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Traditional medicinal plants used for the treatment of diabetes in the Sudan: A review

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The prevalence of metabolic disorders such as diabetes among population is of increasing concern worldwide. Sudan is a developing country, where several areas frequently depend on folk medicine. Several herbal preparations have been used in folklore practice in Sudan for the management of diabetes with claims asserting their hypoglycemic effect. Basic research relating to these plants are reviewed in this paper with the intention to highlight their therapeutic potential for the treatment of diabetes and promote their regular use in Sudan. Ethnobotanical information was obtained by an assessment of the available literature in electronic data bases with publications describing the medicinal plants used for the treatment of diabetes. In this review paper, different parts of 38 plant species, are described that are used in the Sudanese traditional medicine and belong to 35 genera and 23 families. Thirty three plants have been documented in scientific literature to possess in vivo antidiabetic activity and only one was ineffective in lowering blood glucose level, namely Striga hermonthica. Many of the plants in the study review have been studied in in vitro models (such as α-amylase or α-glucosidase inhibition) in an effort to explain some of their biomedical interaction. The role of isolated bioactive compounds like trigonelline and 3, 5-dicafeeylquinic acid in diabetes management is also evaluated in the present review. Ten plants original from Sudan have been already used in clinical trials for the treatment of type 2 diabetes. This review provides useful information on the characterization of such herbal medicines that are utilized in the Sudanese traditional medicine for the control of metabolic syndromes such as diabetes.

**Key words:** Diabetes, Sudan, ethnopharmacology, pharmacology, toxicology.

INTRODUCTION

Diabetes is a metabolic disorder characterized by chronic hyperglycemia which results from insulin deficient secretion or impaired cellular action of the hormone. Insufficient insulin secretion caused by immune destruction of pancreatic β-cells are vital for insulin secretion and consequent development of insulin-
dependent diabetes (Type 1). In case of abnormal insulin cellular action, type-2 diabetes is developed due to a gradual development of insulin resistance and pancreatic β-cell dysfunction. Symptoms are worsened due to obesity and lack of physical activity by the sedentary lifestyle (Gutch et al., 2014).

Diabetes prevalence is on the rise worldwide as a result of accumulating risk factors well pronounced in economically growing nations. An estimated 69% rise is observed for the prevalence of the disease in adults in developing countries versus 20% for adults in developed countries (Shaw et al., 2010). The prevalence rate of diabetes in Sudan ranks the disease high among others that are of raising great concern for the community and for the medical service (Ahmad et al., 2008).

The total population in Sudan is about 34 million, with 70% of it in the Northern parts. Prevalence of type 1 diabetes is estimated at 0.1% among school-age children 7 to 14 years old while type 2 diabetes is estimated at 10.4% among adult population (over 25 year of age) in Northern Sudan (Elamin et al., 1989; Motala et al., 2003).

A complex mechanism involving enzymes and other factors influences the action of insulin in the management of hyperglycemia. Mainstream drugs that are used to control diabetes fall into three main categories. The first category of drugs aims to enhance endogenous insulin availability and includes agents that act on the sulfonyl urea receptors in the pancreas to promote insulin secretion and others that have an impact on the small intestinal mucosal epithelium.

Medicines categorized as group two are directed to potentiate the response to insulin, among them being thiazoline. This group of drugs seems to act as initiator of peroxisomal receptors responsible for regulation of metabolism of carbohydrates, lipids and proteins. The drugs categorized as group three are represented by α-glucosidase inhibitors and are targeted at reducing the metabolism of complex sugars (Sheehan, 2003; Thompson and Davis, 2017).

Current glycemic medications burden patients due to variable contraindications and interactions with other drugs as well as limited efficacy, limited tolerability and significant side effects arising from their complex mechanisms of action (Moller, 2001; Rotenstein et al., 2012). Existing pharmacotherapy is still far from achieving optimal blood sugar control in such patients, as an effect of a dysfunction in insulin secretion, action, or both (Gupta et al., 2016).

A number of reviews from different countries have highlighted the significance of medicinal plants application for the control of diabetes (Ezuruike and Prieto, 2014). Despite the wide-spread traditional use of these plants for diabetes management in Sudan, scientific support for the safe and effective use of such plants does not exist. The aim of the present work was to collect data relevant to the traditional control of diabetes with plants wellknown for their medicinal effects in Sudan.

Traditional medicinal plants for the control of diabetes

Data about the use of plants in folklore medicine for the control of diabetes relates either with previously conducted ethnobotanical studies or published papers demonstrating the antidiabetic impact of certain plants in Sudan. The experimental evidence of the antidiabetic activity is highlighted in this review, along with biochemical analysis (where available). Considering comprehensiveness and increasing interest in antioxidant activity in relation to antidiabetic effect of plants, the antioxidant activity of these plants are also reviewed (Ezuruike and Prieto, 2014). A literature search of electronic databases on these plants was carried out and for each of the identified plants, synonyms (for plants which were not identified with their accepted names in the original publication), family name, local name, and plant part used are compiled in Table 1. Available pharmacological evidence of these plants for their therapeutic use is also summarized, and most toxicological relevant studies are presented in Table 2.

Experimental evidence concerning medicinal plants and their phytoconstituents that are used in management of diabetes

