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A Review on Anti-Diabetic Polyherbale Formulation, Murraya Koenigii (Curry Patta) & Allium Sativam (Garlic)

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ABSTRACT:

The study focuses on polyherbal antidiabetic formulations of different plants used in the treatment of diabetes mixed in different concentrations. In the present study eleven medicinal plants with proven antidiabetic and related beneficial effects were selected for the preparation of five mixtures. The efficacy of prepared mixtures has been tested on streptozotocin- (STZ-) induced diabetic rats and compared with a commercially available drug glibenclamide. The mixtures at the dose levels of 400 mg/kg b.w. produced a significant decrease in blood glucose level by 69.6%, 70.97%, 64.45%, 71.82%, and 64.44% after 21 days of treatment. The elevated level of SGPT, SGOT, and ALP in the diabetic controlled group reflected the significant alteration of liver function by STZ induction and was found to be equipotent to glibenclamide in restoration of the elevated enzyme levels to normal. The elevated lipid levels (triglyceride and total cholesterol) were restored to near normal by these mixtures for all the estimated parameters. The results of the mixtures on treated group were found to restore the glycemic level to the near normal level thereby indicating antihyperglycemic activity of the formulated mixtures.

This article reviews recent literature on the usage and relevance of garlic and its bioactive components in controlling diabetes and diabetes-associated pathologies; and also updates recent patents on the subject. Antidiabetic effect ofgarlic is well documented even in ancient medical literature. Garlic and its active ingredients have been extensively studied for their antidiabetic efficacies in either experimentally induced or genetic animal models of diabetes. Human studiesare also available where hypoglycemic effect of garlic was reported. The beneficial effects of garlic are mainly attributedto the presence of volatile sulfur compounds like alliin, allicin, diallyl disulfide, diallyl trisulfide, diallyl sulfide, S-allylcysteine, ajoene and allyl mercaptan. Garlic and garlic extracts have been shown to be effective in reducing insulin resistance. Therefore, considering the importance of garlic in controlling diabetic complications, several preparations and foodprocesses containing garlic have been patente.

Curry patta (murraya koenigii) leaves are used traditionally in aindian Ayurveda system to treat diabetes.The purpose of the study is to investigate effect of carbazole alkaloidsfrom murraya koenigii leaves on blood glucose and serum lipid profile on streptozotocin induced diabetic rats.ethanolic extract of murraya koenigii showed a significance reduction in blood glucose level at both doses,200&400mg/kg b.w.in addition murraya koenigii exhibited a profound antioxidant effect with decreased GSH level particularly at the 200mg/kg b.w.

Keywords: Antihyperglycemic, antioxidant, metformin, streptozotocin, Diabetes mellitus

Introduction:

Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Metabolic abnormalities in carbohydrates, lipids, and proteins result from the importance of insulin as an anabolic hormone. Low levels of insulin to achieve adequate response and/or insulin resistance of target tissues, mainly skeletal muscles, adipose tissue, and to a lesser extent, liver, at the level of insulin receptors, signal transduction system, and/or effector enzymes or genes are responsible for these metabolic abnormalities. The severity of symptoms is due to the type and duration of diabetes. Some of the diabetes patients are asymptomatic especially those with type 2 diabetes during the early years of the disease, others with marked hyperglycemia and especially in children with absolute insulin deficiency may suffer from polyuria, polydipsia, polyphagia, weight loss, and blurred vision. Uncontrolled diabetes may lead to stupor, coma and if not treated death, due to ketoacidosis or rare from nonketotic hyperosmolar syndrome

