Study the physiological and immunological effects of pumpkin extract on wound healing in diabetic rats

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Abstract

The current study was designated to investigate the effect of pumpkin aqueous extract on blood glucose and wound healing in diabetic-wounded rats. Forty male rats were divided into five groups (8 rats for each). G1: served as healthy control, G2: Positive control, wounded and diabetic rats, G3: Wounded and diabetic rats treated with aqueous extract 200 mg/kg b.wt., G4: Wounded and diabetic rats treated with aqueous extract 200 mg/kg b.wt. and wounds treated locally with aqueous extract 200 mg/kg b.wt. as ointment (mix with inert Vaseline) and G5: healthy (non-diabetic) rats, wound treated locally with aqueous extract 200 mg/kg b.wt. The effects of aqueous extract on some parameters were investigated in normal and diabetic rats such as glucose level, tumor necrosis factor alpha (TNF-α), platelets derived growth factor (PDGF), total and differential count of WBC. The results investigated a significant (P≤0.05) decrease in blood glucose level in all treated diabetic groups compared with positive groups on 8th day and an improvement in healing in wounds in rats after 8 days of treatment with plant extract, the level of TNF-α and PDGF were significantly (P<0.05) increased in the positive control group and other diabetic groups as compared with negative control on the 4th day, then it showed decrease in all treated groups as compared with positive control group on the 8th day, total count of WBC showed a significant increase in the positive control group and other diabetic groups as compared with negative control on the 4th day, then it showed decrease in all treated groups except the positive control group on the 8th day. The percentage of neutrophils and platelets reported a significant (P<0.05) increase in the positive control and other treated groups on the 4th day as compared with the negative control group on 8th day. Moreover, the statistical analysis revealed a significant (P<0.05) increase in the percentage of lymphocyte on the 4th and 8th day in positive and other treated groups compared with control groups at the same period.

Key word: pumpkin, blood glucose, aqueous extract

Introduction

Hyperglycemia is the most symptoms of diabetes that occurs after injury in diabetic patients, and the insulin resistance is the main important factor that causes impairment in the blood glucose control[1]. Some stress mediators such as stress cytokines and hormones are participated in glucose metabolism especially in the liver and skeletal muscle[2]. Hyperglycemia affects blood vessels which cause microvascular dysfunctions, slower circulation and reduced tissue oxygenation which affect wound healing. Impaired wound healing in diabetes patients is associated with a various and complex pathophysiology factors that include biochemical, immune and neuropathic components also the alteration in blood vessel in diabetic patients reduced leukocyte migration into the wound, which becomes more susceptible to infections[3,4]. Other factors correlated with reduction healing process...
include decreased production of growth factors, impaired nitrite oxide synthesis, delayed inflammatory response and excessive protease activity [5,6].

Herbal medicine is highly used in the treatment of numerous diseases, especially diabetes mellitus. These compounds have been contributed in the management of hyperglycemia and diabetes-associated complications by stimulating insulin signaling pathways, improving cellular antioxidant defense mechanisms against oxidative stress, and regulating hormones, peptides and inflammatory pathways. Some extracts of medicinal plants have been reported to promote wound healing, inhibit bacterial growth and prevent bleeding from fresh wounds, [7].

Numerous studies have shown the beneficial effects of herbs in diabetes treatment, the traditional medicinal plant, Cucurbita ficifolia (Cucurbitaceae), known as pumpkin, is one of the most herbal plants used in the treatment of diabetes [8,9], this plant used as functional food or medicine [10], and used in many countries in the treatment of different diseases such as an anti-inflammatory, antiviral, analgesic, antiulcer, and antioxidant [11]. Pumpkin seeds contain carbohydrates, fat, minerals [12], and saturated fatty acids such as arachidic acid, palmitic acid and α-tocopherol [13]. The oil of pumpkin seeds displayed the promising effects on wound healing activities, the high quality of polyunsaturated fatty acids and tocopherols in pumpkin oil accelerate its effect on wound healing [14]. The aim of this current work was to estimate the effect of pumpkin extract and oil in the treatment of blood glucose and wound in alloxan-induced diabetic rats.