Acacia nilotica

A dose of 400 mg/kg body weight (b.w) of an aqueous methanol extract of A. nilotica pods significantly reduced the levels of blood glucose, the plasma total cholesterol (TC), total triglyceride (TG), low-density lipids (LDL), the activity of serum glutamate oxaloacetate (GOT) and pyruvate transaminase (GPT) after one month of treatment in diabetic rabbits compared to the untreated diabetic ones. Furthermore, the same dose also significantly increased the plasma high density lipids (HDL) levels of the treated rabbits but not significant effect on creatinine clearance was observed (Ahmad et al., 2008). Hot water extract of A. nilotica pods decreased significantly the plasma glucose level of alloxan-induced Albino mice after 1 to 2 h of administration (Abd el-aziz et al., 2013). A similar observation was obtained in Wistar albino rats treated with 400 to 800 mg/kg of aqueous extract of A. nilotica pods and 800 mg/kg of ethyl-acetate and n-butanol fractionated from aqueous extract after 12 to 18 h of administration (Auwal et al., 2013). Moreover, Tanko et al. (2013) demonstrated that ethyl acetate fraction obtained from the methanolic extract of A. nilotica leaves had a remarkable hypoglycemic effect in alloxan-induced diabetic rats after treatment with 50 and 100 mg/kg for 7 to 12 days. Modified lignin extracted from the hardwood of A. nilotica exhibited increased glucose binding efficiency as demonstrated by the decreased glucose diffusion and enhanced α-amylase inhibition in
<table>
<thead>
<tr>
<th>S/N</th>
<th>Plant species</th>
<th>Family</th>
<th>Local name</th>
<th>Part used</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Acacia nilotica</em> (L.) Willd. ex Delile</td>
<td>Fabaceae</td>
<td>Garad</td>
<td>Pod, bark</td>
<td>(Gaber et al. 2013)</td>
</tr>
<tr>
<td>2</td>
<td><em>Acacia senegal</em> (L.) Willd.</td>
<td>Fabaceae</td>
<td>Hashab</td>
<td>Fruit</td>
<td>(Hilmi et al. 2014)</td>
</tr>
<tr>
<td>3</td>
<td><em>Aloe sinkatana</em> Reynolds</td>
<td>Xanthorrhoeaceae (Aloaceae)</td>
<td>Sabar</td>
<td>Leaf</td>
<td>(Gaber et al. 2013)</td>
</tr>
<tr>
<td>4</td>
<td><em>Allium cepa</em> L.</td>
<td>Amaryllidaceae</td>
<td>Basal</td>
<td>Bulb</td>
<td>(TajEldin et al. 2009)</td>
</tr>
<tr>
<td>5</td>
<td><em>Allium sativum</em></td>
<td>Amaryllidaceae</td>
<td>Toom</td>
<td>Bulb</td>
<td>(Ebrahim et al. 2012)</td>
</tr>
<tr>
<td>6</td>
<td><em>Ambrosia maritima</em> L.</td>
<td>Asteraceae</td>
<td>Damesisa</td>
<td>Leaf</td>
<td>(Yagi et al. 2013)</td>
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<tr>
<td>7</td>
<td><em>Ammi visnaga</em> (L.) Lam.</td>
<td>Apiaceae</td>
<td>Bizrat al khalla</td>
<td>Fruit</td>
<td>(Hilmi et al. 2014)</td>
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<tr>
<td>8</td>
<td><em>Balanites aegyptiaca</em> (L.) Del.</td>
<td>Zygophyllaceae</td>
<td>Laloub</td>
<td>Fruit</td>
<td>(Gaber et al. 2013)</td>
</tr>
<tr>
<td>9</td>
<td><em>Bauhinia rufescens</em> L.</td>
<td>Fabaceae</td>
<td>Kulkul</td>
<td>Leaf</td>
<td>(El-Ghazali et al. 1997)</td>
</tr>
<tr>
<td>10</td>
<td><em>Capparis decidua</em> (Forssk.) Edgew.</td>
<td>Capparaceae</td>
<td>Tundub</td>
<td>Stem</td>
<td>(Zia-Ul-Haq et al. 2011)</td>
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<tr>
<td>11</td>
<td><em>Catunaregam nilotica</em> (Stapf) Tirven (Syn. Randia nilotica Stapf)</td>
<td>Rubiaceae</td>
<td>Kir Kir</td>
<td>Fruit</td>
<td>(Alamin et al. 2015)</td>
</tr>
<tr>
<td>12</td>
<td><em>Cicer arietinum</em> L.</td>
<td>Fabaceae</td>
<td>Kabbabe</td>
<td>Seed</td>
<td>(Mustafa et al. 2013)</td>
</tr>
<tr>
<td>13</td>
<td><em>Cinnamomum verum</em> J. Presl (Syn. Cinnamomum zeylanicum Blume)</td>
<td>Lauraceae</td>
<td>Gerfa</td>
<td>Stem bark</td>
<td>(Mustafa et al. 2010)</td>
</tr>
<tr>
<td>14</td>
<td><em>Citruscolyntis</em> (L.) Schrad.</td>
<td>Citruscolyntis</td>
<td>Hundal</td>
<td>Seed</td>
<td>(El-Ghazali et al. 1997)</td>
</tr>
<tr>
<td>15</td>
<td><em>Cyperus rotundus</em> L.</td>
<td>Cyperaceae</td>
<td>Sieda</td>
<td>Rhizome</td>
<td>(El-Ghazali et al. 1997)</td>
</tr>
<tr>
<td>16</td>
<td><em>Eucalyptus globulus</em> Labill.</td>
<td>Myrtaceae</td>
<td>El kafour</td>
<td>Leaf</td>
<td>(Houacine et al. 2012)</td>
</tr>
<tr>
<td>17</td>
<td><em>Faidherbia albida</em> (Delile) A. Chev. (Syn. <em>Acacia albida</em> Delile)</td>
<td>Fabaceae</td>
<td>Haraz</td>
<td>Root bark</td>
<td>(Gaber et al. 2013)</td>
</tr>
<tr>
<td>18</td>
<td><em>Foeniculum vulgare</em> Mill.</td>
<td>Apiaceae</td>
<td>Shamar</td>
<td>Fruit</td>
<td>(Anitha et al. 2014)</td>
</tr>
<tr>
<td>19</td>
<td><em>Geigeria alata</em> (Hochst. &amp; Steud. ex DC.) Oliv. &amp; Hiern</td>
<td>Asteraceae</td>
<td>Al Gadad</td>
<td>Root</td>
<td>(Hafizur et al. 2012)</td>
</tr>
<tr>
<td>20</td>
<td><em>Guiera senegalensis</em> J.F.Gmel.</td>
<td>Combretaceae</td>
<td>Ghubeish</td>
<td>Leaf</td>
<td>(Gaber et al. 2013)</td>
</tr>
<tr>
<td>21</td>
<td><em>Hyphaene thebaica</em> (L.) Mart.</td>
<td>Arecaceae</td>
<td>Naban</td>
<td>Epicarp</td>
<td>(Gaber et al. 2013)</td>
</tr>
<tr>
<td>23</td>
<td><em>Lupinus termis</em> Forssk.</td>
<td>Papilionaceae</td>
<td>Turmus</td>
<td>Fruit</td>
<td>(Gaber et al. 2013)</td>
</tr>
<tr>
<td>25</td>
<td><em>Momordica balsamia</em> L.</td>
<td>Cucurbitaceae</td>
<td>Abu el Efin</td>
<td>Leaf &amp; seed</td>
<td>Houacine et al. (2012)</td>
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<td>26</td>
<td><em>Nauclea latifolia</em> Smith</td>
<td>Rubiaceae</td>
<td>Karmadoda</td>
<td>Leaf</td>
<td>Alamin et al. (2015)</td>
</tr>
<tr>
<td>27</td>
<td><em>Nigella sativa</em> L.</td>
<td>Ranunculaceae</td>
<td>Al Haba</td>
<td>Seed</td>
<td>Hilmi et al. (2014)</td>
</tr>
<tr>
<td>28</td>
<td><em>Rhyncchosia minima</em> (L.) DC.</td>
<td>Fabaceae</td>
<td>Irg el Dam</td>
<td>Root</td>
<td>EL-Kamali and EL-amir (2010)</td>
</tr>
<tr>
<td>29</td>
<td><em>Salvia officinalis</em> L.</td>
<td>Lamiaceae</td>
<td>Maaramya</td>
<td>Leaf</td>
<td>Houacine et al. (2012)</td>
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<tr>
<td>30</td>
<td><em>Sclerocarya birrea</em> (A. Rich.) Hochst</td>
<td>Anacardiaceae</td>
<td>Hommaid</td>
<td>Stem bark</td>
<td>Mariod et al. (2012)</td>
</tr>
<tr>
<td>31</td>
<td><em>Senna obtusifolia</em> (L.) H.S. Irwin &amp; Barneby (Syn. <em>Cassia tora</em> L.)</td>
<td>Caesalpinaceae</td>
<td>Kawal</td>
<td>Fermented leaf</td>
<td>EL-Kamali and EL-amir (2010)</td>
</tr>
<tr>
<td>32</td>
<td><em>Sesamum indicum</em> L.</td>
<td>Pedaliaceae</td>
<td>Simsim</td>
<td>Seed</td>
<td>Hilmi et al. (2014)</td>
</tr>
<tr>
<td>33</td>
<td><em>Striga hermonthica</em> (Delile) Benth.</td>
<td>Orobanchaceae</td>
<td>Al-buda</td>
<td>Whole plant</td>
<td>Alamin et al. (2015)</td>
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<td>35</td>
<td><em>Trigonella foenum-graecum</em> L.</td>
<td>Fabaceae</td>
<td>Hilba</td>
<td>Seed</td>
<td>(Gaber et al. 2013)</td>
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<tr>
<td>36</td>
<td><em>Vangueria madagascariensis</em> J.F. Gmel.</td>
<td>Rubiaceae</td>
<td>Soum Eyown</td>
<td>Root</td>
<td>Musa et al. (2011)</td>
</tr>
<tr>
<td>37</td>
<td><em>Zygophyllum coccineum</em> L.</td>
<td>Zygophyllaceae</td>
<td>Tartir</td>
<td>Whole plant</td>
<td>(Gaber et al. 2013)</td>
</tr>
</tbody>
</table>
### Table 2. Toxicological studies on plants used in Sudanese traditional medicine for treatment of diabetes.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Interaction/toxicity studies</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acacia nilotica</em></td>
<td>Co-incubation of 0.01% of the extract in Caco-2 cell monolayers decreased the integrity of the monolayer and the secretory transport of CsA indicating possible inhibition of P-gp</td>
<td>Deferme et al. (2003)</td>
</tr>
<tr>
<td><em>Acacia senegal</em></td>
<td>Toxicity studies of the ethanol extracts of the stem bark revealed that they exhibited no significant toxicity against <em>Artemia salina</em>.</td>
<td>Okoro et al. (2012)</td>
</tr>
<tr>
<td><em>Allium sativum</em></td>
<td>Components of aged garlic extract did not produce significant inhibition of Cytochrome P450 enzymes <em>in vitro</em> and in humans</td>
<td>Markowitz et al. (2003) and Greenblatt et al. (2006)</td>
</tr>
<tr>
<td><em>Ambrosia maritima</em></td>
<td>A single dose of 2000 mg/kg Body weight of the methanolic extract of <em>A. maritima</em> was fatal to rats, but a daily dose of 500 and 250 mg/kg b.w was not fatal.</td>
<td>Afaf et al. (2007)</td>
</tr>
<tr>
<td><em>Ammi visnaga</em></td>
<td>Acute toxicity (LD50) of intraperitoneal administration of aqueous extract of <em>A. visnaga</em> fruit in rats was 3.6 g/kg</td>
<td>Jouad et al. (2002)</td>
</tr>
<tr>
<td><em>Bauhinia rufescens</em></td>
<td>In the brine shrimp lethality assay, ethyl acetate and methanol extracts of the leaves of the plant were found toxic to the <em>Artemia salina</em> with IC50 values of 0.059 mg/mL and 0.389 mg/mL. While, both the petroleum ether and ethyl acetate extracts of the stem bark were not toxic to the larva.</td>
<td>Muhammad and Sira (2013)</td>
</tr>
<tr>
<td><em>Capparis decidua</em></td>
<td>Petroleum ether, chloroform, ethyl acetate and butanol extracts were not toxic against brine shrimps and vero cell lines.</td>
<td>Abdalrahman et al. (2016)</td>
</tr>
<tr>
<td><em>Catunaregam nilotica</em></td>
<td>Aqueous extract of fruits was safe up to a dose of 2000 mg/kg body weight.</td>
<td>Alamin et al. (2015)</td>
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<tr>
<td><em>Citrullus colocynthis</em></td>
<td>A single daily dose of alcoholic extract of <em>C. colocynthis</em> (50, 100, 200, 400 g/kg) can have toxic effects on liver cells which may induce hepatocyte necrosis and liver fibrosis</td>
<td>Dehghani and Shahin (2006)</td>
</tr>
<tr>
<td><em>Cyperus rotundus</em></td>
<td>Ethanol extract of rhizomes was safe up to the dose 2000 mg/kg to Wistar rats</td>
<td>Jebasingh et al. (2012)</td>
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<tr>
<td><em>Eucalyptus globulus</em></td>
<td>Ethanolic leaf extract have shown moderate brine shrimp lethality with LC50 value of 55.95 μg/mL.</td>
<td>Houacine et al. (2012)</td>
</tr>
<tr>
<td><em>Faidherbia albida</em></td>
<td>Ethanolic stem bark extract of <em>F. albida</em> is relatively safe when used sub-acutely in rats.</td>
<td>Oluwakanyinsola et al. (2010)</td>
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<tr>
<td><em>Guiera senegalensis</em></td>
<td>Ethanolic leaf extract have shown moderate brine shrimp lethality with LC50 value of 26.94 μg/mL.</td>
<td>Houacine et al. (2012)</td>
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<td><em>Kigelia africana</em></td>
<td>Fruits of the plant given to the experimental rats at doses 100, 200 and 400 mg/kg/day orally were toxic but not fatal.</td>
<td>Adam et al. (2013)</td>
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<td><em>Lupinus termis</em></td>
<td>Lupins contain certain secondary compounds including toxic alkaloids, such as lupinine</td>
<td>Rahma and Narasinga (1984)</td>
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<td><em>Mitragyna inermis</em></td>
<td>Alkaloid rich extract derived from <em>M. inermis</em> induced a strong inhibition of protein synthesis in mammalian cells but did not exhibit mutagenic or genotoxic activity</td>
<td>Traore et al. (2000)</td>
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<tr>
<td><em>Momordica balsamina</em></td>
<td><em>In vitro</em> toxicity results raise concern for chronic use</td>
<td>van de Venter et al. (2008)</td>
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<tr>
<td><em>Nauclea latifolia</em></td>
<td>An alkaloid rich extract derived from <em>N. latifolia</em> could interact <em>in vitro</em> with DNA of bacteria and mammalian cells, leading to G2-M cell cycle arrest and heritable DNA-damage, as well as inducing <em>in vivo</em> single-strand breaks in liver, kidney and blood cells</td>
<td>Traore et al. (2000)</td>
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<tr>
<td><em>Rhynchosia minima</em></td>
<td>The plant is reported as toxic to fish. The seeds are bitter and poisonous and seed extract shows specific agglutinating action on human RBC</td>
<td>Mali and Mahale (2008)</td>
</tr>
</tbody>
</table>
Table 2. Cont’d.