Garlic (Allium sativum) under the family Liliaceae, is a well-known herb with medicinal value and has been used forboth nutritional and medicinal purposes since ancient times. It is native to central Asia and cultivated in other parts of Asia, Africa, and Europe . It was also known to ancient Egyptians . For centuries, garlic is being used for culinary purposes, and its health benefits have been known since 1500 BC when ancient Chinese and Indians used it as a blood-thinning agent [4]. There are reports that during the earliest Olympics in Greece, garlic was fed to the athletes for raising strength and performance [5]. Ancient medical traditions of India like Tibbi, Unani and Ayurveda, made extensive use of garlic, as a central part of the healing efficacy.Recent studies also reveal the beneficial effects of garlic or its preparations, in combating various diseases and thus validate the ancient literature with experimental proofs.Hypolipidaemic, antiatherosclerotic, anticoagulant, antihypertensive, antimicrobial, anticancer, antidote, hepatoprotective and immunomodulatory activities of garlic are now well established from animal and human studies. The salutary effect of garlic in controlling diabetes is

also well documented. Diabetes mellitus is a complex metabolic disorder characterized by impaired insulin release from pancreas and variable degrees of insulin resistance leading to high blood glucose levels. According to World Health Organization(WHO) estimations, the death ratio of the people with diabetes will get doubled by 2030 [8]. The international diabetes

CLASSIFICATION OF DIABETES MELLITUS

Although classification of diabetes is important and has implications for the treatment strategies, this is not an easy task and many patients do not easily fit into a single class especially younger adults[1,4-6] and 10% of those initially classified may require revision[7]. The classical classification of diabetes as proposed by the American Diabetes Association (ADA) in 1997 as type 1, type 2, other types, and gestational diabetes mellitus (GDM) is still the most accepted classification and adopted by ADA[1]. Wilkin[8] proposed the accelerator hypothesis that argues "type 1 and type 2 diabetes are the same disorder of insulin resistance set against different genetic backgrounds"[9]. The difference between the two types relies on the tempo, the faster tempo reflecting the more susceptible genotype and earlier presentation in which obesity, and therefore, insulin resistance, is the center of the hypothesis. Other predictors of type 1 diabetes include increased height growth velocity[10,11] and impaired glucose sensitivity of β cells[12]. The implications of increased free radicals, oxidative stress, and many metabolic stressors in the development, pathogenesis and complications of diabetes mellitus[13-18] are very strong and well documented despite the inconsistency of the clinical trials using antioxidants in the treatment regimens of diabetes[19-21]. The female hormone 17-β estradiol acting through the estrogen receptor-α (ER-α) is essential for the development and preservation of pancreatic β cell function since it was clearly demonstrated that induced oxidative stress leads to β-cell destruction in ER-α knockout mouse. The ER-α receptor activity protects pancreatic islets against glucolipotoxicity and therefore prevents β-cell dysfunction[22].

Type 1 Diabetes Mellitus

Autoimmune type 1 diabetes This type of diabetes constitutes 5%-10% of subjects diagnosed with diabetes[23] and is due to destruction of β cells of the pancreas[24,25]. Type 1 diabetes accounts for 80%-90% of diabetes in children and adolescents[2,26]. According to International Diabetes Federation (IDF), the number of youth (0-14 years) diagnosed with type 1 diabetes worldwide in 2013 was 497100 (Table 1) and the number of newly diagnosed cases per year was 78900[27]. These figures do not represent the total number of type 1 diabetes patients because of the high prevalence of type 1 diabetes in adolescence and adults above 14 years of age. One reported estimate of type 1 diabetes in the United States in 2010 was 3 million[28,29]. The number of youth in the United States younger than 20 years with type 1 diabetes was estimated to be 166984 in the year 2009[30]. The prevalence of type 1 diabetes in the world is not known but in the United States in youth younger than 20 years was 1.93 per 1000 in 2009 (0.35-2.55 in different ethnic groups) with 2.6%-2.7% relative annual increase[26,31]. Type 1 diabetes is mainly due to an autoimmune destruction of the pancreatic β cells through T-cell mediated inflammatory response (insulitis) as well as a humoral (B cell) response[25]. The presence of autoantibodies against the pancreatic islet cells is the hallmark of type 1 diabetes, even though the role of these antibodies in the pathogenesis of the disease is not clear. These autoantibodies include islet cell autoantibodies, and autoantibodies to insulin (IAA), glutamic acid decarboxylase (GAD, GAD65), protein tyrosine phosphatase (IA2 and IA2β) and zinc transporter protein (ZnT8A)[32]. These pancreatic autoantibodies are characteristics of type 1 diabetes and could be detected in the serum of these patients months or years before the onset of the disease[33]. Autoimmune type 1 diabetes has strong HLA associations, with linkage to DR and DQ genes. HLA-DR/DQ alleles can be either predisposing or protective[1]. This autoimmune type 1 diabetes is

