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AMELIORATIVE EFFECT OF HYDROALCOHOLIC FLOWER EXTRACT OF NERIUM OLEANDER EXTRACT ON DIABETES ASSOCIATED COMPLICATIONS

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ABSTRACT

INTRODUCTION:

Diabetes mellitus is a common metabolic disorder resulting from impaired insulin action.It's complications include Diabetic Neuropathy,nephropathy and retinopathy and cardiovascular diseases. Nerium oleander, commonly known as oleander, is a flowering shrub that contains various bioactive compounds.The aim of this study is to investigate the potential benefits of nerium oleander in mitigating the complications associated with diabetes.

MATERIALS AND METHODS:

The flowers of Nerium oleander collected were shade dried and then coarsely powdered. About hundred grams of dried N.oleander flowers were weighed and packed in a Soxhlet extractor with 200 ml of 70% hydro alcohol (70% ethanol and 30% water). Extraction was carried out using a hot extraction procedure for 18-20 hours and filtered. Filtrate was concentrated under gentle heat to give a concentrated material.

RESULTS:

The extract of Nerium oleander inhibited the activity of reductase and prevents the accumulation of sorbitol which contributes to pathological process associated with diabetes.The study's findings indicate that the hydroalcoholic flower extract of Nerium oleander has potential ameliorative effects on diabetes-associated complications, including neuropathy and nephropathy.

CONCLUSION:

The results of the study demonstrated promising findings. The administration of hydroalcoholic flower extract of Nerium oleander showed beneficial effects in attenuating the progression or alleviating the symptoms of diabetes-associated complications.

Keywords: Nerium oleander, diabetes,hydro alcoholic,Inhibitory activity

INTRODUCTION

Diabetes mellitus is a metabolic disorder due to hyperglycemia resulting from defects in insulin secretion or action .Millions of individuals around the world are impacted by this pervasive and growing global health issue. Long-term health issues from diabetes can have negative consequences on quality of life and place a greater burden on healthcare systems.A large proportion of people with diabetes continue to have consequences, including heart disease, neuropathy, nephropathy, and retinopathy, among others, in spite of these efforts.The current management strategies for diabetes typically focus on glycemic control by dietary modifications, oral anti-diabetic drugs, and insulin therapy.(1)

In recent years, there has been a surge of interest in exploring complementary and alternative therapeutic options derived from natural sources.The potential of herbal treatments and plant extracts to treat a variety of ailments, including diabetes and its consequences, has been thoroughly investigated. Nerium oleander, an Apocynaceae plant, is one of these intriguing species.

Nerium oleander, commonly known as oleander, is an evergreen shrub that is native to the Mediterranean region. It is a herb that grows in dry areas and has a variety of colourful blossomsDespite its toxic nature, it has been traditionally used in folk medicine for a wide range of ailments, including diabetes. (2)Various parts of the plant, such as leaves and flowers, contain a diverse array of bioactive compounds, including cardiac glycosides, flavonoids, and terpenoids, which have been attributed to its potential medicinal properties.The pink or white salver-shaped flowers are unscented.This plant's parts are used as a folk remedy for a variety of conditions, including dermatitis, abscesses, eczema,psoriasis, sores, warts,herpes, skin cancer, asthma, dysmenorrhea, epilepsy.(3)The hydroalcoholic floral extract of Nerium oleander is particularly intriguing because it has demonstrated promising pharmacological properties in preclinical tests, such as antioxidant, anti-inflammatory, and cardioprotective actions. These characteristics suggest that Nerium oleander extract may have therapeutic potential for reducing problems related to diabetes.

In conclusion, this research has the potential to provide light on the possible therapeutic advantages of Nerium oleander hydroalcoholic floral extract in issues related to diabetes. The identification of efficient complementary treatments derived from natural sources could provide fresh and insightful information for enhancing the lives of people with diabetes, as diabetes continues to be a huge public health concern.(4)

MATERIALS AND METHODS

Plant material

Fresh flowers of Nerium oleander were collected from Chennai,Tamil Nadu. The N.oleander flowers were then shade dried at ambient temperature. Thereafter the dried flowers were pulverised into a coarse powder and ready for extraction.