<table>
<thead>
<tr>
<th>Plant Name</th>
<th>Description</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salvia officinalis</em></td>
<td>Ethanolic leaf extract have shown moderate brine shrimp lethality with LC₅₀ value of 37.21 μg/mL</td>
<td>Houacine et al. (2012)</td>
</tr>
<tr>
<td><em>Sclerocarya birrea</em></td>
<td>Stem-bark aqueous and methanolic extracts are relatively safe but in vitro toxicity test has raised concerns over chronic use of <em>S. birrea</em> extracts</td>
<td>Ojewol (2003)</td>
</tr>
<tr>
<td><em>Senna obtusifolia</em></td>
<td>The leaves can cause marked toxic effects on rats, and the processing of the leaves by fermentation to produce kawal did not alter the toxic activity of the ingredients in the leaves</td>
<td>Yagi et al. (1998)</td>
</tr>
<tr>
<td><em>Sesamum indicum</em></td>
<td>Sesame oil or its lignans, due specifically to their methylenedioxyphenyl group, could interact with the P450 isozymes and affect the drug metabolisms or dispositions in human</td>
<td>Gokbulut (2010)</td>
</tr>
<tr>
<td><em>Striga hermonthica</em></td>
<td>Aqueous extract of whole plant was safe up to a dose of 2000 mg/kg body weight</td>
<td>Alamin et al. (2015)</td>
</tr>
<tr>
<td><em>Tinospora bakis</em></td>
<td>Aqueous extract of seeds was safe up to a dose of 2000 mg/kg body weight.</td>
<td>Alamin et al. (2015)</td>
</tr>
<tr>
<td><em>Trigonella foenum-graecum</em></td>
<td>Short-term (90 days) and long term (24 week) feeding of fenugreek seeds to rats at levels equivalent to 2 and 4 times the therapeutic dose recommended for humans (25 g/day) produced no toxic effects.</td>
<td>Udayasekhara et al. (1996) and Sharma et al. (1996)</td>
</tr>
</tbody>
</table>

comparison to the controls (Barapatre et al., 2015).

**Acacia senegal**

Administration of 200 and 400 mg/kg b.w of ethyl acetate extract from the stem bark of *A. senegal* significantly lowered the levels of blood glucose, serum TC, serum TTG, serum LDL, serum urea and creatinine, and increased the serum HDL level in alloxan-induced diabetic albino rats on day 16 after the administration (Batra et al., 2013). Treatment of CCl₄-induced acute hepatotoxicity in albino Wistar rats with 400 and 800 mg/kg/day of the hydroalcoholic (70% ethanol) extract of *A. senegal* pods, orally for 7 days, significantly reduced the liver damage and the symptoms of liver injury by restoration of architecture of liver as indicated by lower levels of serum bilirubin and prevention of hepatic damage (Pal et al., 2014). The components extracted by ethanol from the leaves of *A. Senegal* decreased the activity of sucrose enzyme and appeared to support the control of carbohydrate hydrolysis, and consequently reduces the rise of postprandial blood glucose in diabetics (Abdelhady and Youns, 2014).

**Aloe sinkatana**

The effects of aqueous extracts of *A. sinkatana* leaves on blood glucose and lipid profile in type 2 diabetic patients was evaluated by Gaber et al. (2013). The volunteers of the experimental group received the aqueous extract (500 g/l) at a dose of 5 mL/day. A significant reduction in levels of fasting blood sugar, TTG, TC and LDL and a significant increase in HDL levels was observed.

**Allium cepa**

A detailed review on the positive antidiabetic activity effect of *A. cepa* in different animal models, and its antioxidant activity as well as clinical studies on diabetic patients was presented by Akash et al. (2014).

**Allium sativum**

Administration of aqueous extract of *A. sativum* to induced diabetic rats reduced the blood glucose level, total serum lipids and cholesterol (Thomson et al., 2007; Ozougwu and Eyo, 2010; Badole et al., 2013; Thomson et al., 2016).

**Ambrosia maritima**

Administration of water, 50% ethanolic, ether or petroleum ether extracts of *A. maritima* whole plant to albino rats significantly reduced blood glucose after 1.5 and 2 h, however without significant changes in insulin.
levels (Ammar et al., 1993). Alloxan-induced diabetic albino rats treated orally with 28.5 mg/kg b.w. of aqueous extract of A. maritima aerial parts twice/day showed significant improvement in most of biochemical parameters (levels of fasting blood glucose, serum insulin, total proteins, albumin, globulin, HDL, aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, creatinine, uric acid, serum TC, TGG and LDL) (Helal et al., 2014).

**Ammi visnaga**

Aqueous extract of A. visnaga at the dose of 20 mg/kg b.w significantly reduced blood glucose in induced-streptozotocin diabetic rats after repeated oral administration for nine days (Jouad et al., 2002).

**Balanites aegyptiaca**

The addition of 10 % whole or extracted pulp of B. aegyptiaca fruits instead of starch in the basal diet of alloxan-induced albino rats, for 20 days, caused a significant decrease in serum glucose level and inhibited the activities of serum GOT and GPT (El-Saadany et al., 1986). Aqueous extract of mesocarps of the fruits exhibited a prominent antidiabetic activity when offered orally in streptozotocin-induced diabetic mice (Kamel et al., 1991). Administration of fruits aqueous extract (1.5 g/kg b.w daily for 45 days) in streptozotocin-induced Wistar albino diabetic rats significantly reduced the mean plasma glucose and malondialdehyde levels, and significantly increased the mean plasma insulin, liver-pyruvate kinase, and total antioxidant capacity levels. An obvious increase in the weight of the pancreas and the size of the islets of Langerhans, and improvement in the histoarchitecture were also evident in the treated groups compared to untreated ones (Khallil et al., 2016). The antidiabetic activities of different fruit extracts and fractions of B. aegyptiaca were tested in cultured C2C12 skeletal muscle cells and 3T3-L1 adipocytes. An 18 h treatment with 200 μg/mL of the sugars fraction, dichloromethane (E) and ethyl acetate (F) successive extracts increased basal glucose uptake in muscle cells. Only E and F extracts accelerated the triglyceride accumulation in pre-adipocytes undergoing differentiation (Motaal et al., 2012). Dichloromethane and ethyl acetate extracts of the fruit were standardized by high-performance liquid chromatography to contain 0.031 and 0.239% of rutin, and 0.007 and 0.004% of isorhamnetin, respectively (Abdel Motaal et al., 2012). Trigonelline (3-carboxy-1-methyl pyridinium) was identified in the fruits (8 and 13 mg in the peel and pulp respectively) in addition to the flavonoids quercetin, isorhamnetin flavonol and epicatechin (Farag et al., 2015). Saponins, 26-O-beta-D-glucopyranosyl-(25R)-furost-5-ene-3 beta, 22, 26-triol 3-O-[alpha-L-rhamnopyranosyl-(1-2)]-beta-D-xylopyranosyl-(1-3)]-alpha-L-rhamnopyranosyl- (1-4)]-beta-D-gluco.pyranoside and its 22-methyl ether, 26-O-beta-D-glucopyranosyl-(25R)-furost-5-ene-3 beta,22,26-triol 3-O-(2,4-di-O-alpha-L-rhamnopyranosyl)-beta-D glucopyranoside and its methyl ether were also isolated and identified. It was revealed that the individual saponins did not show antidiabetic activity, while their combination resulted in significant activity.

**Bauhinia rufescens**

The oral administration of 200, 300, and 400 mg/kg b.w methanol extract from the leaves of B. rufescens (once a day, for four weeks) significantly lowered the blood glucose levels in alloxan-induced diabetic rats in a dose dependent manner (Aguh et al., 2013).

**Catunaregam nilotica**

Acute and chronic treatment of streptozotocin-induced diabetes rats with aqueous extracts of C. nilotica (Syn. Randia nilotica) fruit at 400 mg/kg significantly lowered blood glucose, serum lipid and creatinine levels, and brought back the activity of AST enzyme to normal level. Histopathological studies showed that the aqueous extracts of the plant reinforced the protection of liver (Alamin et al., 2015). Methanolic extracts of leaves, bark and seedcake of C. nilotica possess good antioxidant activity and high phenolic content (Mariod et al., 2012).

**Capparis decidua**

Fruits of C. decidua decreased the lipid peroxidation and altered free radical scavenging enzymes such as superoxide dismutase and catalase in erythrocytes, liver, kidney and heart in alloxan induced diabetic rats (Agarwal and Chavan, 1988; Yadav et al., 1997). Moreover, the fruit extract showed satisfactory inhibitory effect on α-amylase and α-glucosidase enzymes, followed by flowers and leaves extracts (Zia-Ul-Haq et al., 2011).

**Cicer arietinum**

Administration of petroleum ether extract (400 mg/kg) of the seed to alloxan-induced diabetic mice reduced significantly the serum glucose level in both acute and subacute studies (Yadav et al., 2009). The seed showed significant diphenylpicrylhydrazyl (DPPH) nitric oxide and hydrogen peroxide activity (Vadnere et al., 2013).