Type 2 Diabetes Mellitus

The global prevalence of diabetes in adults (20-79 years old) according to a report published in 2013 by the IDF was 8.3% (382 million people), with 14 million more men than women (198 million men vs 184 million women), the majority between the ages 40 and 59 years and the number is expected to rise beyond 592 million by 2035 with a 10.1% global prevalence. With 175 million cases still undiagnosed, the number of people currently suffering from diabetes exceeds half a billion. An additional 21 million women are diagnosed with hyperglycemia during pregnancy. The Middle East and North Africa region has the highest prevalence of diabetes (10.9%), however, Western Pacific region has the highest number of adults diagnosed with diabetes (138.2 millions) and has also countries with the highest prevalence (Figure 1)[27]. Low- and middleincome countries encompass 80% of the cases, "where the epidemic is gathering pace at alarming rates"[27]. Despite the fact that adult diabetes patients are mainly type 2 patients, it is not clear whether the reported 382 million adults diagnosed with diabetes also include type 1 diabetes patients.

In addition to diabetes, insulin resistance has many manifestations that include obesity, nephropathy, essential hypertension, dyslipidemia (hypertriglyceridemia, low HDL, decreased LDL particle diameter, enhanced postprandial lipemia and remnant lipoprotein accumulation), ovarian hyperandrogenism and premature adrenarche, non-alcoholic fatty liver disease and systemic inflammation[6,54]. The presence of type 2 diabetes in children and adolescence who are not obese[59-61], the occasional severe dehydration and the presence of ketoacidosis in some pediatric patients with type 2 diabetes[55] had led to the misclassification of type 2 to type 1 diabetes.

Some patients with many features of type 2 diabetes have some type 1 characteristics including the presence of islet cell autoantibodies or autoantibodies to GAD65 are classified as a distinct type of diabetes called latent autoimmune diabetes in adults (LADA)[62]. People diagnosed with LADA do not require insulin treatment. In a recent study, Hawa et al[63] reported 7.1% of European patients with type 2 diabetes with a mean age of 62 years, tested positive for GAD autoantibodies and the prevalence of LADA was higher in patients diagnosed with diabetes at a younger age. This classification of LADA as a distinct type of diabetes is still controversial .