Preparation of plant extract

The flowers of Nerium oleander collected were shade dried and then coarsely powdered. About hundred grams of dried N.oleander flowers were weighed and packed in a Soxhlet extractor with 200 ml of 70% hydro alcohol (70% ethanol and 30% water). Extraction was carried out using a hot extraction procedure for 18-20 hours and filtered. Filtrate was concentrated under gentle heat to give a concentrated material. The extracts were concentrated and used for further experiments.

Chemicals and reagents

Aminoguanidine hydrochloride, Metformin and was procured from TCI Chemicals, India. DL-glyceraldehyde, D-glucose, Fructose, lithium sulphate, NADPH, NADP, dimethyl sulphoxide (DMSO), sorbitol, bovine serum albumin, perchloric acid, ammonium sulphate, Tris-HCl, EDTA, sucrose and sorbitol dehydrogenase were purchased from Sigma aldrich (St Louis, MO, USA). All other chemicals of analytical grade were obtained from Himedia, India and SRL chemicals, India.

Advanced Glycation end product (AGE) assay (Harris et al., 2011)

Advanced glycation end products (AGEs) are formed by non-enzymatic glycosylation of proteins that enhance vascular permeability in both micro and macro vascular structures by binding to specific macrophage receptors. The hydroalcoholic extract of Nerium flowers were evaluated for its activity on AGEs formation at different concentrations of 2.5-25µg/ml. AGE reaction mixture was constituted as follows; 1 mg/mL bovine serum albumin in 50mM sodium phosphate buffer (pH 7.4) and 0.02% sodium benzoate into 0.2M fructose and 0.2M glucose. The reaction mixture (2.75mL) was treated with different concentrations of Nerium flowers extract (2.5-25µg/ml). Amino guanidine was used as positive control. After incubating at 37°C for 3 days, the fluorescence intensity of the reaction was determined at excitation and emission wavelengths of 350 nm and 450 nm, respectively, using Biotek synergy multi-mode reader, USA. The percentage activity was calculated with respect to solvent control.

Determination of Aldose Reductase Inhibition (Reddy et al., 2011)

A total of 531µL of 0.1 M potassium buffer (pH 7.0), 90µL of NADPH solution (1.6 mM in potassium buffer), 90µL of recombinant human aldose reductase (AR) (6.5U/mg) (Sigma, USA - SRP6371-100UG), 90µL of

ammonium sulphate solution (4 M in potassium buffer), and 90 µL of DL-glyceraldehyde (25 mM in potassium buffer) were mixed with 9µL of different concentrations of Nerium flower extract (2.5-25µg/ml) in a cuvette, and the activity of AR was assessed spectrophotometrically by measuring the decrease in NADPH absorbance at 340 nm for 3 min using a spectrophotometer (Biotek Synergy H4 multimode reader, USA). Metformin was used as positive control. The inhibition of AR (%) was calculated using the following equation: (1 − (△A sample/min) – (△A blank/min)/(△A control/min) − (△A blank/min)) × 100%, where △A sample/min is the decrease in absorbance over 3 min with reaction solution, test sample, and substrate, and △A control/min without the test sample.'

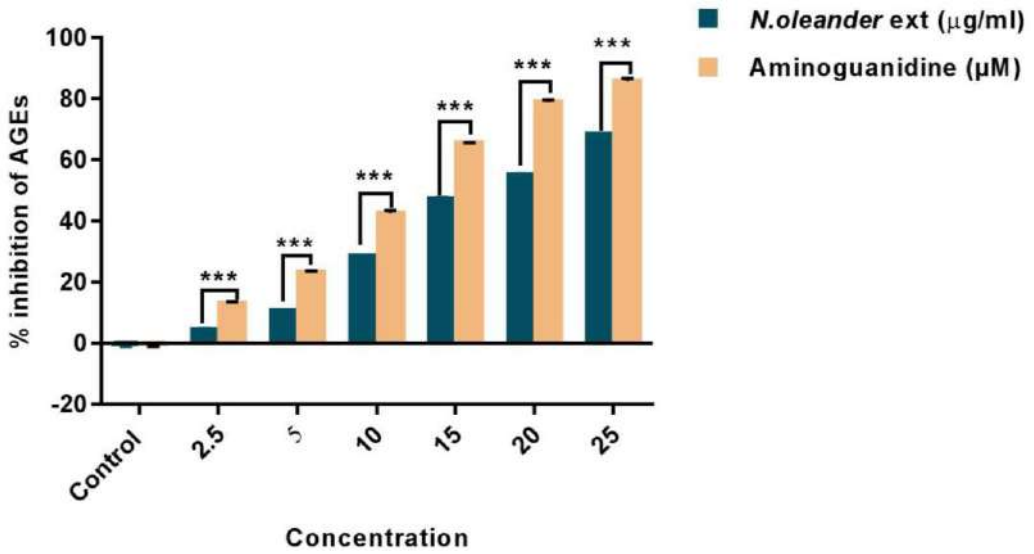
Sorbitol accumulation inhibition assay (Malone et al., 1980)