**Cinnamomum verum**

Administration of 200 mg/kg b.w of cinnamon aqueous extract to alloxan-induced diabetic rats lowered
significantly the levels of fasting blood glucose, TC, HDL, LDL and TG (El-Desoky et al., 2012). Moreover, administration of bark aqueous extract of cinnamon containing 45 and 75% gallic acid equivalents of polyphenol to streptozotocin-induced diabetic rats at 200 mg per kg b.w. for 30 days displayed hypoglycemic and hypolipidemic effects (IM et al., 2014). The bark is rich in volatile oil and polyphenols including rutin, catechin, quercetin, kaempferol and isorhamnetin have been isolated (Yang et al., 2012).

**Citrus colocynthis**

Administration of roots aqueous extract (2000 mg/kg) to alloxan-induced diabetic rats showed hypoglycemic effect and improved serum levels of urea and lipid (Agarwal et al., 2012). Moreover, hydroethanol extract (300 mg/kg bw) of the seed reduced significantly the blood glucose level in alloxan-induced diabetic rats (Oryan et al., 2014). Petroleum ether extract (300 and 500 mg/kg bw) of fruit pulp showed significant hypoglycemic effect in streptozotocin-induced diabetes albino rats (Jayaraman et al., 2009).

**Cyperus rotundus**

The ethanolic extract of *C. rotundus* rhizomes at dose of 250 and 500 mg/kg b.w, for 3 weeks, revealed significant antidiabetic activity and resulted in improvement of body weight and reduction in the levels of biochemical parameters such as GPT, GOT, TC and TTG in streptozotocin-induced diabetic mice (Singh et al., 2015).

**Eucalyptus globulus**

Administration of leaf aqueous extract of *E. globulus* at a dose of 150 mg/kg b.w decreased the blood glucose and lipid levels in alloxan induced diabetic rats (Patra et al., 2009). Aqueous ethanolic leaf extract at a dose of 400 mg/kg b.w reduced also the blood glucose level in glucose loaded rats (Houacine et al., 2012). Incorporation of *E. globulus* leaf in diet (20 g/kg) and drinking water (2.5 g/L) had hypoglycemic effect and reduced oxidative stress in streptozotocin-induced diabetic rats (Nakhae et al., 2009).

**Faidherbia albida**

The administration of an aqueous extract from the stem bark of *F. albida* at dose 125 to 500 mg/kg b.w to alloxan-induced diabetic rats decreased significantly the fasting blood glucose level in a dose dependent manner and ameliorated the serum markers of the liver, feed and fluid intake, body weight and packed cell volume (Umar et al., 2014).

**Geigeria alata**

Diabetic rats orally treated with 250 mg/kg of *G. alata* root aqueous methanolic extract for 2 h (acute) appeared to have significantly lower blood glucose levels after 120 min. Constant treatment for 14 days of diabetic rats with 250 mg/kg of *G. alata* extract resulted in a significant decrease in blood glucose level (7.34±0.33 mmol/l) closer to that of nondiabetic rats. At the same time, it significantly decreased serum TTG levels, increased serum insulin levels, improved β-cell function, and the antioxidant status. *G. alata* also showed strong antioxidant and α-glucosidase inhibitory activities in *in vitro* assays (Hafizur et al., 2012).

**Guiera senegalensis**

A dose-dependent significant reduction in blood glucose levels which was more remarkable at the dose of 400 mg/kg was observed after the application of *G. senegalensis* leaves ethanolic extract (Houacine et al., 2012).

**Hyphaene thebaica**

Oral administration of aqueous extract of *H. thebaica* mesocarp experimentally caused a significant decrease in blood glucose level in Wistar albino rats, at 12 to 18 h post administration (Auwal et al., 2012). Aqueous extract improved glucose and insulin tolerance, and significantly lowered blood glycosylated hemoglobin levels. Chrysoeriol and 7-O-β-D-galactopyranosyl(1→2)-α-L-arabinofuranoside, which were isolated in the aqueous extract reduced significantly AST and ALT levels of liver and improved the kidney function (Salib et al., 2013).

**Khaya senegalensis**

The antidiabetic activity of *K. senegalensis* butanol fraction of the root ethanolic extract in type 2 diabetes model of rats was examined by Ibrahim and Islam (2014). The orally administered extract, at 300 mg/kg b.w, significantly reduced blood glucose level, improved oral glucose tolerance ability and β-cell function (HOMA-β), decreased insulin resistance (HOMA-IR), stimulated hepatic glycogen synthesis, ameliorated serum lipids alterations and prevented hepatic and renal damages compared to untreated diabetic rats. Additionally, the fraction tended to improve weight gain, decrease feed and fluid intake, stimulate insulin secretion and lower
serum fructosamine concentrations. Polyphenolic compounds such as catechin, rutin and procytanidins with significant antioxidant activities were also identified in different parts of the plant (Atawodi et al., 2009).

**Kigelia africana**

In streptozotocin-induced diabetic rats, daily administration of the defatted methanolic extract of *K. africana* flower at the doses of 250 and 500 mg/kg b.w for 21 days reduced significantly the blood glucose and the TC and TTG levels as well (Kumar et al., 2012). Similarly, methanolic extract from the leaves was found to significantly decrease (P<0.01) serum glucose level in alloxan-induced diabetic rats after the 21 days of oral treatment (Priya et al., 2014). The ethanolic extract, together with compounds catalpol, specioside and minecoside (10 μM) isolated from the n-butanol fraction exhibited significant stimulation of GLUT4 translocation to cell surface from intracellular compartments (Khan et al., 2012). Acetone, ethanol, chloroform, and water extracts of the leaves caused a significant α-amylase inhibitory effect (Dhriti et al., 2014). The root, stem bark, fruit and leaves were found to possess antioxidant activity (Atolani et al., 2011; Sikder et al., 2011; Agyare et al., 2013; Akanni et al., 2014).

**Lupinus termis**

A dose (75 mg/100 g b.w) of aqueous suspension from *L. albus* orally administered daily to alloxan-diabetic rats restore the changes in the levels of glucose, urea, creatinine and bilirubin and the enzymic activities of AST, ALT and lactate dehydrogenase (LDH) to their normal levels after 4 weeks of treatment (Mansour et al., 2012). In contrast, Sewani-Rusike et al. (2015) reported that the use of *L. albus* may not be effective in treating hyperglycaemia in type 1 diabetes but effective for treating diabetes induced dyslipidemia. They found that *L. albus* demonstrated significant hypoglycaemic effects in normal rats but not in diabetic rats after acute and long term treatment. Normal treated rats showed higher insulin levels compared to normal controls but insulin remained very low in diabetic rats. However, *L. albus* was effective in reducing atherogenic lipid levels.

**Mitragyna inremis**

Oral administration of aqueous extracts from *M. inremis* fruits at the level of 400 mg/kg to streptozotocin-induced diabetes rats, for 14 days, resulted in a significant antihyperglycemic effect and have the capacity to correct the metabolic disturbances associated with diabetes. Histopathological studies showed that the aqueous extracts of the plant reinforced the protection of liver (Alamin et al., 2015).

**Momordica balsamina**

Aqueous extract of *M. balsamina* seeds at the level of 500 mg/kg b.w dose caused a significant increase in the blood glucose levels of streptozotocin-induced diabetic rats. Furthermore, after three weeks of treatment of the diabetic animals with the aqueous extract (500 mg/kg b.w) blood sugar level was significantly higher compared to untreated diabetic rats; at the same time, lipid profile and body weight were improved (Bhardwaj et al., 2010). Moreover, aqueous and organic extracts of *M. balsamina* was screened against chang liver, C2C12 muscle and 3T3-L1 adipose cells using a glucose utilization assay. Results showed that *M. balsamina* extracts were active in myocytes and stimulated glucose utilisation in hepatocytes (van de Venter et al., 2008).

**Nauclea latifolia**

Aqueous leaves extracts of *N. latifolia* at the level of 200 mg/kg b.w significantly lowered glucose levels of the alloxan-induced diabetic rats within 4 h (Gidado et al., 2005). Moreover, the aqueous and ethanolic extracts significantly lowered the fasting blood glucose levels of the streptozotocin-diabetic Wistar rats in a dose-dependent manner after 1-6 h of administration (Gidado et al., 2008). The same results were observed when ethanolic extracts (100, 200 and 400μg/kg b.w) of the leaves were provided orally for 45 days to streptozotocin-induced diabetic rats (Abubakar et al., 2009). Significant reduction was found in the fasting blood glucose, lipid profile (TG and LDL) levels in diabetic rats administered 150 and 300 mg/kg b. w. of n-hexane and methanolic leaves fractions of *N. latifolia* (Effiong et al., 2014). Treatment of Swiss albino mice with 200 mg/kg b.w of ethanolic extract of leaves, twice a day for 21 days, decreased significantly blood glucose in diabetic animals and caused significant decrease (p<0.05) in TC, LDL level and ALT and AST activities (Sylvester and Dan, 2015).

**Nigella sativa**

Several studies demonstrated the hypoglycemic effect of *N. sativa* seed (Benhaddou-Andaloussi et al., 2011; Sathiavelu et al., 2013; Ikram and Hussain, 2014; El Rabey et al., 2017). The seed was shown to ameliorate biochemical and histopathological changes caused by diabetes, decrease oxidative stress, elevate level of insulin, reduce resistance of insulin and hepatic gluconeogenesis, enhance renewal of β-cells of islets of
Langerhans and create direct insulin-like effects at the cellular and molecular levels in various organs. Seed volatile oil and thymoquinone were found to possess the highest antidiabetic activity (Bamosa, 2015).

**Salvia officinalis**

The hypoglycemic effect of the aqueous ethanolic extract of *S. officinalis* leaves at the dose of 200 to 400 mg/kg is revealed as a dose-dependent significant reduction of blood glucose levels (Houacine et al., 2012).