- ***Garlic as anti- diabetes:***

Diabetes mellitus is a complex metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Abnormalities in the metabolism of carbohydrate, protein, and fat are also key factors for diabetes and related complications. Garlic has been shown to play an important role in the control of blood glucose levels and lessen diabetes-associated complications. Several studies documented the efficacy of garlic in reducing blood glucose levels in various animal models of type 1 and type 2 diabeteell as in humans16]. The hypoglycemic effect of garlic and different preparations of garlic has been attributed to the presence of allicin and other organosulfur compounds [15]. Garlic has been shown to reduce blood glucose levels in streptozotocin (STZ)-induced and alloxan-induced diabetes models (Type 1) in rats and mice (Table 1 & 2). Although both streptozotocin and alloxan-induced type-1 diabetic animal models are mostly used for experimental purposes, more than 70% studies utilised streptozotocin model for evaluating the antidiabetic efficacy of garlic. Many experimental studies showed that garlic and its different forms can reduce hyperglycemia in diabetic mice [17, 18], rats [19-21] and rabbits [22]. Garlic oil was found to be effective in seven different type-1 diabetic animal studies whereas garlic homogenate, aqueous garlic extract and garlic powder, containing allicin as major bioactive compounds, were found to be effective in eleven studies. S-allyl cysteine (SAC) and S-allyl cysteine sulfoxide (SACS), sulfur containing amino acid present in garlic preparation have been shown to reduce diabetes and related complications due to antioxidant effect [7, 17, 20, 23]. Cardiac complications, vascular reactivity, oxidative stress in liver and kidney, and cataract due to diabetes were shown to be attenuated after garlic treatment (Table 1). Different hepatic injury markers like increased serum acid, alkaline phosphatase, alanine and asparate transferases and serum amylase in diabetic rats was decreased after garlic oil treatment [23]. Aged garlic extract is also effective to reduce adrenal hypertrophy, hyperglycemia and corticosterone levels in hyperglycemic mice induced by immobilization stress [24]. Garlic (6.25%) in diet reduced hyperphagia and polydipsia but did not alter hyperglycemia and hypoinsulinaemia in streptozotocininduced diabetic mice after 12 days [25]. Experimental evidence also shows that ingestion of garlic juice improved glucose utilisation in glucose tolerance test performed in rabbits [26, 27]. Different extracts of garlic i.e., ethyl alcohol, petroleum ether and ethyl ether extracts produced a significant reduction in blood glucose levels in rabbits [28]. A single component of garlic, allicin at a dose of 250 mg/kg in alloxan-induced diabetic rabbit is 60% as effective as tolbutamide [26]. Garlic was also tested in different animal models of type 2 diabetes like fructose fed insulin resistance model, streptozotocin plus nicotinamide induced model, and genetic models i.e., db/db and KK-A(y) (Table 2). Increased sensitivity to insulin along with a decrease in oxidative stress was observed in all those models after administration of aqueous garlic extract or garlic homogenate rich in allicin. Out of 11 type-2 diabetic animal studies, 8 of them utilised 'fructose fed insulin resistance model' for evaluating the efficacy of garlic (Table 2). Duration of garlic intervention for most of the studies was 4 to 8 weeks. Nine studies out of 11 administered garlic homogenate or garlic powder, containing allicin as bioactive compound, to reduce diabetes and its complications in experimental animals. Different animal species were utilised to test the efficacy of garlic. While 29 studies out of 33 used rat as an experimental animal for type-1 diabetic study, 8 studies out of 11 used rats for type 2 diabetic studies (Table 1 and Table 2). Another important issue is the proper dose of garlic for antidiabetic activity. A wide range of doses (10-500 mg/kg) of garlic or its different preparations were used for their antidiabetic activities by different laboratories (Table 1). However, Banerjee et al., reported that garlic homogenate with more than 500 mg/kg/day dose is not safe [29]. Similarly rodent diet containing 6.5% or more percentage of garlic may be higher than the effective dose and need more safety and toxicity studies. Oral route was preferred as route of administration for evaluating antidiabetic efficacy of garlic in both type 1 and type 2 diabetic models. Although there are good numbers of experimental studies showing antidiabetic efficacies of garlic and its preparations in various animal models, the same is not being well explored in humans. However, few studies in humans indicated controversial results and therefore warrant further examination (30-34). While administration of garlic tablet (Kwai and Allicor), garlic powder, garlicin and garlic oil [30-34] showed significant reduction in blood glucose levels, some other studies using ethylacetate extract, fresh garlic and garlic powder [35-37] showed no significant change in blood glucose levels. Thus the role of garlic, its different preparations and duration of garlic administration in diabetic patients are yet to be confirmed. Similar negative data also exist for antihyperlipidemic effect of garlic [1]. The mechanism/s of antidiabetic property of garlic was explored by different studies. Nevertheless, in-vivo [7, 38] and in-vitro [7] studies showed that garlic may act as an insulin secretagogue. Increasing either the pancreatic secretion of insulin from the beta cells or its release from bound insulin was proposed as a probable mechanism of antidiabetic effect of garlic [28]. Padiya et al showed that chronic consumption of raw garlic attenuated insulin resistance in fructose fed rats [39]. Antioxidant effect of raw garlic and isolated compounds from garlic may also contribute to garlic's beneficial effect in diabetes and its associated complications [7, 39]. CURRENT & FUTURE DEVELOPMENTS Diabetes is a complex metabolic disease associated with multiple risk factors. Hyperglycemia, hyprelipidemia, hyperuricemia, insulin resistance, ketoacidosis and organ damages are major features of diabetes. Antidiabetic drugs approved till now are not sufficient to reduce diabetic