Materials and methods

1. Preparation of plant extract

The pumpkin extract was prepared by mixing pumpkin powder with distilled water (1:4 w/w) at 50°C for three hours, centrifuged at 3000 r.p.m. for 15 minutes, the sediment was mixed with 95% ethanol for 24 hours at 4°C, while the supernatant was collected as a water-soluble extract followed by centrifugation at 3000 r.p.m. for 10 minutes, the sediment was discarded while the supernatant (ethanol-water soluble extract) was used in the experiment. The mixture of the water-soluble and ethanol extracts was named pumpkin crude extract.

2. Experimental design

Forty albino male rats weighing (180-210) grams were used in this study, divided into five groups (8 rats/group): G1: healthy control, G2: Positive control, wounded and diabetic rats, G3: Wounded and diabetic rats treated with aqueous extract 200 mg/kg b.wt., G4: Wounded and diabetic rats treated with aqueous extract 200 mg/kg b.wt. and wounds treated locally with aqueous extract 200 mg/kg b.wt. as ointment (mix with inert Vaseline) and G5: healthy (non-diabetic) rats, wound treated locally with aqueous extract 200 mg/kg b.wt.

3. Induction of diabetes

Diabetes was induced in overnight fasting rats using single dose of alloxan monohydrate 90 mg/kg b.wt. (BDH chemicals, England), immediately 5 ml of 5% glucose was injected to the rats in order to overcome decrease in blood glucose (hypoglycemia), after starvation for 12 hours fasting blood glucose were measured by using glucometer (Rosmax, Germany) and the rats with blood sugar over 250 mg/dl were considered to be hyperglycemic.

4. Wound model

All rats were anesthetized with halothane via inhalation, shaved with hair removal cream (for 5 minutes) and cleaned with 70% ethanol to maintain aseptic conditions, then the general anesthesia of the rats were done by intraperitoneal injection of Ketamine 5% at a dose (90 mg/kg) and Xylazine 2%
at a dose (10 mg/kg) mixed in the same syringe, wound at a distance of about 2 cm was done in each rat. The extract was administered once daily for eight days.

5. Blood collection

At the end of the eight days, the rats were fasted for 12 h, blood samples were collected under anaesthetized condition, immediately centrifuged at 3000 r.p.m for 10 min the serum was used for further analyses.

6. Immunological test

Measurement of TNF-α was done by using (TNF-α ELISA Kit, Elabscience, China), PDGF by (PDGF ELISA Kit, Elabscience, China). Total and differential count of W.B.C were estimated by automated digital counter machine from Bengaluru, India.

7. Statistical Analysis

The data of the experiment were calculated by using one-way analysis of difference and the group differences were calculated using Duncan multiple range test, data are presented as mean±SM, the different big letters investigate a significant difference (P<0.05).

Results

The results obtained from this study demonstrated a significant (P<0.05) increase in initial and final glucose level in diabetic positive group, but after treatment by pumpkin extract, there was a significant (P<0.05) decrease in blood glucose level and essential improvement in wound healing in diabetic groups compared to control as shown in table 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial glucose level mg/dl</th>
<th>Final glucose level mg/dl</th>
<th>Initial distance wounds/cm</th>
<th>Final distance wounds/cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>90.32±4.32</td>
<td>85.52±2.63</td>
<td>2</td>
<td>0.9</td>
</tr>
<tr>
<td>G2</td>
<td>355.45±10.43</td>
<td>390.22±6.44</td>
<td>2</td>
<td>1.5</td>
</tr>
<tr>
<td>G3</td>
<td>366.21±7.72</td>
<td>220.23±4.23</td>
<td>2</td>
<td>0.7</td>
</tr>
<tr>
<td>G4</td>
<td>320.09±5.09</td>
<td>200.05±4.20</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td>G5</td>
<td>89.45±3.47</td>
<td>83.21±2.80</td>
<td>2</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Differences big letters are significant (P<0.05) as compression between columns