**Sclerocarya birrea**

Following acute treatment, relatively moderate to high doses of *S. birrea* stem-bark aqueous extract (25 to 800 mg/kg b.w) induced a dose-dependent, significant reduction in the blood glucose concentrations of fasted streptozotocin-treated diabetic rats (Ojewole, 2003). Results from male Wistar rats subjected to oral load of glucose (4g/kg) after receiving a dose of 35 mg/kg of aqueous extracts of fresh leaves and barks of *S. birrea* showed that the extracts caused significant antihyperglycemic effects after 2 and 4 h (François et al., 2014). Aqueous and methanolic extracts of the stem bark inhibited the activities of α-amylase and α-glucosidase in a concentration dependent manner. Both extracts possess antioxidant activity, with the methanolic extracts displaying the strongest free radical scavenging capacity. Extracts also significantly increased glucose uptake in C2C12 myotubes, 3T3-L1 adipocytes and HepG2 hepatocarcinoma cells. However, insulin secretion from RIN-m5F cells was not affected (Mousinho et al., 2013). Crude *S. birrea* stem bark methanolic and acetone extracts inhibited human urinary α-amylase more potently than acarbose. Crude hexane extract displayed a strong inhibition of α-glucosidase and weak inhibition of α-amylase. Furthermore, the hexane extract significantly suppressed the rise in postprandial glucose level after oral administration of sucrose but failed to induce similar effects after oral administration of starch and glucose in both normal and diabetic rats (Mogale et al., 2011).

**Sesamum indicum**

Treatment of streptozotocin-induced diabetic rats with 500 mg/kg b.w ethanolic extract of *S. indicum* seeds for 8 weeks increased significantly the blood glucose and glycosylated hemoglobin levels but decreased significantly the serum insulin and hemoglobin levels. The liver glycogen level was significantly decreased in diabetic rats closer to normal revealing its potential effect to control hyperglycemia (Bhuvaneswari and Krishnakumari, 2012). Alloxan-induced diabetic rats provided with 10% and 20% seeds either raw or roasted as supplemented diet had significantly (p<0.05) lower levels of blood glucose, lipids and some serum enzymes (Akanya et al., 2015). Takeuchi et al. (2001) found that hot-water extract from defatted sesame seed and its methanolic fraction had a reductive effect on the plasma glucose concentration of KK-Ay mice, and this effect is suggested to have been caused by the delayed glucose absorption. Amutha and Godavari (2016) demonstrated that *S. indicum* can be used to reduce the postprandial hyperglycemia by inhibiting carbohydrates metabolizing enzymes α- amylase and α-glucosidase, and also to combat the free radicals due to its antioxidant activity. It has been reported that sesame seeds can improve oxidative status due to the activities of their contents including sesamin, sesamolin, sesamol, and sesame (Wichitsranoi et al., 2011).

**Striga hermonthica**

Daily oral administration of *S. hermonthica* whole plant aqueous extract (400 mg/kg b.w) to streptozotocin-induced diabetic rats for 14 days appeared to increase the blood glucose level, and did not improve the levels of TC, LDL, HDL, urea and blood urea nitrogen indicating that it has no antihyperglycemic effect (Alamin et al., 2015). Kiendrebeogo et al. (2005) found that the aqueous extract of *S. hermonthica* whole plant possessed antioxidant activity and they suggested that the isolated luteolin could be responsible for this activity.

**Tinospora bakis**

Acute and chronic treatment of streptozotocin-induced diabetes rats with aqueous extracts of *T. bakis* seeds at 400 mg/kg significantly lowered blood glucose levels, and had the capacity to correct the metabolic disturbances associated with diabetes. Histopathological studies showed that the aqueous extracts of the plant reinforced the healing of liver (Alamin et al., 2015).

**Trigonella foenum-graecum**

Animal’s standard diet supplemented with seeds of *T. foenum-graecum* (5%) for 30 days to alloxan induced diabetic rats significantly decreased the levels of glucose, TG, TC and LDL-CH and increased the level of HDL-CH. Also it reduced the oxidative stress by improving the superoxide dismutase, catalase and glutathione peroxidase activities both in serum and in pancreas homogenate (Beji et al. 2016). Aministration of *T. foenum-graecum* seeds (2.5 and 5 g) for 4 weeks to Tunisian type 2 diabetic patients, improved blood glucose
level in dose-dependent and the dose of 5 g reduced significantly TC and TG levels and serum α-amylase activity (Khlifi et al., 2016).

Zygophyllum coccineum

A dose of 1.5 mL of aqueous suspension of Z. coccineum herb/100 g b. w (equivalent to 75 mg/100 g b.w), orally administered daily to alloxan-diabetic rats for 4 weeks, restored significantly (P<0.05) the changes at the levels of glucose, urea, creatinine and bilirubin and the activities of AST, ALT, LDH and alkaline phosphatase enzymes in plasma, liver and testes (Mansour et al., 2002). Moreover, 1.5 mL of water soluble extract/kg b.w of the herb, administered orally to alloxan-induced diabetic rats daily for 4 weeks, significantly decreased the blood glucose level and the activity of cytochrome P450, NADPH-cytochrome C reductase, aryl hydrocarbon (benzo(a)pyrene) hydroxylase (AHH), N-nitrosodimethylamine N-demethylase I (NDMA-dl), NADPH-cytochrome C reductase, and detoxified by glutathione S-transferase (GST) and glutathione (GSH) enzymes in the liver of diabetic rats (Sheweita et al., 2002). The leaves were found to possess antioxidant activity (El-Shora et al., 2016). Various compounds from the leaves of Z. coccineum were identified by gas chromatography–mass spectrometry (GC/MS) like 1-nonadecene, 9-octadecenoic acid, 2-methyl propanoic acid, β-sitosteryl, tricosane and tetracosenes, Stigmast-5-en-3-ol, docosene, 1-ecosanol, hexacosane, heptacosane, nonacosane, 6-Ethyl-5-hydroxy-2,3,7-trimethoxynaphthoquinone and pentacosane (El-Shora et al., 2016).

DISCUSSION

Sudan is a developing country that frequently depends on folk medicine in all areas of the country. Several herbal preparations have been used in folklore practice for the management of diabetes with claims asserting their hypoglycemic effect. In this paper, an effort was made to refer to the different parts of 38 plant species that are used in the Sudanese traditional medicine (Table 1). Interestingly, some of these plants have already been reported in previous studies originated from other countries like Algeria (Houacine et al., 2012), Iran (Mikaili et al., 2013), Egypt (Helal et al., 2014), India (Singh et al., 2015), Nigeria (Auwal et al., 2012) and Saudi Arabia (Bamosa, 2010). The reviewed plants have been evaluated, in in vivo experiments with diabetic animals that were induced either by alloxan or streptozotocin (Fröde and Medeiros, 2008) in addition to genetically mutated in vivo models such as KK-Ay mice. Ten of the characterized plants (Acacia nilotica, Catunaregam nilotica, Cicer arietinum, Cinnamomum verum, Geigeria alata, Guiera senegalensis, Khaya senegalensis, Mitragyna inremis, Momordica balsamina and Tinospora bakis) tested effective in animal models for their antidiabetic potential from samples collected from Sudan.

In vitro pharmacological evidence

From the 38 plants reviewed in this paper, only four of them were not tested for hypoglycemic activity, either in vivo or in vitro. Only one from the 34 plant species was ineffective in lowering blood glucose level, namely Striga hermonthica (Alamin et al., 2015), suggesting lack of antidiabetic effect. Ezuruike and Prieto (2014) reported that the absence of an in vivo antihyperglycemic effect of some plants would not be a reason to stop their use as antidiabetics, since they may used in multicomponent preparations because of their benefits in co-morbid conditions or possibly be the foundation for comprehensive control of the disease and consequent complications. In fact, components aqueously extracted from S. hermonthica whole plant reduced the TG level, improved several liver parameters (reduced ALT activity) and possessed high antioxidant activity (Alamin et al., 2015; Kiendrebeogo et al., 2005). Many of the plants described in the present review have been studied in in vitro models that could possibly explain some of their mechanisms of action. Information on the mechanism of action would be an important element in implementing a therapeutic plan for diabetes, considering the likely benefit of the synergy of medicinal plants (Ezuruike and Prieto, 2014). Four plants (Acacia nilotica, Capparis decidua, Geigeria alata and Sclerocarya birrea) have inhibitory effects against either α-amylase or α-glucosidase enzymes; Seven plants (Ambrosia maritima. Balanites aegyptiaca, Geigeria alata, Hyphaene thebaica, Khaya senegalensis, Sclerocarya birrea and Sesamum indicum) induce secretion of insulin from β-cells of the pancreas; five plants (Balanites aegyptiaca, Cinnamomum verum, Kigelia africana, Momordica balsamina and Trigonella foenum-graecum enhance glucose absorption in muscles or liver or increase GLUT4 gene expression leading to enhanced glucose absorption by muscle and fat tissue and one plant decrease the activity of sucrose enzyme and offer a support to control carbohydrate hydrolysis in diabetic disease.

Bioactive compounds

A number of active compounds have been identified from the plants in this review paper but their role in diabetes management was not proved for most of them. However, trigonelline (3-carboxy-1-methyl pyridinium) was identified in Balanites aegyptiaca fruits (8 and 13 mg in the peel and pulp respectively) by Farag et al. (2015). Its discovery provides novel insight into the balanite fruits
antidiabetic properties as the compound is known for a pronounced hypoglycemic effect (Farag et al., 2015). More recently, 3, 5-dicaffeoylquinic acid was found to be the dominant acylquinic acid in Geigeria alata roots (25.96±2.08 mg/g dry weight) and ameliorated significantly (P < 0.05) the blood glucose and liver biochemical parameters in streptozotocin-induced (40 mg/kg, i.p.) diabetic normotensive Wistar rats and spontaneously hypertensive rats (Simeonova et al., 2016).

