Effects of the Extracts of Olive and Morus Alba Leaves on Experimentally STZ Induced Diabetes in Male Rats

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Abstract: Fifty adult male albino rats (weighting 150 -200 g) were used and divided into 5 groups (10 rats of each). The first group was served as control group. The remaining groups were injected (i.p) by streptozotocin (STZ) at 45 mg/kg b.wt to induce diabetes. The 2nd diabetic group was used as control diabetic group. The third diabetic group was treated with Cidophage (500 mg/kg, orally). While, the fourth and fifth diabetic groups were treated with alcoholic Olive leave and Morus alba leave extracts (500 mg/kg b.wt, 600 mg/kg b.w orally, respectively). Treatment was performed daily for successive 30 days. The levels of blood glucose, albumin, total protein and creatinine were measured at 1 day, 1 and 2 weeks after the end of treatments of diabetic groups. The rats were sacrificed at the end of 4th and 5th diabetic groups were treated with alcoholic Olive leave and Morus alba leave extracts (500 mg/kg b.wt, 600 mg/kg b.w orally, respectively). Treatment was performed daily for successive 30 days. The levels of blood glucose, albumin, total protein and creatinine were measured at 1 day, 1 and 2 weeks after the end of treatments of diabetic groups. The rats were sacrificed at the end of 30days post treatment. The obtained results showed that the use of Olive leave and morus alba leave extracts improved the levels of blood glucose, albumin, total protein and creatinine of diabetic rats. Moreover, Olive leave extract and morus alba leave extractsare capable of improving the impaired above parameters of STZ diabetic animals.

Keywords: Olive leaves, Morus alba leaves and diabetic rats

1. Introduction

Diabetes mellitus is a group of metabolic disorder characterized by chronic effects of carbohydrate, fat and protein metabolism that results from defects in both insulin secretion and/or insulin action. The disease is associated with reduced quality of life and increased risk factors for morbidity and mortality. The long term hyperglycemia is an important factor in the development and progression of micro- and macrovascular complication, which include cerebrovascular diseases (Strojek, 2003), nephropathy and cardiovascular (Shim, et al., 2011).

Anti-diabetes drugs such as biguanide, sulfonylurea and thiazolidinedione are now available to diminish hyperglycemia in diabetes mellitus (Matsui, et al., 2006). The use of these drugs related with a number of side effects (Noor, et al., 2008).

Some of the plants that are being used for the treatment of diabetes have received scientific or medicinal scrutiny and even the World Health Organization’s expert committee on diabetes recommends that area warrants further attention (WHO, 1980). Medicinal plants provide a useful source of oral hypoglycemic compounds for the development of new pharmaceutical leads as well as dietary supplements to existing therapies (Kavishankar, et al., 2011).

Olive leave extract lowers blood pressure (e.g. systolic and diastolic) and dilate the coronary arteries surrounding the heart (Khyyal, et al., 2002), inhibits the oxidation of LDL and the development of atherosclerosis (Somovaet al., 2003), reduces blood sugar, enhance the immune system (Kubo, et al., 1985), has hypouricemia effect and antidiarrhea effect (Duke, et al., 2002).

Morus alba, known as mulberry, has been considered to possess many different medicinal properties such as diuretic, expectorant and antidiabetic effect (Hikino, et al., 1985). refer to that the Oral administration of mulberry leaves powder could decrease blood and urine glucose, triacylglycerid (TG), LDL-cholesterol and VLDL-cholesterol and fatty acid in type-2 diabetes patients (Andalluet al., 2009) and potent antihyperglycemic effect (Nakagawa, et al., 2010).

The present study aimed to investigate the effects of both extracts on some biochemical parameters (glucose, albumin, total protein and creatinine) in STZ diabetic rats. Moreover, the effects of treated extracts on some histopathological finding in the pancreas of treated rats was performed.

2. Material and Methods

1- Materials

a- Olive leave and Morus alba leave extracts
Olive leave and Morus alba Leaves were collected from Arboretum of Agriculture College, Mansoura University Egypt, cleaned, washed with tap water, dried and stored in dry atmosphere. The alcoholic extracts of Olive leave and Morus alba Leaves were suspended in distilled water according to the method of Harborne (1984) by the use of Soxhlet apparatus. Olive leaves extract was given orally to the animals at a dose of 500 mg/ kg b.wt (Eidi, et al., 2009), while Morus alba leaves extract was given at 600 mg/kg b.wt (Jamshid and Prakash 2012), by stomach tube daily for 30 days.

b- Drugs
1- Streptozotocin (STZ): was purchased from Sigma Company (USA). Induction of diabetes was done by using
streptozotocin (STZ) at 45mg/kg b.wt in rats according to El-Seifi, et al., (1993).