5ml of blood was collected into heparinized tubes from healthy volunteers after an overnight fast. The blood was immediately centrifuged at 2000rpm for 5min, 4oC to separate the erythrocytes from the plasma. After discarding the plasma and buffy coat, add isotonic saline (0.9% NaCl) equal to twice the volume of the erythrocytes and centrifuged at 2000 rpm for 10min. Washed RBCs were suspended in Hank's balanced salt solution [HBSS] (pH 7.4) to a ratio of 1:10. Samples were incubated at 37°C for 3 h under normal (5.5mM) and high glucose (55mM) conditions. The effect of Nerium flowers extract on sorbitol accumulation was evaluated by incubating the RBC with different concentrations of the extract (2-5-25µg/ml). At the end of incubation periods, RBC were centrifuged, washed with saline and again centrifuged. Red cell was precipitated with cold 6% perchloric acid to the ratio of 1:3. The homogenate was centrifuged at 2000 rpm at 4°C for 10 min and the pH of the supernatant was adjusted to 3.5 with 0.5M potassium carbonate. The sorbitol content of the supernatant was measured by fluorometric method. In brief, the reaction mixture contained the appropriate protein-free supernatant, 50mM glycine buffer (pH 9.4), 0.2mM NAD+, and 1.28U/ml sorbitol dehydrogenase. The mixture was incubated at 37°C for 30 min, and the relative fluorescence due to NADH was measured by a fluorescence spectrometer at an excitation wavelength of 366 nm and an emission wavelength of 452 nm.