Clinical studies

Clinical evaluation, involving human subjects, of biologically active plants is necessary towards the progress of incorporation of medicinal plant products in the health service system (Ezuruike and Prieto, 2014). In this review, 10 plants sourced from Sudan were subjected to clinical trials in Type 2 diabetic patients and results showed that the order of effectiveness of the aqueous extracts of the studied plants to lower fasting blood sugar level was Lupinus albus > Balanites aegyptiaca > Allium Sativum > Allium cepa > Guiera senegalensis > Aloe sinkatana > Hyphaene thebaica > Trigonella foenum-graecum (Gaber et al., 2013). Capsules containing Nigella sativa seeds were administered orally to human volunteers, in Saudi Arabia, in a dose of 1, 2 and 3 g/day for three months. The dose of 2 g/day caused significant reduction in fasting blood glucose levels, while β-cell function was increased after 12 weeks of treatment (Bamosa, 2010).

Toxicological evidence

Assessment of the safety and toxicity profile of herbal medicine is essential to ensure its therapeutic potential. A summary of the studies that describe the toxicological effects of medicinal plants is presented in Table 2. It has been noted that the majority of the investigations corresponded mainly to the determination of acute toxicity and safe dose and included very limited information concerning toxicological and herb–drug interactions. However, some of the plants listed in Table 2, like Allium cepa, A. sativum, Cinnamomum verum and Trigonella foenum-graecum are actually consumed frequently in Sudan and other countries, and are usually perceived as safe. However, Zaid et al. (2010) reported that garlic and onion bulbs share many similar active compounds (for example, allyl propyl and diallyl sulfide) and decrease blood glucose levels also by normalizing liver hexokinase and glucose-6-phosphatase activities and increase insulin secretion from the pancreas but excessive consumption of these two bulbous plants might lead to harmful effects. T. foenum-graecum and C. verum exhibited cytotoxic effects at concentrations higher than 500 µg/mL (Kadan et al., 2013). Moreover, consumers are usually aware of possible health hazards occurring after consumption of certain plants and the necessity of their proper process to remove toxicants before utilization. For example, the toxic lupinine found in Lupins (Lupinus termis) is removed through debittering process, including soaking in water and daily replacement of water until bitterness disappears before the seeds could be safely consumed. Although, people in Sudan and other African countries consume kawal (fermented fresh leaves of Senna obtusifolia), studies have shown that fermentation has not altered the toxic activity of the ingredients in the leaves (Yagi et al., 1998). Thus, toxicological evaluation of medicinal plants is equally significant as their evaluation for efficacy and there is an urgent need for a vibrant pharmacovigilance system to ensure their use in therapeutic management (Shaw et al., 2012).

Conclusion

The quest for control of diabetes has led to an increasing research at different fronts, among which is medicinal plants. Given the observation of an increasing use of medicinal plants for diabetes in Sudan, this necessitates validation of efficacy and safety. In vitro experiments are carried out to ascertain the mechanism of action of medicinal plants. The hypoglycemic effect of certain plants arises as a side effect of their in vivo toxicity (Marles and Farnsworth, 1995). However, a risk may be posed by the fact that such hypoglycemic effect is probably partially exhibited via an unfavourable physical mechanism overriding a physiological one. As for the validation of experiments, ethical considerations concerning animal use are increased (Festing and Wilkinson, 2007), and therefore the use of non-animal models should be seriously considered. A number of standardization measures, such as reference pharmacopeial monographs, are necessary to assert the medicinal value of these herbal medicines as reliable and therapeutically effective. Case studies involving standardized medicinal plant products should be carried out in order to validate the usefulness of plant preparations in diabetes management, which will give support to the pre-clinical results.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Evaluation of antihypertensive and vasorelaxant effects of *Heimia salicifolia* (family: Lythraceae)

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There are reports of the presence of vasodilatory alkaloids in *Heimia salicifolia* extracts. The action mechanisms of the potential antihypertensive effect of such alkaloids have not however, been elucidated. The objective of the present study was to corroborate the antihypertensive and vasorelaxant activities of the isolated alkaloids from *H. salicifolia*, and to characterize their vasorelaxant actions. A chloroform extract (HSCE) was obtained from *H. salicifolia*. The alkaloids were separated by thin layer chromatography, extracted from silica gel, and evaluated using spectroscopy. The alkaloid with the most potent vasorelaxant activity was identified as lythrine. The antihypertensive effect of the HSCE was corroborated on normotensive rats and on animals with Nω-nitro-L-arginine-methyl ester (L-NAME) induced hypertension. The action mechanisms of the HSCE and lythrine were studied on isolated and perfused rat mesenteric vascular bed (MVB) preparations. HSCE administration produced a concentration-dependent relaxation, but in preparations without vascular endothelium, the relaxant response to HSCE was significantly diminished. In MVB preparations with intact vascular endothelium, pre-contracted with phenylephrine or L-NAME, perfusion with lythrine produced concentration-dependent relaxation; a similar effect was produced by acetylcholine. Wortmannin (phosphatidylinositol 3-kinase inhibitor) and methylene blue (guanylate cyclase inhibitor) decreased the relaxant effect of lythrine. These data suggest that the vascular relaxation induced by alkaloids isolated from *H. salicifolia* is dependent on the activation of the nitric oxide/guanylate cyclase pathway.

Key words: *Heimia salicifolia*, lythrine alkaloid, hypertension, Nω-nitro-L-arginine-methyl ester (L-NAME), mesenteric vascular bed.

INTRODUCTION

Hypertension can be defined as chronic elevation of systolic and/or diastolic blood pressure above 140/90 mmHg.
Hg. It is a major risk factor for cardiovascular diseases such as congestive heart failure, coronary artery disease, stroke, and renal disease. Despite progress in prevention, detection, treatment, and control of high blood pressure, hypertension remains an important public health problem (Go et al., 2014), and its prevalence has been gradually increasing worldwide. The World Health Organization (WHO) reported in 2013 that hypertension is a major public health issue and it is the cause one in every eight deaths. Globally, cardiovascular diseases account for approximately 17 million deaths/year, and complications of hypertension account for 9.4 million deaths/year worldwide (World Health Organization, 2013). In Mexico, hypertension is considered a national health problem (Balam-Ortiz et al., 2014). Hypertension prevalence in Mexican adults ≥ 20 years was 23.8% in 1993, 30.8% in 2006, and 31.5% in 2012 (Campos-Nonato et al., 2013). Many synthetic antihypertensive drugs have been commonly used for hypertension control in developed countries. Some of these drugs have many side effects. Complementary and alternative medicine may have the potential for hypertension treatment (Magos et al., 2017; Baharvand et al., 2017). Herbal medicines still remain the most popular choice in certain developing countries. The extensive use of herbal remedies in such nations has led to extensive research in this area to determine their potential efficacy. Several modern cardiovascular drugs are now available as natural/herbal products (Tirapelli et al., 2010; Luna et al., 2013).

There are several regions in Mexico with many plants used as herbal remedies that may represent an opportunity for the discovery of new antihypertensive drugs (Hernández et al., 2013). Also, it has been demonstrated that a partial separation of *H. salicifolia* alkaloids produced antihypertensive effects on angiotensin II-caused acute hypertension. Nevertheless, the pharmacological properties of *H. salicifolia* have not yet been widely studied. The aim of the present study was to investigate the antihypertensive and vasorelaxant activity of the isolated alkaloids from *H. salicifolia* and to characterize its possible vasorelaxant action.

**MATERIALS AND METHODS**

**Study species**

*Heimia salicifolia* (H.B&K) Link & Otto (Lythraceae family) is a wild flowering shrub distributed over Mexico, Western Texas, El Salvador, Jamaica, Uruguay, and Argentina (Malone and Rother, 1994). This plant is known as sun opener (Graham, 1997). In Mexico, the most common name is sinicuichi (Guzman et al., 2006). *H. salicifolia* has been used in folk medicine in several countries for different indications, as an emetic, haemostatic, tonic laxative, diuretic, anti-inflammatory, and to treat some syphilis symptoms (Malone and Rother, 1994; Aguilar et al., 1994). Due to its psychotomimetic activity, native people in Central America and Mexico have used the plant for shamanic purposes (Graham, 1997). After the administration of alcohol decoction or plant juice, the subjects experiment a variety of effects such as yellow-colored vision and auditory distortion in which bells or voices sound as if they were originated in farther distances (Malone and Rother, 1994). Phytochemical analyses have shown that *H. salicifolia* contain bisphenylquinolizidine lactone alkaloids (Malone and Rother, 1994). More than 20 of these alkaloids such as vertine, sinicuichine, lythrine, nesodine, lyfoline, dehydrocodeine, and demethyllasubine I, have been isolated (Blomster et al., 1964; Malone and Rother, 1994). Vertine, lyfoline, lythrine, and nesodine seem to be the primary source of the traditional effects of *H. salicifolia*. It also has been reported that these alkaloids possess cardiovascular effects; nesodine produced a fall in blood pressure (Malone and Rother, 1994; Kaplan and Malone, 1966). Another reported effect for cryogenine and nesodine is the inhibition of prostaglandin synthase, which may support the folklore anti-inflammatory usage of *H. salicifolia*. Cryogenine has been reported to have anti-inflammatory activity similar to that of aspirin (Lema et al., 1986). Rumalla et al. (2008) isolated two more alkaloids from *H. salicifolia* and elucidated its chemical structure using spectroscopic techniques. The new alkaloids showed antimarial activity. There are no ethnomedical reports of *H. salicifolia* being used as an antihypertensive drug. It has been found that the aqueous extract from *H. salicifolia* leaves decreased the systolic blood pressure in anaesthetized normotensive rats.

**Chemicals and drugs**

Phencythrine bitartrate (Phen), acetylcholine chloride, N-nitro-L-arginine methyl ester (L-NAME), atropine, methylene blue, wortmannin and diphenhydramine were purchased from Sigma-Aldrich Co. (St Louis, MO, USA). All other reagents were analytical grade from Merck, Germany.