complications and mortality linked to cardiovascular dysfunctions, stroke and kidney failure. This limitation has driven the research community to continue their search to find effective antidiabetic agents or products with improved safety profiles. Garlic is being used worldwide as a prophylactic for different diseases based on both ancient and modern scientific literature. Adequate amount of experimental evidence on garlic usage as an antidiabetic has been obtained from animal studies. We found 33 and 11 animal studies, respectively, to find the efficacy of garlic against Type 1 and Type 2 diabetes (date of retrieving was October 3, 2012). One third of Type-1 diabetic studies utilised aqueous garlic or garlic powder containing allicin as a major component to reduce the diabetes and its related complications. In case of type-2 diabetes, all studies except one utilised garlic aqueous homogenate or garlic powder to alleviate the disease. Similarly, out of eight human studies five of them utilised garlic.

- ***Identification of Phytochemicals in Garlic Extract***

The polyphenols in garlic bulb extract were identified using a massspectrometer (ESI-micrOTOF-QTM, Bruker Daltonics, Bremen, Germany). Negative ion mode was used to acquire the spectra over a mass range of 50–3000 m/z. The other parameters were capillary voltage 2700 V; dry gas temperature 180 ◦C; dry gas flow 4.0 L/min; nebulizer pressure 0.4 bar; and spectra rate 1 Hz.Bruker Data Analysis 4.0 software (Bruker Daltonics Inc., Billerica, MA, USA) was used to analyze the data. The native phenolic compounds were assigned based on the matching of molecular weight, isotope pattern, ion mode collected, and considering the polarity of compounds information available in the literature and plant database.

- ***Material and methods***

- ***Chemical reagents***

Sodium phosphate buffer, α-amylase solution, starch solution, dinitrosalicylic acid colour reagent,α-glycosidase enzyme solution, intestinal acetone powder, saline, potassium phosphate buffer, ρ-nitro phenyl α-d-glucopyranoside solution, sodium carbonate, phosphate buffer, 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), acetylthiocholine iodide (ATChl), Ellman solution, acetyl-cholinesterase solution (AChE) and butyrylthiocholineterase (BChE) were purchased from Sigma-Aldrich (Steinem, Germany).

- ***Sample preparation***

5 kg of fresh leaves of M. Koenigii was purchased from Ukay Nursery (Kuala Lumpur, Malaysia) and identified at the For-est Research Institute Malaysia (FRIM) with the voucher number of 031/18. The dirt of the leaves was cleaned of using distilled water. The bone drying weight of M. Koenigii leaves was obtained using an oven dryer dried at 105 °C for 24 h, according to the ASTM standard (D1348-94) [17].

- ***Drying of fresh Murraya koenigii leaves***

Fresh M. koenigii whole leaves were dried using four drying methods such as a convective hot-air drying (CD), a single stage hybrid drying microwave vacuum-drying (MVD), a two-stage hybrid convective hot-air pre-drying followed by vacuum microwave fnishing-drying (CPD-MVFD) and freeze-drying . Freeze-dried samples were used as the control samples. For weight loss measurement of CD, the weight loss was collected at 5-min interval for the first hour, 30-min interval for the next four hours and an hour interval until the mass loss diference was 0.05 g or less using an analytical balance . In the case of MVD, the weight loss of the leaves was recorded for every 4-, 3- and 2-min interval for 6, 9 and 12 W/g, respectively, until the diference in the mass loss was 0.05 g or less using an analytical balance.

Convective hot‑air drying

Approximately 40 g of fresh M. koenigii whole leaves was dried using a convective hot-air dryer at 40, 50 and 60 °C. The leaves were placed on a wire mesh tray and spreadevenly.