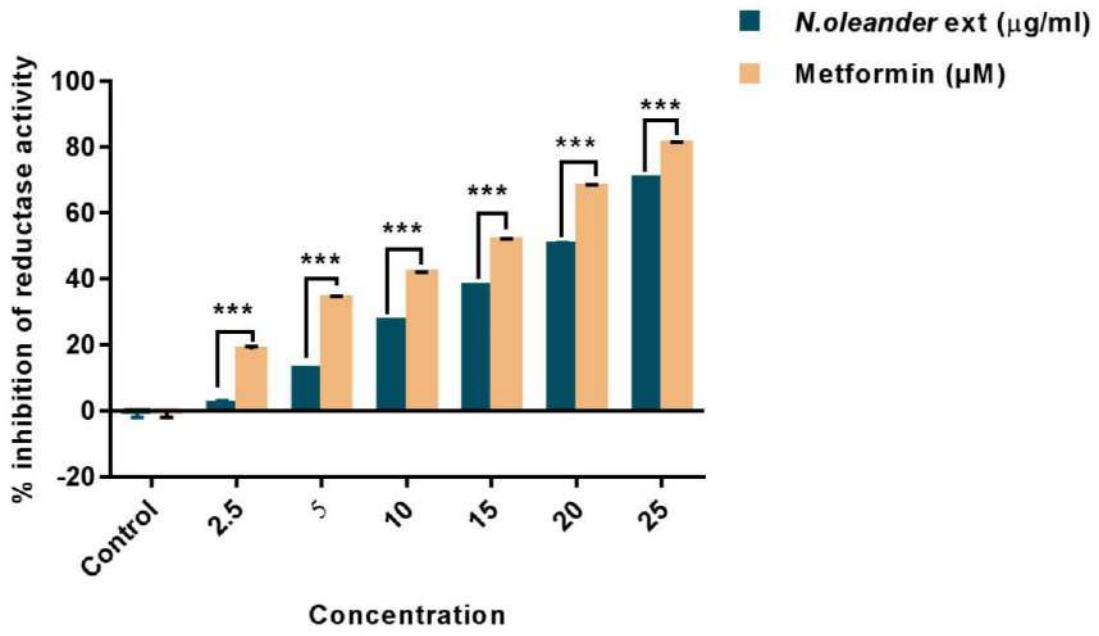
Statistical analysis

Data were analysed using Graphpad prism (version 7.0). The results were expressed as Mean±SEM and the IC50 values were obtained from the linear regression plots. Two-way ANOVA was used to assess differences between means at $p<0.001$ level of significance. The means were compared with standard groups using the Holm-Sidak Test.

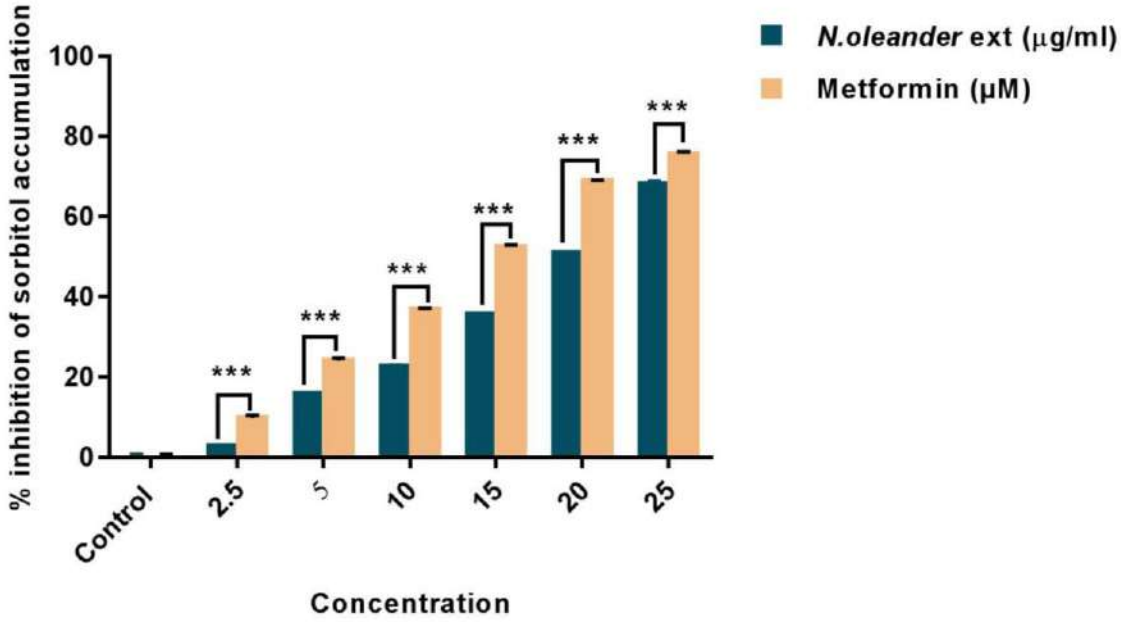
RESULTS



Graph 1: Advanced glycation end product assay



Graph 2: Aldose reductase assay



Graph 3: Sorbitol accumulation assay

Graph 1: It shows the results of advanced glycation end product assay. Nerium oleander was compared with aminoguanidine which is the standard drug for AGE inhibition. Nerium oleander shows almost the same results as the standard drug in inhibiting AGE.

Graph 2: It shows the results of aldose reductase inhibition assay. Here the extract is compared with Metformin. As the concentration of the extract increases the inhibitory activity also increases.This also almost gave the same results as the standard drug.

Graph 3: It shows the results of sorbitol accumulation assay .Nerium oleander exhibited a greater inhibition of sorbitol accumulation which is similar to the standard drug Metformin.

DISCUSSION

The present study aimed to investigate the potential ameliorative effects of hydroalcoholic flower extract of Nerium oleander on diabetes-associated complications. The findings of this study provide insights into the therapeutic potential of Nerium oleander extract as an adjunctive therapy for managing the complications associated with diabetes.