**Experimental animals**

The experiments were performed in male Wistar rats (300-350 g) breed in the Animal Facility of the Facultad de Estudios Superiores Iztacalca (FESI), Universidad Nacional Autonoma de Mexico (UNAM). Female animals were not studied to avoid physiological changes due to oestrus cycle. Animals were housed under conditions of controlled temperature (24±0.5°C) and with 12 h light/12 h dark photoperiod. They were fed rat chow (Ralston-Purina) and water *ad libitum*. All animal procedures and protocols were conducted in accordance with the Federal Regulations for Animal Experimentation and Care (NOM-062-ZOO-1999, Ministry of Agriculture, Mexico) on care and use of laboratory animals, and approved by the institutional ethics review board.

**Plant material**

Dried leaves of *H. salicifolia* were obtained from the Sonora Market, a medical plants selling place in Mexico City. The plant’s botanical identity was verified at the IZTA Herbarium of the Botanic Department, Facultad de Estudios Superiores Iztacalca and a voucher specimen numbered 41653 was deposited at the same herbarium for reference.

**Preparation of chloroform extract and alkaloids isolation**

A published method for chloroform extract preparation and alkaloids isolation from *H. salicifolia* was used (Blomster et al., 1964). Briefly, 5 kg of *H. salicifolia* leaves were dried at 45°C and powdered. Afterwards, to remove fat, 4 L of petroleum ether (J.T. Baker) was added; then the plant material was macerated in 5 L of methanol
(J.T. Baker) during 24 h, and concentrated in a rotary evaporator (Buchi Rotavapor model Mp60) under reduced pressure to get a final volume of 50 mL. Alkaloids detection was carried out with Dragendorff reagent. Methanol concentrate was dried at 50°C for 24 h, in addition to 100 mL distilled water and acidified to pH 2.0 with 10% hydrochloric acid solution (Tec. Chem.), and filtered through celite (Sigma Chem.); the precipitate was washed with distilled water. The aqueous acidic filtrate was further defatted in a continuous extractor with 500 mL ethyl ether. The pH was adjusted to 9 with 28% ammonium hydroxide solution, continuously extracted with 200 mL chloroform. This H. salicifolia chloroform extract (HSCE) was dried in vacuum at 40°C with a yield of 15 g (approximately 0.3%). HSCE was used for biological experiments and for alkaloids isolation.

Alkaloids isolation from HSCE

Dried HSCE was dissolved in chloroform, adsorbed on basic alumina (J.T. Baker), dried, and placed on the top of a column (70 x 2 cm) of basic alumina (J.T. Baker). Elution was done with a mixture of methanol/chloroform (1:1) and finally with methanol. The effluent was collected in three fractions. Each fraction was dried in vacuum and chemical tests were done for alkaloids. Thin-layer preparative chromatography of the fractions was performed on silica gel G plates (Merck), with chloroform/methanol (3:2) (v/v); the chromatograms were observed with an ultraviolet lamp (UVG-L-25) at 250 nm. The separated fractions were marked, eluted from the plate and examined again for alkaloids with 4-nitroanilindiazothized reagent; thereafter, four principal alkaloids were separated and assayed in biological tests. The alkaloid with higher biological activity was the alkaloid 3 (Guzman et al., 2006), which was isolated and prepared for chemical identification by NMR studies.

Alkaloid identification

1H-NMR spectrum was recorded at 500 MHz, 13C-NMR at 75 MHz, on a Varian Inova; chemical shifts (PPM) were relative to (CH3)4 Si as internal reference, and CDCl3 (Aldrich) as solvent. The 1H NMR and 13C NMR data were obtained on a Varian Unity 300 instrument. Comparisons were made with spectra known of these alkaloids using the Organic Chemistry Data Base program.

Effect of HSCE on blood pressure in normotensive rats

The rats were randomly allocated into groups of six each. The Group 1 (control) received vehicle (carboxymethyl cellulose), whereas Groups 2, 3, and 4 received 10, 20, and 40 mg/kg of HSCE for 10 days p.o., respectively. At the beginning and end of treatment, systolic arterial blood pressure (SBP) was measured non-invasively using a tail-cuff computer-aided monitoring device (Automatic Blood Pressure Computer, Model LE 5007; Letica Scientific Instruments, Barcelona, Spain) as follows. The rat was restrained in a size-matched plastic container, and an inflatable latex ring containing a sensor was placed over a tail artery while the rat was kept warm (37°C) in the same device. The rats were trained to be inside the container with the cuff placed on the tail and to get used to the inflation and deflation of the latex ring. The SBP measurements were recorded for each group, and the mean of three measurements obtained in one session was considered.

Effect of HSCE on blood pressure during chronic hypertension caused by inhibition of nitric oxide synthase

Rats were randomly allocated into groups of six each. Group 1 (control) received vehicle; Group 2: Nω-nitro-L-arginine-methyl ester (L-NAME) (70 mg/Kg, Sigma-Aldrich, St. Louis, MO, USA); Group 3: L-NAME + enalapril (5 mg/kg/day, Sigma-Aldrich, St. Louis, MO, USA); Group 4: L-NAME + HSCE (10 mg/kg/day); and Group 5: L-NAME + HSCE (20 mg/kg/day) for 10 days. All drugs were administered in carboxymethyl cellulose and L-NAME in the drinking water. The actual doses in each group were calculated from the daily water intake. SBP measurement was done using the aforementioned technique.

Phenylephrine-induced vasoconstriction

To measure the MVB vasoconstrictor response, concentration-response curves to phenylephrine (Phen) were constructed. Increasing Phen doses (0.5 to 80 µg) were injected in bolus with a 50 µL Hamilton syringe. The injection volume was 0.1 mL. The interval between injections was 5 min or the time needed for the perfusion pressure to return to its initial value, which was never longer than 10 min. In other preparation, the MVB was constricted with Krebs solution containing Phen (10-3 M) to induce submaximal vasoconstriction (about 90%) and allowed to reach a plateau. When the MVB constriction was stable (30-45 min), concentration-response curves to acetylcholine (ACh) were performed; ACh was injected in 0.1 mL volumes and 10-90 µg doses, causing a dose-dependent relaxation, recorded as a decrease in perfusion pressure. The injection duration time was 10 s. Concentration-response curves to HSCE (10-90 µg) were drawn using the same method. In some preparations, the endothelium was removed by perfusion with distilled water for 10 min (Criscone et al., 1984). Concentration-response curves were constructed using the previously described parameters with the MVB pre-contracted with Phen. A bolus injection of ACh (80 µg) was applied. Vascular endothelium removal was confirmed by the absence of a relaxation response. To study the possible vasorelaxant mechanism exerted...
by the alkaloid 3 isolated from HSCE, MVB with intact endothelium was perfused for 10 min with L-NAME (10^{-4} M), (unspecific nitric oxide synthase inhibitor), methylene blue (10^{-3} M) (soluble guanylate cyclase inhibitor), wortmannin (10^{-5} M) (phosphatidylinositol 3-kinase inhibitor) and/or diphenhydramine (10^{-5} M) (histamine H_{1} receptor antagonist) and concentration-response curves to alkaloid 3 (20-80 µg in bolus) were performed. A maximum of 3 curves per preparation was constructed.

**Statistical analysis**

Data are expressed as mean ± standard error of the mean (SEM) of at least five experiments. Means were compared by unpaired Student’s \(t\)-test; concentration-response curves by two-ways ANOVA and Bonferroni’s post-hoc test. Differences were considered to be statistically significant with \(p \leq 0.05\).

**RESULTS**

**HSCE effects on SBP of normotensive and hypertensive rats**

Oral HSCE administration of 10 and 20 mg/kg for 10 days decreased the SBP in normotensive rats (from 115±3 to 85.5±4.5, and 96.8±4.2 mm Hg respectively); the 40 mg/kg dose did not cause SBP reduction (Figure 1a). Chronic NO inhibition by L-NAME was associated with a progressive rise in the SBP, reaching a maximum of 164±2 mm Hg at Day 10, as compared with the control group (115±3 mm Hg). HSCE administration (10 and 20 mg/kg, v. o) produced SBP reduction (148±3 mm Hg). Enalapril also reduced SBP to 131±2.5 mm Hg (Figure 1b).

**Effect of HSCE on MVB from normotensive rats with and without endothelium**

Baseline perfusion pressure of isolated and perfused MVB from normotensive rats was 50±2.2 mm Hg. Phen (0.5-80 µg) bolus administration induced a concentration-dependent vasoconstriction; 40 µg of Phen increased the perfusion pressure to 84±1.2 mm Hg. Constant perfusion with Phen (10^{-5} M) added to the Krebs’ solution increased the perfusion pressure to 84±1.2 mm Hg. When the perfusion pressure reached a plateau (Figure 2), an ACh bolus injection produced concentration-dependent relaxation, with 90 µg of ACh reducing the perfusion pressure to 25±1 mm Hg (Figure 3a). HSCE administration (10-90 µg) produced concentration-dependent relaxation whereas 70 µg of HSCE decreased the perfusion pressure to 27±3 mm Hg (Figure 3b). When vascular endothelium was removed and pre-contracted with Phen, the relaxant response to ACh and HSCE was significantly diminished (Figures 3a and 3b).
**Figure 2.** Phenylephrine-dependent vasoconstriction of isolated perfused mesenteric vascular bed of rats, with intact endothelium. Each point represents the mean ± S.E.M., from 6 experiments. *p< 0.05.

**Figure 3.** Effect of acetylcholine (a) and HSCE (b) on the relaxation of isolated perfused mesenteric vascular bed of rat phenylephrine ($10^{-5}$ M) pre-contracted in the presence (+E) or absence (−E) of functional endothelium. Each point represents the mean ± S.E.M., from 6 experiments. *p< 0.05.
Figure 4. Chemical structure obtained with $^{13}$C NMR and spectrum $^1$H NMR from purification of alkaloid 3 obtained from *H. salicifolia* chloroform extract (HSCE). This chemical structure corresponds to lythrine.