Microwave vacuum‑drying

40 g of fresh M. koenigii leaves were dried using a microwave vacuum dryer (Plazmatronika, Wroclaw, Poland) using 6, 9 and 12 W/g microwave power. The leaves were first placed in an organic glass container which was attached to a vacuum system with a constant rotating speed of 6 rpm throughout the drying process to prevent local overheating of leaves. The temperature of the leaves was measured immediately after the samples were taken out of the dryer.

Convective pre‑drying followed by vacuum

microwave fnishing‑drying (CPD‑MVFD) Fresh M. koenigii leaves were partially dried using CPD in a convective hot-air dryer at 50 °C for 2 h until the moisture ratio reached 0.4727, which the surface moisture was mostly evaporated. Then, the samples were completely dried using a microwave vacuum dryer (Plaz-matronika, Wrocław, Poland) at 9 W/g. The convective hot-air temperature at 50 °C and microwave wattage at 9 W/g were used in CPD-MVFD to ensure the better quality of the dried product .

Freeze‑drying

The M. Koenigii whole leaves were dehydrated using a freeze dryer (OE-950, Hungary) at a vacuum pressure of 65 Pa. The freezing temperature was − 60 °C, while the heating plate was set to 30 °C for the sublimation process.

Methods of curry patta (murraya koenigii):

Methods

Plant Material. Fresh plant material was obtained from the local wet market in Kuantan, Pahang, Malaysia. Specimen sample was authenticated by a Taxonomist and deposited in the Faculty's Herbarium. Isolated fresh leaves were dried and pulverized to powdered using the Fritsch universal cutting mill (AZM-160-23) and stored in a desiccator at 20 ºC for subsequent use in the experiment. Preparation of aqueous extract. The powdered leaves (600 g) were subjected to cold maceration in 2 L of distilled water on three occasions, with intermittent stirring at 48 hour intervals. The extract was filtered and the yield was 550 mL. The extract was concentrated using a rotary vacuum evaporator (BUCHI R-205) to a final adjusted volume of 500 mL. The concentrated water soluble extract (500 mL) was frozen at – 70 ºC and were immediately freeze dried for a continuous two week period until the extract was completely dried, with the final extract weighing 78 g. The extract was then preserved in the laboratory chiller at 2 ºC for subsequent use. The final concentration of the aqueous extract was adjusted to 100 mg/mL.

α-amylase.1 inhibition:

The α-amylase inhibitory activity was performed using the method described by Gonzalez-Munoz et al. [34]. 0.5 ml of leave extract was mixed with 0.5 mL of 0.02 M sodium phosphate bufer and adjusted to pH 6.9 using 0.006 M of NaCl to give 0.5 mg/mL of α-amylase solution. The mixture was incubated at room temperature for 10 min. Then,0.5 mL of 1% starch solution in 0.02 M of sodium phosphate bufer was added into the mixture and incubated for 10 min. Then, 1 mL of dinitrosalicylic acid colour reagent was added into the mixture, and the mixture was incubated for a duration of 10 min in a water bath which was heated to 100 °C and allowed to cool to room temperature.Lastly, the mixture was diluted by adding 15 mL of water,and the absorbance value was measured at 540 nm using a UV-2401 PC spectrophotometer (Shimadzu, Kyoto, Japan).All samples were performed in duplicate, and the result was expressed as inhibitor % and IC50.

α-glucosidase inhibition

The α-glucosidase inhibitory activity was conducted2.6.2 α‑glucosidase inhibitionThe α-glucosidase inhibitory activity was conducted with slight modifcation based on previous works [4]. The α-glucosidase enzyme solution was prepared by dissolving 0.5 g of intestinal acetone powder in 10 mL of saline (0.9%w/v) and sonicated 12 times at 30-s intervals in an ice bath.Thereafter, the mixture was centrifugated at 3000 rpm for 30 min at 4 °C, and the supernatant was diluted as the enzyme solution two times with 0.1 M potassium phosphate bufer (pH 6.9). For the α-glucosidase inhibition, 50µL of leave extract was mixed with 50 µL of enzyme solution and incubated at room temperature for 10 min. Theenzyme reaction was initiated by adding 50 µL of 5 mMρ-nitrophenyl-α-d-glucopyranoside solution into the mixture and incubated at 37 °C for 20 min. Lastly, 100 µL of 0.1 M sodium carbonate solution was added into the mix-ture, and the absorbance value was measured at 405 nm using a UV-2401PC spectrophotometer (Shimadzu, Kyoto,Japan). All samples were done in duplicate, and the result was expressed in inhibitor % and IC50.

