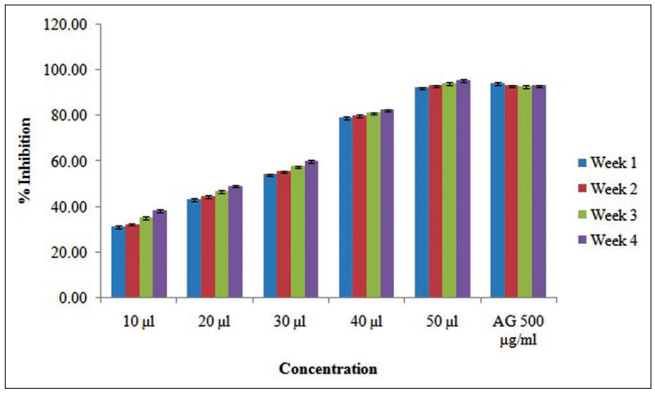

Figure 6: The effects of *Gymnema sylvestre* Mother tincture on the formation of fluorescent advanced glycation end-products in Bovine serum albumin incubated with fructose. Values are mean ± standard deviation for *n* = 3 Aminoguanidine

to accumulation of sorbitol in cells. This leads to cellular injury, formation of ROS, oxidative stress and eventually diabetic complications. Prevention of intracellular sorbitol accumulation would ameliorate the diabetic complications. This study revealed the significant inhibitory effect of Mother tincture, 6C and 30C of *G. sylvestre* on sorbitol accumulation [Figure 9]. IC_{50} of Ascorbic acid was found to be 186.08 μg/ml and that of Mother tincture, 6C and 30C of *G. sylvestre* was found to be 27.55 μl, 197.96 μl and 1.009 ml respectively.

Aldose Reductase Inhibitory Activity

In chronic hyperglycemia, ALR converts excess glucose to sorbitol using NADPH and accumulation of excess sorbitol leads to cellular and organ damage.[26] Agents inhibiting the ALR activity would lead to the attenuation of diabetic complications. ALR1 was partially purified from kidney of Wistar rat and the activity of ALR1was measured spectrophotometrically by monitoring the oxidation of NADPH at 340 nm. IC_{50} of quercetin (standard) was found to be 5.17μg/ml and that of Mother tincture, 6C and 30C of *G. sylvestre* was found to be 28.10 μl, 159.71 μl and 0.82 ml respectively [Figure 10].

CONCLUSION

Results obtained in this study suggested that Mother tincture, 6C and 30C of *G. sylvestre* have a higher amount of phenols, which are known to scavenge free radicals. *G. sylvestre* was also effective in DPPH, H_2O_2, NO and superoxide dismutase scavenging activity. Further, the study reflects the ability of *G. sylvestre* to inhibit the formation of AGEs and sorbitol accumulation. This profound protective effect of *G. sylvestre* may suggest its extensive use as antioxidant and future studies are directed for their *in vivo* evaluation in diabetic complications.

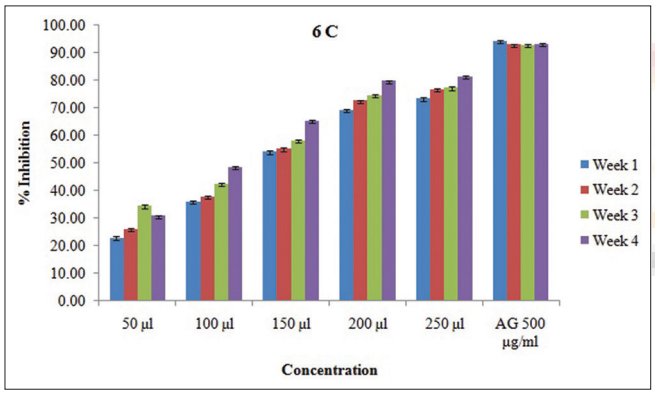

Figure 7: The effects of *Gymnema sylvestre* 6C on the formation of fluorescent advanced glycation end-products in Bovine serum albumin incubated with fructose. Values are mean ± standard deviation for *n* = 3 Aminoguanidine