Table 1. $^1$H and $^{13}$C NMR chemical shift assignments of alkaloid 3 (lythrine) in CDCl₃.

<table>
<thead>
<tr>
<th>Position</th>
<th>$^1$H (J/Hz)</th>
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<td>3</td>
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<td>7.07dd 8.2, 1.4</td>
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<td>5</td>
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Alkaloids isolation and identification

Four alkaloids were separated by thin layer chromatography; their retention factors ($R_f$) were as follows: alkaloid 1, $R_f = 0.392$; alkaloid 2, $R_f = 0.87$; alkaloid 3, $R_f = 0.55$; and alkaloid 4, $R_f = 0.35$. The alkaloids were extracted of silica gel and assayed in normotensive rats and in animals that developed hypertension by angiotensin II administration. Alkaloid 3 showed high anti-hypertensive activity (Guzman et al., 2006), so it was isolated and prepared for structural chemical identification. Spectroscopic studies identified alkaloid 3 as lythrine, with its chemical structure similar to that of the quinolizidine alkaloids group (Figure 4). The spectroscopic data of the active compound were UV ($\lambda_{max}$) 279.9; $^1$H and $^{13}$C NMR (Table 1), corresponding to those reported for lythrine (Rumalla et al., 2008). Thus, in the following experiments, alkaloid 3
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Figure 5. Effect of L-NAME (10⁻⁵M) (panels a and b), wortmannin (10⁻⁵M) (panels c and d), and methylene blue (10⁻⁵M) (panels e and f) administration on the endothelium-dependent relaxation induced by acetylcholine (panels a, c and e) or lythrine (panels b, d and f) in phenylephrine (10⁻⁵M) pre-contracted rat mesenteric vascular bed. Each point represents the mean ± S.E.M., from 6 experiments. *p< 0.05.

is referred as lythrine alkaloid.

Effect of lythrine on endothelium-derived NO-cyclic GMP pathway in MVB

A concentration-response curve for the lythrine alkaloid on the isolated and perfused MVB from normotensive rats with intact endothelium was constructed. Lythrine did not modify the baseline perfusion pressure (50±2.2 mm Hg). In Phen pre-contracted preparations, constant perfusion with lythrine (20-80 μg) produced concentration-dependent relaxation (8.8±0.6, 20.4±1.9, 23.6±1.7 and 23.6±1.7 mm Hg respectively). In all the concentration-response curves, ACh was the control drug producing similar relaxant effect as lythrine (Figures 5). When the MVB was perfused with 10⁻⁴ M L-NAME, the vasodilatation induced by lythrine alkaloid decreased (perfusion pressure 7±2 mm Hg, Figure 5b). A similar effect was observed with ACh (Figure 5a).

In the presence of 10⁻⁵ M wortmanin, the relaxant effect of lythrine (20-80 μg) was only 1.6±2 mm Hg (Figure 5d), similar to that observed with ACh (Figure 5c).

The perfusion with 10⁻⁵ M methylene blue decreased the relaxant effect of lythrine. The maximum relaxant effect was 7.4±1.3 mm Hg (Figure 5f), similar to that observed with ACh (Figure 5e).

These data suggest that lythrine exerts its relaxant effect on functional endothelium through the nitric oxide synthesis pathway.
Figure 6. Effect of atropine (10⁻⁵ M) administration on the endothelium-dependent relaxation induced by acetylcholine (panel a) or lythrine (panel b) in phenylephrine (10⁻⁵ M) precontracted rat mesenteric vascular bed. Each point represents the mean ± S.E.M., from 6 experiments. *p<0.05.

Lythrine-induced vasorelaxation in MVB treated with atropine and/or diphenhydramine

In an isolated perfused MVB preparation with intact endothelium pre-contracted with Phen in the presence of 10⁻⁵ M atropine, the relaxant effect of lythrine (20-80 μg) was decreased (4.8±0.7, 4.8±0.7, 7±1.8, 7±1.5 mm Hg, respectively) (Figure 6b). A similar concentration-response curve was obtained with ACh (Figure 6a), suggesting that the vasodilatory effect of lythrine requires binding to a muscarinic receptor and an intact pathway of the nitric oxide synthesis.

In an isolated perfused MVB preparation pre-contraction with Phen, histamine (20-80 μg) produced a relaxant effect (18±1.5, 22±1.03, 24±0.68 and 24±0.51 mm Hg respectively) (Figure 7a). When the preparation was perfused with 10⁻⁵ M diphenhydramine, the histamine relaxant effect was blocked. On the other hand, the lythrine relaxant effect was not affected by diphenhydramine (Figure 7b), suggesting that the lythrine vasodilatory effect is independent of the H₁ histamine receptors.

DISCUSSION

*H. salicifolia* contains alkaloids with antihypertensive activity. Four alkaloids with vasorelaxant effect were obtained from *H. salicifolia* chloroform extract (HSCE). The alkaloid with the highest vasorelaxant activity, lythrine, was isolated a decade ago (Rumalla et al., 2008), but pharmacological studies on the compound have not been reported. The results suggest that the HSCE vasorelaxant effect depends on the release of nitric oxide (NO) or a NO-mediated substance. HSCE administration (10 and 20 mg/kg) to normotensive rats or animals with L-NAME-induced hypertension decreased the blood pressure. In MVB preparations with the endothelial cells eliminated by perfusion with distilled water (Criscone et al., 1984), HSCE-induced vasodilatation was significantly reduced, suggesting that it is mediated by the release of endothelium-derived substances. Vascular tone and therefore blood pressure is determined by the contractile state of vascular smooth muscle cells within the blood vessel wall. The role of endothelium is of particular importance since it regulates the vascular leiomyocytes tone by releasing potent vasoconstrictor molecules such as endothelin-1 and vasodilator substances such as NO (Godo and Shimokawa, 2017). NO is an important regulator of vascular tone and blood pressure. It has been reported that the pharmacological reduction of NO can lead to hypertension in normotensive rats (Raghavan and Dikshit, 2004; Wang et al., 2017). L-NAME administration decreases NO synthesis and develops hypertension in rats (Leo et al., 2015). In this study, animals receiving
Figure 7. Effect of diphenhydramine (10^{-5}M) administration on the endothelium-dependent relaxation induced by histamine (panel a) or lythrine (panel b) in phenylephrine (10^{-5}M) precontracted rat mesenteric vascular bed. Each point represents the mean ± S.E.M., from 6 experiments. *p< 0.05.

simultaneous administration of L-NAME and HSCE showed lower systolic blood pressure as compared with animals receiving only L-NAME. Similar data were reported for Nitraria sibirica (Senejouxa et al., 2012), Bidens pilosa (Bilanda et al., 2017), Ulmus wallichiana (Syeda et al., 2016), Antidesma thwaitesianum (Kukongviriyapan et al., 2015), plants with NO-mediated hypotensive effect. The in vitro studies using the MVB preparation pre-contracted by Phen also support that the vasorelaxant mechanism of lythrine is likely to be mediated by activation of NO release from vascular endothelium, since the removal of endothelial cells led to absence of the response to HSCE. H. salicifolia exerted an important vasorelaxant effect both in vivo and in vitro. The major compound identified in HSCE was the lythrine alkaloid as its vasorelaxant effect has not been previously described. In this study, its action mechanism was elucidated using in vitro MVB preparations. In the preparation of isolated perfused MVB pre-contracted with phenylephrine and intact endothelium, the effect of the lythrine seemed to be endothelium dependent, as the vasorelaxant action of lythrine was abolished by L-NAME perfusion.

The involvement of cGMP in the relaxant and hypotensive effects of lythrine was verified. The soluble guanylate cyclase inhibitor, methylene blue, completely abolished lythrine-induced vasodilatation, an effect similar to that produced by Orthosiphon stamineus (Yam et al., 2016) and Praeruptorin A (Xua et al., 2010).

Activation of endothelial nitric oxide synthase (eNOS) depends on the formation of calcium–calmodulin complex (Su et al., 2014). However, eNOS can also be activated by phosphorylation with PI3K, a calcium-independent mechanism (Zhang et al., 2014). The PI3K-dependent activation of eNOS by wine-derived polyphenolic compounds has been reported (Ziberna et al., 2013). In the present study, the MVB was pre-contracted with phenylephrine and wortmannin, a PI3K inhibitor. Wortmannin strongly reduced lythrine-induced vasodilatation, suggesting that PI3K-dependent activation of eNOS is an important underlying mechanism in the vasodilator effect of lythrine, similar to that reported for Tapirira guianensis (Rodrigues et al., 2017).

Atropine reduced lythrine-induced vasodilatation, suggesting that the vasodilator effect of lythrine is mediated through the interaction with cholinergic muscarinic receptors. A similar action mechanism has been reported for other herbal remedies, such as O. stamineus (Yam et al., 2016) and Cymbosema roseum (Rocha et al., 2015). To verify if the effect of lythrine is mediated by muscarinic receptors with some degree of specificity, the MVB was perfused with phenylephrine...
plus diphenhydramine, an H₁ receptor antagonist.

Lythrine showed vasodilatory effect both in the presence and absence of diphenhydramine, suggesting that it is mediated by muscarinic receptors and not by histamine H₁ receptors.

**Conclusion**

This study demonstrated that lythrine alkaloid isolated from *Heimia salicifolia* induces vascular relaxation in isolated MVB, which could explain its hypotensive effect in normotensive and hypertensive rats. These effects appear to be dependent on the activation of the NO/guanylate cyclase pathway. The development of pharmacological strategies capable of stimulating or maintaining endogenous NO production may contribute to improved management of several pathological conditions, such as hypertension. These findings have shown the potential of *Heimia salicifolia* as an herbal remedy, and contribute to providing the pharmacological basis for its use in folk medicine.

**CONFLICT OF INTERESTS**

The authors have not declared any conflicts of interests.

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