AChE and BChE inhibition

The AChE and BuChE inhibition were performed using the methods described by G. Hasbal et al. [35]. The Ellman solution consisted of phosphate bufer (pH 7.5) with 318 µM DTNB and 955 µM of ATChl. 20 µL of the sample solution and 220 µL of Ellman solution were mixed, and the absorbance value

was measured using a UV-2401PC spectrophotometer (Shimadzu, Kyoto, Japan) at 412 nm for the duration of 10 min. Then, 10 µL of acetylcholinesterase (AChE) solution (0.5 U/mL) was added into the mixture,and the absorbance value of the samples was measured at 412 nm for 10 min using UV-2401PC spectrophotometer (Shimadzu, Kyoto, Japan). The percentage of inhibition can be calculated using Eq. (1). The BuChE inhibition was performed similarly by replacing 10 µL of AChE solution to butyrylthiocholine (BChE) solution (0.5 U/mL). 20 µL of sample solution was replaced with distilled water as a control. All samples were done duplicated, and the results were expressed in % inhibition.

Statistical analysis

Results were expressed as mean ± standard deviation,and the error bars in the fgures indicate the standard deviation. The diferences between means were analysed using one-way ANOVA test through SPSS 23 (IBM, USA).

Materials and Methods (Garlic)

Plant Material

Garlic bulbs (*Allium sativum* L.) were purchased from a local market in Mexico City, Mexico. Garlic bulbs were frozen using liquid nitrogen and crushed into a fine powder. The fine powder was immediately used for biomolecule extraction.

Chemicals and Reagents

HPLC grade methanol, dimethyl sulfoxide, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and a human DPP-4 inhibitor screening kit were purchased from Sigma Aldrich (St. Louis, MO, USA).

Preparation of Garlic Extract

A digital ultrasonic bath (G.T.Sonic brand, Model: VGT-1730, Power: 100 W, GuangDong GT Ultrasonic Co., Ltd., Shenzhen, China) was used for the extraction purpose in this study with the frequency of 40 kHz. The extraction was carried out in methanol:water (8:2 *v*/*v*) using a ratio garlic powder:solvent of 1:10 in a 100 mL glass beaker. The beaker was incubated for 30 min in an ultrasonic bath and then filtered using syringe filters of 0.45 µm. The solvent was evaporated under vacuum using an Eppendorf Concentrator 5301 (Eppendorf, Hamburg, Germany), and the resulting residue was dissolved in dimethyl sulfoxide (DMSO) for biological activity assays.

Fourier-Transform Infrared Spectroscopy (FTIR)

Fourier-transform infrared spectroscopy (FTIR) spectra of garlic extract in aqueous methanol and DMSO were recorded at room temperature using a Perkin Elmer Frontier spectrum spectrophotometer, scanning over the frequency range of 4000 to 400 cm^{-1}. The spectrum of each sample was acquired using spectrum software version 5.3.1 and a diamond ATR (Perkin Elmer, MA, USA) and each spectrum was an average of 64 scans, with a resolution of 4 cm^{-1}. The aqueous methanol aliquots and DMSO aliquots of 10 µL were uniformly spread directly onto the ATR crystal before each spectrum was collected. Functional group analysis was carried out using Know-it-all software (Bio-Rad, Life Science, Hercules, CA, USA.