The results suggest that the hydro alcoholic flower extract of nerium oleander inhibits AGE, sorbitol accumulation and also aldose reductase which causes diabetic retinopathy,neuropathy and nephropathy.

One of the key processes implicated in the onset and progression of several diabetic problems, such as nephropathy, retinopathy, and neuropathy, is advanced glycation. Exogenous AGE exposure at high levels has been linked to vascular and renal problems in animal studies AGEs frequently amass intracellularly. These intracellular AGEs have significant effects on intracellular signalling networks and alter the way intracellular proteins operate. Most locations of diabetes problems, such as the kidney, retina, and atherosclerotic plaques, accumulate AGEs.(5) The results of the advanced glycation end product assay shows that the extract of nerium oleander inhibited the accumulation of AGE in the body.

The first step in the polyol pathway, which converts glucose to sorbitol, is catalysed by aldose reductase (AR), an aldo-keto reductase. Secondary diabetes problems such retinopathy, nephropathy, and neuropathy have been linked to the accumulation of osmotically active sorbitol caused by the polyol pathway.

Aldose reductase inhibitors have been proven to prevent or delay significantly diabetic complications.AR inhibitors inhibit the accumulation of AGEs, enhance the effects of aminoguanidine (an inhibitor of non-enzymic glycosylation)(6)As shown in the graph 2 : the nerium oleander extract shows a great inhibitory effect of aldose reductase same as metformin which taken as a standard aldose reductase inhibitor .

Numerous problems of diabetes mellitus have been linked to intracellular sorbitol buildup as an underlying cause.Diabetes causes the sorbitol route, which cells do not need insulin for, to become more active in organs like the retina, kidney, peripheral nerves, and blood vessels. Because sorbitol diffuses slowly through cell membranes, it builds up and damages osmotic balance (7). In graph 3: Metformin is used as a standard drug and the nerium oleander extract is compared with it . Nerium oleander shows almost the same rate of inhibition as metformin.

From the results of all these assays we can conclude that nerium oleander can be used for diabetes associated complications

Previous studies provided evidence that Nerium oleander possess hypoglycaemic potential as well as ameliorates diabetes associated hyperlipidemic and nephropathic complications in a dual way i.e. by improving carbohydrate metabolism. The studies also examined the impact of Nerium oleander extract on cardiovascular function in diabetic rats.(8) The findings suggested that the extract had beneficial effects on the heart, possibly reducing the risk of diabetic cardiovascular complications (9)

Another study evaluated the preventive effects of glimepiride and Nerium oleander extract on body growth rate, lipid profile, and renal function in streptozotocin-induced diabetic rats. (13) In streptozotocin-induced diabetic rats compared to controls, serum aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase activity were all considerably higher. Glimepiride or Nerium oleander extract therapy enhanced the activity of liver enzymes in diabetic rats (10)

While the study showed promising effects of Nerium oleander extract on diabetes-related complications, further research is necessary to fully understand its mechanisms of action and confirm its safety and efficacy in humans. (14) Preclinical studies provide valuable initial insights, but clinical trials involving human subjects are required to validate these findings and establish appropriate dosages and treatment regimens.(11)

Nerium oleander extract could offer a novel and complementary approach to managing diabetes and reducing the burden of associated complications, ultimately improving the quality of life of individuals with diabetes. (12)

CONCLUSION

In conclusion, the findings of this study suggest that the hydroalcoholic flower extract of Nerium oleander holds promise as a potential adjunctive therapy for managing diabetes-associated complications.The extract showed potential cardioprotective effects, which may contribute to reducing the risk of diabetic cardiovascular complications. Moreover, the extract demonstrated renoprotective effects, indicating a possible role in preventing diabetic nephropathy. It also contributes to reducing the risk of retinopathy.So Nerium oleander extract could represent a novel and promising natural approach to complement existing therapies.

FUTURE SCOPE:

Further research is needed to elucidate the specific molecular mechanisms by which Nerium oleander extract exerts its ameliorative effects on diabetes-associated complications and to identify and isolate the bioactive compounds within Nerium oleander extract responsible for the beneficial effects.

CONFLICT OF INTEREST:

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported

ETHICAL CLEARANCE:

Since it is an in-vitro study ethical clearance is not required.

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