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 W.B.C. The results investigated a significant (P<0.05) decrease in blood glucose level in all treated diabetic groups compare with positive groups on 8th day and an improvement 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 relaveled 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.

**Keywords**: Pumpkin, blood glucose, aqueous extract.

### Introduction

Hyperglycemia is the most symptoms of diabetes that occurs after injury in diabetic patients, and theinsulin resistance is the main important factor that causes impairment in the blood glucose control (Mahmoodpoor et al., 2016), some stress mediators such as stress cytokines and hormones are participated in glucose metabolism especially in the liver and skeletal muscle (Van Cromphaut, 2009). Hyperglycemia affects blood vessels which cause microvascular dysfunction, 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 (Dinh et al., 2011; Greenhalgh, 2003). Other factors correlated with reduction healing process include decreased production of growth factors, impaired nitrate oxide synthesis, delayed inflammatory response and excessive protease activity (Mangoni et al., 2015; Perez-Gutierrez et al., 2006).

Herbal medicine is highly used in the treatment of numerous diseases, especially diabetes mellitus. These compound have been contributed in the management of hyperglycemia and diabetes-associated complications by stimulating insulin signaling pathway, improving cellular antioxidant defense mechanism against oxidative stress, and regulating hormones, peptides and inflammatory pathways, also the extracts of medicinal plants have been reported to promote wound healing, inhibits bacterial growth and prevent bleeding from fresh wounds, (Lau et al., 2008).

Numerous studies have shown the beneficial effects of herbs in the diabetes treatment, the traditional medicinal plant, Cucurbita ficifolia (Cucurbitaceae), known as pumpkin, is one of the most herbal plant used in the treatment of diabetes (Liu et al., 2008; Vieira et al., 2019), this plant used as functional food or medicine (Caili et al., 2006), and used in many countries in the treatment of different diseases such as an anti-inflammatory, antiviral, analgesic, antiulcer, and antioxidant (Ratnam et al., 2017). Pumpkin seeds contain carbohydrates, fat, minerals (Gryzbe et al., 2016), and saturated fatty acids such as linoleic acid, palmitic acid and α-tocopherol (Karanja et al., 2013), 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 (Bardaa et al., 2016). 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**

**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 rpm 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 rpm 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.

**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.

**Induction of diabetes**

Diabetes was induced in overnight fasting rats using single dose of alloxan monohydrate 90 mg/kg h.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.

**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.

**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.

**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 1:** The effect of pumpkin extract and oil on blood glucose level and wound healing in rats

<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 ± 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 A</td>
<td>3.57 ± 0.41 A</td>
</tr>
<tr>
<td>G4</td>
<td>889.12±20.27 A</td>
<td>687.22±16.10 B</td>
<td>7.84±1.90 A</td>
<td>5.34±0.57 C</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: The effect of pumpkin extract on total and differential count of WBC

<table>
<thead>
<tr>
<th>Groups</th>
<th>WBC total count Cellsx10³ 4 days</th>
<th>WBC total count Cellsx10³ 8 days</th>
<th>Neut.% 4 days</th>
<th>Neut.% 8 days</th>
<th>Lymph.% 4 days</th>
<th>Lymph.% 8 days</th>
<th>Platelet Cellsx10³ 4 days</th>
<th>Platelet Cellsx10³ 8 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>5.44±0.78 C</td>
<td>5.03±0.93 A</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 C</td>
<td>688.16 ± 4.79 D</td>
<td>701.6 ±20.2 B</td>
</tr>
<tr>
<td>G2</td>
<td>7.43±1.63 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 A</td>
<td>32.10±1.30 A</td>
<td>843.43±10.88 A</td>
<td>836.2±9.4 A</td>
</tr>
<tr>
<td>G3</td>
<td>7.93±0.69 A</td>
<td>5.50±0.45 A</td>
<td>64.45±3.11 AB</td>
<td>55.45±2.63 C</td>
<td>33.55±1.20 C</td>
<td>39.52±1.42 C</td>
<td>820.22±1.43 B</td>
<td>684.3±7.10 C</td>
</tr>
<tr>
<td>G4</td>
<td>6.44±2.44 B</td>
<td>5.90±1.02 A</td>
<td>62.22±4.10 B</td>
<td>52.17±3.54 B</td>
<td>42.27±2.63 A</td>
<td>42.27±2.63 A</td>
<td>750.43±6.43 C</td>
<td>690.10±5.39 C</td>
</tr>
<tr>
<td>G5</td>
<td>6.43.45±1.09 B</td>
<td>5.34±0.32 B</td>
<td>65.20±2.09 A</td>
<td>54.32±3.52 B</td>
<td>29.33±2.34 B</td>
<td>40.27±1.05 A</td>
<td>758.34±10.32 C</td>
<td>634.1±7.2 D</td>
</tr>
</tbody>
</table>

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

Discussion

The results of the current research indicated 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 b-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 (Hu et al., 2010; Zhao et al., 2011), also inhibit the activity of α-amylase and α-glucosidase (Kushawaha et al., 2016), as well as Pumpkin extract contain D-chiro-Inositol and this compound act as insulin sensitizer to increase insulin action (Xia and Wang, 2006).

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 (Kovalik et al., 2014; Tamri et al., 2014), 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 (Musalmah et al., 2002). 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 (Thakur et al., 2011), as well as the Pumpkin oil demonstrated antibacterial activity against a wide range of pathogenic bacteria which evaluate wound healing Further more total mucilage content of pumpkin peel was increase wound healing by inducing autolytic debridement, migration of keratinocytes, angiogenesis and re-epithelialization (Clark, 2007).

TNF-α has been revealed to play an important role in the development of several inflammatory diseases and activate inflammatory cells (Hussaana et al., 2019). In the epidermal cells, TNF-α stimulates the initial stages of the response to injury or inflammation (Broussard et al., 2013), 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 release...
several cytokine such as interferon (IFN)-γ and tumor necrosis factor (TNF-α) (Trefzer et al., 1991). TNF-α leads to increase the expression of inflammatory mediators by stimulating various signaling pathways that includemitogen activated protein kinases and caspases (Chen et al., 2002). 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 (Swaroop et al., 2012)

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-1β and TNF-α as well as inflammatory cells. Our data showed a reduction of TNF-α in diabetic rats treated with pumpkin extract cream (Badr et al., 2016) this effect may be related to the presence of several antioxidants compounds such as anthocyanins, which have potent antioxidants activity that have been proven to inhibit TNF-α level (Belwal et al., 2017). Proinflammatory cytokines are mainly involved in the cell proliferation and differentiation during the wound healing process (Freedberg et al., 2001). PDGF is growth factor secreted bymacrophages, 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 (Heldin et al., 1999).

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 (Hamzah et al., 2019).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 susceptibleness to infection (Simsek et al., 2009). The WBC count was given to be accompanied with type two diabetes and insulin resistance (Mohamed et al., 2009). Pumpkin seed contain many phytochemical compound such as flavonoids which has various benefits to immune system by activating the immune cells (Marbun et al., 2018). To copherols 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 (Gropper et al., 2005). It also enhanced the proliferation of natural killer cells and splenic lymphocytes cells which shows an immuno modulatory activity for the plant (Xia et al., 2003).

Neutrophils cells is the most immune cells present in the wound, their concentration were highly increased and these cells release chemo attractant mediators to stimulate migration of further neutrophil cell to wound area (Su et al., 2015). Platelets cell 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 (Nami et al., 2016), this the improvement of WBCs and platelets due to its antioxidant activity of pumpkin extract (Mariam et al., 2013). 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**