The results in table 2 reported a significant (P<0.05) increase in serum level of TNF-α and PDGF in the positive control group and other diabetic groups in relation with negative control on the 4th day, then it showed gradual decrease in all treatments groups as compared with positive control group on the 8th day.
Table (2): The effect of pumpkin extract on TNF-α and PDGF level in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>TNF-α pg/ml (Mean±SD) 4 days</th>
<th>TNF-α pg/ml (Mean±SD) 8 days</th>
<th>PDGF ng/ml (Mean±SD) 4 days</th>
<th>PDGF ng/ml (Mean±SD) 8 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>578.75± 29.97 E</td>
<td>569.58± 26.73 C</td>
<td>4.11 ± 0.09 B</td>
<td>3.97 ± 0.06 C</td>
</tr>
<tr>
<td>G2</td>
<td>849.16± 18.99 B</td>
<td>878.33± 20.97 A</td>
<td>7.20 ± 0.05 A</td>
<td>6.34.12± 0.15 A</td>
</tr>
<tr>
<td>G3</td>
<td>750.16± 5.03 C</td>
<td>538.58± 17.38 D</td>
<td>5.19 ± 0.15 B</td>
<td>3.57 ± 0.41 C</td>
</tr>
<tr>
<td>G4</td>
<td>889.12±20.27 A</td>
<td>687.22±16.10 A</td>
<td>7.84±1.90 A</td>
<td>5.34±0.57 A</td>
</tr>
<tr>
<td>G5</td>
<td>722.16 ± 29.20 D</td>
<td>570.91 ± 13.90 C</td>
<td>4.91 ± 0.03 B</td>
<td>3.16 ± 0.08 C</td>
</tr>
</tbody>
</table>

Differences big letters are significant (P<0.05) as compression between columns

Table 3 revealed a significant (P≤0.05) increase in total count of WBC in the positive control group and other diabetic groups as compared with negative control on the 4th day, then it showed decrease in all treated groups except the positive control group on the 8th day. The percentage of neutrophils and platelets reported a significant (P<0.05) increase in the positive control and other treated groups on the 4th day as compared with the negative control group on 8th day. Moreover, the statistical analysis revealed a significant (P≤0.05) increase in the percentage of lymphocyte in the 4th and 8th day in positive and other treated groups compared with control groups at the same period.

Table 3: The effect of pumpkin extract on total and differential count of WBC

<table>
<thead>
<tr>
<th>Groups</th>
<th>WBC total count Cellsx10^9 4 days</th>
<th>WBC total count Cellsx10^9 8 days</th>
<th>Neut. % 4 days</th>
<th>Neut. % 8 days</th>
<th>Lymph. % 4 days</th>
<th>Lymph. % 8 days</th>
<th>PlateletCellsx10^9 4 days</th>
<th>PlateletCellsx10^9 8 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>5.44±0.78 C</td>
<td>5.03±0.93 B</td>
<td>63.99 ± 1.05 B</td>
<td>59.95 ± 0.24 C</td>
<td>24.95 ± 1.17 C</td>
<td>28.99 ± 0.40 D</td>
<td>688.16 ± 4.79 D</td>
<td>701.6 ±20.2 B</td>
</tr>
<tr>
<td>G2</td>
<td>7.43±1.03 A</td>
<td>8.63±1.78 A</td>
<td>66.24±3.34 A</td>
<td>63.83±2.29 A</td>
<td>30.32±0.94 B</td>
<td>32.10±1.30 C</td>
<td>843.43±10.88 A</td>
<td>836.2±9.4 A</td>
</tr>
<tr>
<td>G3</td>
<td>7.95±0.69 A</td>
<td>5.50±0.45 B</td>
<td>64.45±3.11 AB</td>
<td>55.45±2.63 C</td>
<td>33.55±1.20 A</td>
<td>39.52±1.42 B</td>
<td>820.22±8.43 B</td>
<td>684.3±7.10 C</td>
</tr>
</tbody>
</table>
Discussion

The results of the current research indicate that, the administration of pumpkin extract and oil reduced blood glucose level and accelerates wound healing (Table 1). These results might be related to that, this extract elevated insulin secretion from pancreatic β-cells, restore and repair the impaired islets or enhance the insulin sensitivity of target tissues such as adipose tissue and muscle. Pumpkin polysaccharides may act an essential role in the recovery of glucose utilization by improving liver function [15,16], also inhibit the activity of α-amylase and α-glucosidase [17], as well as Pumpkin extract contain D-chiro-Inositol and this compound act as insulin sensitizer to increase insulin action [18].