2- Cidophage (Metformin hydrochloride 500 mg) CID Company (CID, Giza, Egypt) and it was administrated orally by stomach tube in a dose 500 mg/kg b.wt (Pagano, et al., 1983).

c- Chemicals
NaCl 0.9%, and sodium citrate, citric acid ethyl alcohol 95% were purchased from El- Gomhoria Company. The test reagent Kits for blood glucose, albumin and total protein purchased from Linear Spain (Barcelona) Company, while creatinine purchased from Spectrum company Egypt.

d- Experimental Animals:
A total of fifty (50) adult healthy males rats (8-10 weeks) with weight range between 150-200 grams, were used in this study. Animals purchased from the animal house in Mansoura city, and housed in Department of Pharmacology, Faculty of Veterinary medicine, Mansoura University. Animals were left for one week to acclimatize the place. Animals were kept in cage in a controlled environment, maintained under a 20-25°C and light period of 12 hours daily and 50-70 % humidity. Rats provided with standard diet and water ad-libitum. The animals were housed in plastic cages. Care was taken to avoid any unnecessary stress. The cages were cleaned twice a week. After one week period of acclimatization in cages condition, rats were divided into 5 groups (each of 10 rats) as follows:

Group I: (control clinically healthy)treated with 0.2 ml distilled water orally.

Group II: non diabetic treated with 500 mg /kg b.wt Olive leaves extract orally daily for 30 days (Eidi, et al., 2009).

Group III: non diabetic treated with 600 mg /kg b.wt Mours alba leaves extract orally daily for 30 days. (Jamshid and Prakash, 2012)

Group IV: diabetic treated with 500 mg /kg b.wt Olive leaves extract orally daily for 30 days (Eidi, et al., 2009).

Group V: diabetic treated with 600 mg /kg b.wt Mours alba leaves extract orally daily for 30 days. (Jamshid and Prakash, 2012).

e- Sampling:
1- Blood sample:
Blood samples (from 5 rats of each group) were collected after 1 day, 1 week and 2 week from the last dose into clean centrifuge tubes. The blood samples were centrifuged at 3000 r. p. m for 20 minutes for serum collection. The obtained serum samples were stored at -20°C until assayed.

2- Histopathological sample:
After 1 day post dosing and at the end of study (30 days post dosing), 5 rats from each group were sacrificed. Specimen from pancreas, liver and kidney of control and treated groups were taken and fixed in 10 % formalin for examination.

2 - Methods
a- Biochemical studies:
- Serum samples were assayed for estimation of glucose (Trinder, 1969), total protein and albumin (Young, 2000), and creatinine (Tietz, et al., 1986) levels.

b- Histopathological examination:
- Specimens from the pancreas of control and treated groups were taken after the end of treatment. Specimens were fixed in 10 % neutral formalin to be used for histopathological changes (Lillie and Fulman, 1976).

Statistical Analysis:
Data were subjected to statistical analysis using statistical software program (SPSS for Windows, version 18, USA). The standard errors for each variable were estimated. Differences between means of different groups were carried out using one way ANOVA with Duncan various comparison tests. Dissimilar superscript letters in the same column demonstrate a significance (P<0.05) (Snedecor and Cochran, 1981).

3. Results
1. The effect of Olive leave and morus alba leave extractson serum glucose Level:
It was observed clearly from Table (1) and Figure. (1) that serum glucose level was significantly increased (P<0.05) in diabetic group at 1 day, 1st and 2nd weeks (376.2 ± 23.961, 434.4 ± 11.543, 375.8 ± 20.303 respectively) in comparison with the control group (91.4 ± 4.76, 94.8 ± 4.498, 93.4 ± 3.6, respectively) after treatment. Meanwhile serum glucose level was significantly decreased (P<0.05) in all diabetic treated groups and were 174.6 ± 26.676, 140 ± 3.162, 136.8 ± 8.731 (Cidophage), 177 ± 31.643, 166.4 ± 4.523, 142 ± 19.222 (Olive leave alcoholic extract) and 181 ± 11.193, 157.4 ± 11.634, 135.2 ± 7.831 (Morus alba leave alcoholic extract) at1day, 1st and 2nd weeks, respectively post dosing, in comparison with control diabetic group.