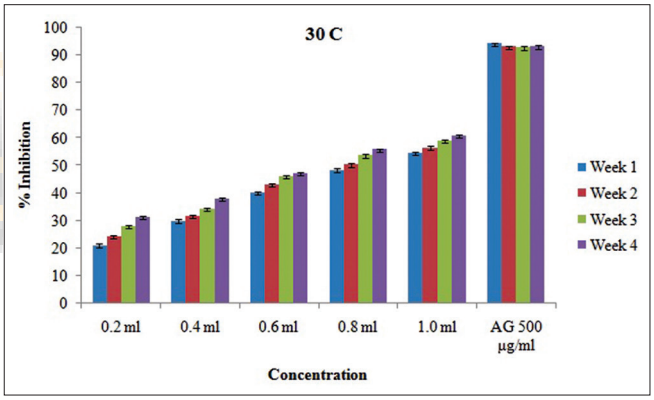

Figure 8: The effects of *Gymnema sylvestre* 30C on the formation of fluorescent advanced glycation end-products in Bovine serum albumin incubated with fructose. Values are mean ± standard deviation for *n* = 3 Aminoguanidine

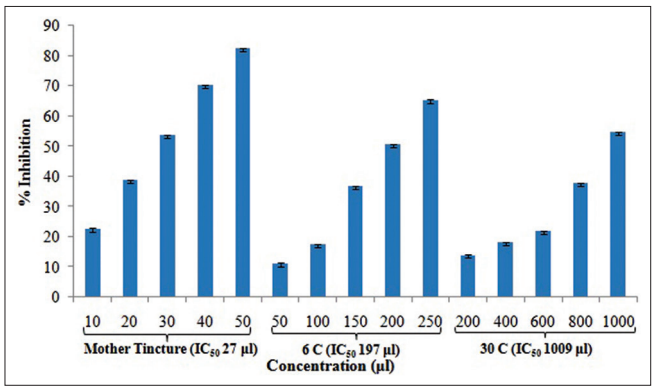

Figure 9: Effect of *Gymnema sylvestre* on erythrocyte sorbitol accumulation inhibition assay in homoeopathic formulation. Values are mean ± standard deviation for *n* = 3

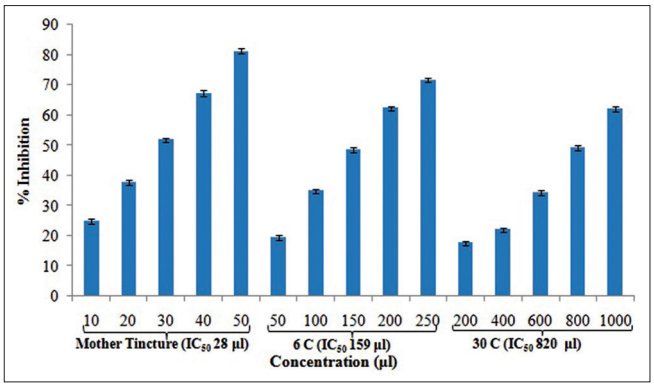

Figure 10: Effect of *Gymnema sylvestre* on aldose reductase inhibitory assay in homoeopathic formulation. Values are mean ± standard deviation for *n* = 3

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Conflicts of Interest

There are no conflicts of interest.

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होम्योपैथिक नुस्खों में एजीई, सार्बिटोल एकत्रीकरण और एल्डोस रिडक्टेस की गतिविधि के प्रतिकूल जिमनेमा सिल्वेस्टर एल. का संरक्षित प्रभाव

सारः

पृष्ठभूमिः

प्रतिक्रियाशील आक्सीजन प्रजातियों के विनिर्माण और निष्कासन के बीच उत्पन्न असन्तुलन के कारण आक्डिेटिव तनाव घटित होता है। पुराने रोगों जैसे– मधुमेह और इससे संबंधित जटिलताओं के उभार में चिरकालीन उच्च रक्तशर्करा के साथ आक्सिडेटिव तनाव एक महत्वपूर्ण भूमिका निभाते हैं।

उद्देश्यः

आक्सिडेटिव तनाव के विरूद्ध जिमनेमा सिल्वेस्टर एल. के संरक्षी प्रभाव के मूल्याकंन के लिये इस अध्ययन की रचना की गई।

सामग्री और विधियाँः

शक्तिकृत जी. सिल्वेस्टर एल डा. विल्मर श्वाबे इंडिया प्राइवेट लिमिटेड से प्राप्त की गयी। कृत्रिम परिवेश में जी. सिल्वेस्टर एल की प्रतिउपचायक क्षमता का मूल्यांकन बहुत सी कृत्रिम परिवेशीय आक्सीकरण रोधी तकनीकों के प्रयोग से किया गया।