The effect of wound healing of pumpkin oil will be attributed to the presence of phenolic compounds since this materials was reported to make an essential role in wound healing of diabetic rats [19,20]. Pumpkin oil provide a connective tissue matrix by inducing the migration of fibroblasts to wound area during reepithelialization, also the higher level of β-sitosterol compound in pumpkin oil, revealed a higher collagen density in diabetic treated group since these compound were contributed with the estimation of collagen content which lead to enhance healing process [21]. Oxidative stress at a high level can affect wound healing by inducing cellular damage and impair lipids, proteins and DNA, pumpkin extract contain large amount of antioxidant compound such as flavonoids, saponins, triterpenes and alkaloids, that play a key roles in promoting wound healing by scavenging free radical activity [22], as well as the Pumpkin oil demonstrated antibacterial activity against a wide range of pathogenic bacteria which evaluate wound healing [23]. Furthermore, total mucilage content of pumpkin peel increase wound healing by inducing autolytic debridement, migration of keratinocytes, angiogenesis and re-epithelialization [24].

TNFα has been revealed to play an important role in the development of several inflammatory diseases and activate inflammatory cells [25]. In the epidermal cells, TNF-α stimulates the initial stages of the response to injury or inflammation [26,27,28,29], the elevated level of TNF-α and PDGF in positive control group and other diabetic groups (Table 2), may be related to that, after injury skin keratinocyte expresses and releases several cytokine such as interferon (IFN)-γ and tumor necrosis factor (TNF)-α [30]. TNF-α leads to increase the expression of inflammatory mediators by stimulating various signaling pathways that included TNF-α-induced protein kinases and caspases [31]. TNF-α activates the growth of insulin by inducing adhesion molecules such as intracellular adhesion molecule-1 that stimulates the growth of insulin, this the elevated level of TNF-α in the current study is associated with the development of diabetes and insulin resistance [32,33].

Furthermore, diabetic wounds are able of inducing a decrease in the expression of anti-inflammatory cytokines such as IL-10 and TGF-β, and an increase in the expression of proinflammatory cytokines such as IL-1/β and TNF-α as well as inflammatory cells. Our data showed a reduction of TNF-α in diabetic rats treated with pumpkin extract cream [34,35], this effect may be related to the presence of several antioxidant compounds such as anthocyanidins, which have potent antioxidant activity that have been proven to inhibit TNF-α level [36,37]. Proinflammatory cytokines are mainly involved in cell proliferation and differentiation during the wound healing process [38]. PDGF is growth factor secreted by macrophages, platelets, epidermal keratinocytes and smooth muscle cells of damaged arteries.
also plays a role in wound healing. As a response to inflammation, PDGF stimulates the fibroblasts cells to produce and secrete a large amount of collagenase that related to evaluate and accelerate the process of wound healing [39].

According to the results presented in table 3, the higher level of WBC, neutrophils, lymphocytes and platelets in diabetic groups might be due to the increased inflammatory response [40,41]. Elevated leukocytes count is reflect the slight inflammation and the reduction in the leukocytes count in diabetic treated groups mention the anti-inflammatory process of pumpkin extracts, diabetes in mice may causes a moderate neutrophils, elongated circulation times of monocytes and neutrophils as well as decreased circulation time of lymphocytes that elevates the susceptibilities to infection [42,43]. The WBC count was given to be accompanied with type two diabetes and insulin resistance [44]. Pumpkin seed contain many phytochemical compound such as flavonoids which has various benefits to immune system by activating the immune cells [45,46]. Tocopherols compound play an important role in maintaining immune system function by decreasing lipid peroxidation and hindering dangerous alteration in vitro that are thought to keep a few infections [47]. It also enhanced the proliferation of natural killer cells and splenic lymphocytes cells which shows an immunomodulatory activity for the plant [48].

Neutrophils cells is the most immune cells present in the wound, their concentration were highly increased and these cells release chemoattractant mediators to stimulate migration of further neutrophil cell to wound area [49,50,51]. Platelets contribute in the modulation of the innate and adaptive immune responses by increasing cell-cell interaction, microbial recognition and stimulating the release of growth factors such as PDGF and TGF-β1 that interact with neutrophils, monocytes, dendritic cells, promoting neutrophil activation and accelerate pathogen detection [52,53], this the improvement of WBCs and platelets due to its antioxidant activity of pumpkin extract [54]. The current study investigated the beneficial and positive effect of pumpkin extract and oil in the treatment of blood glucose and wound in diabetic rats.

Conclusion

From the results obtained in this present study, it could be safely concluded that both pumpkin extract and oil be useful in formulating strategies for therapeutic of hyperglycemia and diabetic wound.

References