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Results and Discussion

The use of traditional medicine utilizes a broad range of plants and herbs for the treatment of diabetes mellitus in Mexico and other countries for many years. These plants are considered to play a significantly important role in providing alternative medicine, preventive agents, and as a source of new leads in the process of drug discovery and drug development. Metformin is one of the widely used natural therapeutic drugs to control blood glucose levels in diabetes mellitus, isolated from the Galega officinalis plant [48] and, indeed, oral delivery of metformin improves incretin effects by inhibiting the DPP-4 enzyme

In recent years, DPP-4 has emerged as an important therapeutic drug target to treat diabetes mellitus, and great effort by academics and the pharmaceutical industry have naturally led to the development of DPP-4 inhibitors with few side effects in comparison to current FDA approved therapeutics. During the process of plant screening, garlic was found to be more attractive because of its antidiabetic activity, even though the mechanism of action is unknown. A highly specific fluorometric assay was utilized in this study to determine precisely the DPP-4 inhibition activity by garlic bulb extract. The DPP-4

inhibitory activity of prepared garlic bulb extract in this study is shown in . It is observed that the garlic extract showed 60.5% DPP-4 inhibition at 100 µg/mL and the inhibition capacity is concentration-dependent. The results obtained demonstrated a stronger activity in DPP-4 inhibition by prepared garlic bulb extract whose IC50 value is 70.9 µg/mL. Diabetes mellitus carries a significant risk of complications, extended hospital stays, and mortality in COVID-19 infected patients. Therefore, insulin is preferred to oral hypoglycemic medications in the management of hospitalized COVID-19 infected diabetic subjects. This cohort has recommended frequent blood sugar checks and prompt management of hypoglycemia, hyperglycemia, and DKA.

Conclusion:

1. Research methods and procedure: In the present study, oral administration of garlic extract (0.1, 0.25 and 0.5 g/kg body wt.) for 14 days on the level of serum glucose, total cholesterol, triglycerides, urea, uric acid, creatinine, aspartate amino transferase (AST) and alanine amino transferase (ALT) in normal and streptozotocin-induced diabetic rats were evaluated.
2. The present study demonstrated that mahanimbine exhibited anti-diabetic and hyolipidemic effects in streptozotocin-induced diabetic rats. Therefore, mahanimbine could be used as anti-diabetic agent in the management of diabetes associated with abnormalities of lipid profiles.

Reference

1. Mitra A. Some salient points in dietary and life style of rural Bengal particularly tribal populace in relation to rural diabetic prevalence. Studies on Ethno-Med 2008, 2: 51-56.
2. world journal of diabetes: the epidemic of the centry by Akram T Kharroubi, Hisham M Darwish.
3. Oman medical journal (2012) vol.27,No.4:269-273 DOI 10.5001/omj.2012.68
4. Diabetes & Metabolic Syndrome: Clinical Research & Reviews journal homepage: www.elsevier.com/locate/dsx.
5. Journal of Ayurveda and Integrative Medicine Journal homepage: http://elsevier.com/locate/jaim.
6. https://www.researchgate.net/publication/233998662
7. Efectiveness of polyherbal formulation for the treatment of typr-2 Diabetes mellitus – A systemic review and meta analysis by Renuks suvarna,Revathi P. Shenoy, Basavaraj S. Hadapad, Anupama V. Nayak.
8. Research Article | Open Access Volume 2013 |Article ID 934797 | https://doi.org/10.1155/2013/934797
9. Article Phytochemicals in Garlic Extract Inhibit TherapeuticEnzyme DPP-4 and Induce Skeletal Muscle Cell Proliferation: A Possible Mechanism of Action to Benefit the Treatment of Diabetes Mellitus Poonam Kalhotra , Veera C.S.R. Chittepu , Guillermo Osorio-Revilla 2 and Tzayhri Gallardo-Velazquez 1,*
10. https://scholar.google.co.in/scholar?q=murraya+koenigii+in+antidiabetic&hl=en&as_sdt=0&as_vis=1&oi=scholart#d=gs_qabs&t=1651029259814&u=%23 p%3D2NTqwTT7QqUJ