परिणामः

सम्पूर्ण फिनॉल 2124,998 और जी. सिल्वेस्टर एल के मूल अर्क, 6सी और 30सी शक्तियों के समतुल्य गैलिक एसिड 546 मि.ग्रा./ग्रा. और समग्र आक्सीकरण रोधी क्षमता 2940802 और एस्कार्बिक एसिड 559 म्यू एम/जी क्रमशः समतुल्य पाया गया। जी. सिल्वेस्टर एल. के मूल अर्क, 6सी और 30सी में शक्तिशाली घटन शक्ति डीपीपीएच रेडिकल, हाइड्रोजन पैराक्साइड, नाइट्रिक आक्साइड और सुपर आक्साइड मौलिक सफाई सक्रियता पायी गयी। जिमनेमा सिल्वेस्टर एल. के मूल अर्क (10–15 मि.ली.), 6सी और 30सी में एजीई निर्माण निषेध का प्रतिशत क्रमशः 38.66 से 95.80 प्रतिशत, 30.93 से 81.48 प्रतिशत और 31.34 से 60.92 प्रतिशत पाया गया। जिमनेमा सिल्वेस्टर एल. के मूल अर्क, 6सी और 30सी ने सार्बिटोल एकत्रीकरण के विरूद्ध संरक्षी प्रभाव दिखाया जो कि क्रमशः आईसी 50 मूल्य 27.55 म्यू एल, 197.96 म्यू एल और 1.009 मि.ली. था। जबकि एल्डोज रिडक्टेज अवरोध जांच में आईसी 50 का मूल्य क्रमशः 28.10 म्यू एल 159.71 म्यू एल और 0.82 मि.ली. था।

निष्कर्षः

इन परिणामों से स्पष्ट होता है कि जी. सिल्वेस्टर एल. के होम्योपैथिक उत्पादों में शक्तिशाली प्रतिउपचायक और प्रतिशर्करा कार्यकलाप हैं।

Efecto protector de *Gymnema sylvestre L*. frente a los AGE, la acumulación de sorbitol y la actividad de la aldosa reductasa en la formulación homeopática

RESUMEN

Fundamento: El estrés oxidativo se produce debido a un desequilibrio entre la producción y la eliminación de especies reactivas de oxígeno. La hiperglucemia crónica junto con el estrés oxidativo desempeña un papel importante en la agravación de trastornos crónicos, como la diabetes y sus complicaciones.

Objetivo: Este estudio se diseñó para evaluar el efecto protector de *G. sylvestre* L. frente al estrés oxidativo.

Material y métodos: Dr. Willmar Schwabe India Pvt. Ltd suministró las potencias de *G. Sylvestre*. El potencial antioxidativo *in vitro* de *G. sylvestre* se evaluó aplicando diferentes métodos antioxidativos *in vitro*.

Resultados: Se constató que, en la tintura madre y las potencias 6C y 30C de *G. sylvestre*, el contenido total en fenoles se situó en 2124, 998 y 546 mg/g de equivalentes de ácido gálico, mientras que la capacidad antioxidativa fue de 2940, 802 y 559 µM/g de equivalentes de ácido ascórbico, respectivamente. Se observó que la tintura madre y las potencias 6C y 30C de *G. sylvestre* poseían una potente capacidad de reducción de los radicales DPPH (2,2-*difenil*-1-picrilhidrazilo), el peróxido de hidrógeno, el óxido nítrico y la actividad de barrido los radicales superóxidos. La inhibición porcentual de la formación de AGE (por sus siglas en inglés *Advanced Glication End-product*) mediante la tintura madre y las potencias 6C y 30C de *G. sylvestre* (10-50 µl) fue del 38,66 al 95,80%, del 30,93 al 81,48 % y del 31,34 al 60,92 %, respectivamente. La tintura madre y las potencias 6Cy 30C de G. sylvestre mostraron un efecto inhibidor frente a la acumulación de sorbitol con un valor de CI50 de 27,55 µl, 197,96 µl y 1,009 ml, respectivamente, mientras que el ensayo de inhibición de la aldosa reductasa dio lugar a un valor CI_{50} de 28,10 µl, 159,71 µl y 0,82 ml, respectivamente.

Conclusiones: Estos resultados indican que los preparados homeopáticos de *G. Sylvestre* poseen una actividad antioxidante y antiglicación potente.