2. The effect of Olive leave and Morus alba leave extracts on serum albumin Level:
It was clearly evident from Table (2) and Figure.(2) that serum albumin level was significantly increased (P<0.05) in diabetic group (5.2 ± 0.240) in comparison with the control group (4 ± 0.141) after treatment. Meanwhile serum albumin level was significantly decreased (P<0.05) in all diabetic treated groups at 1 day, 1st and 2nd weeks and were 3.8 ± 0.170, 4.2 ± 0.114, 3.9 ± 0.044 (Cidophage), 3.74 ± 0.087, 4.12 ± 0.06, 4.132 ± 0.08 (Olive leave alcoholic extract) and 3.84 ± 0.176, 4.16 ± 0.05, 3.84 ± 0.097 (Morus alba leave alcoholic extract) at1day, 1st and 2nd weeks, respectively post dosing, in comparison with control diabetic group.
3. The effect of Olive leaves and Morus alba leaves extracts on serum total protein level:
The obtained data from Table (3) and Figure.(3) that serum total protein level was significantly decreased (P<0.05) in diabetic group (6.88 ± 0.058) in comparison with the control group (7.44 ± 0.294) after treatment. On the other hand serum total protein level was significantly increased (P<0.05) in all diabetic treated groups at 1 day, 1st and 2nd weeks and were 7.02 ± 0.066, 7.2 ± 0.130, 7.12 ± 0.124 (Cidophage), 6.96 ± 0.067, 7.23 ± 0.111, 7.46 ± 0.206 (Olive leave alcoholic extract) and 7.6 ± 0.170, 7.66 ± 0.092, 7.54 ± 0.304 (Morus alba leave alcoholic extract) respectively at1 day, 1st and 2nd weeks, post dosing, in comparison with control diabetic group.

4. The effect of Olive leaves and Morus alba leaves extracts on serum Creatinine level:
It was clearly evident from Table (4) and Figure. (4 ) that serum creatinine level was significantly increased (P<0.05) in diabetic group (0.906 ± 0.027) in comparison with the control group (0.728 ± 0.025) after treatment. Meanwhile serum creatinine level was significantly increased (P<0.05) in all diabetic treated groups at 1 day, 1st and 2nd weeks and were 0.804 ± 0.021, 0.73 ± 0.013, 0.766 ± 0.026 (Cidophage), 0.810 ± 0.010, 0.766 ± 0.020, 0.742 ± 0.038 (Olive leave alcoholic extract) and 0.786 ± 0.015, 0.752 ± 0.021, 0.722 ± 0.034 (Morus alba leave alcoholic extract) respectively at1 day, 1st and 2nd weeks, post dosing, in comparison with control diabetic group.

Histopathological findings:
The histopathological effect of Olive leaves and Morus alba leaves extracts on pancreas was recorded in Figure. (5), (6), (7), (8), (9), (10), (11) and (12).

Table 1: Effect of the extracts of Olive leaves and Morus alba leaves on serum glucose levels in diabetes and non-diabetic rats.(Mean ± SE) (n=5)

<table>
<thead>
<tr>
<th>No.</th>
<th>Parameters</th>
<th>Group</th>
<th>Serum Glucose Level (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1st day</td>
<td>1st week</td>
</tr>
<tr>
<td>1</td>
<td>G1</td>
<td>91.4 ± 4.76c</td>
<td>94.8 ± 4.498c</td>
</tr>
<tr>
<td></td>
<td>(Control given 0.2ml normal saline)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>G2</td>
<td>376.2 ± 23.961a</td>
<td>434.4 ± 11.543a</td>
</tr>
<tr>
<td></td>
<td>(Diabetic by 45 mg/kg b. wt STZ)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>G3</td>
<td>174.6 ± 26.676b</td>
<td>140 ± 3.162b</td>
</tr>
<tr>
<td></td>
<td>(Diabetic treated with Cidophage at 500 mg/kg b. wt)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>G4</td>
<td>177 ± 31.643b</td>
<td>166.4 ± 4.523b</td>
</tr>
<tr>
<td></td>
<td>(Diabetic treated with alcoholic extract of Olive leaves at 500 mg/kg b. wt)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>G5</td>
<td>181 ± 11.193b</td>
<td>157.4 ± 11.634b</td>
</tr>
<tr>
<td></td>
<td>(Diabetic treated with alcoholic extract of Morus alba leaves) at 600 mg/kg b. wt)</td>
<td></td>
<td></td>
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</tbody>
</table>

Figure 1: Effect of the extracts of Olive leaves and Morus alba leaves on serum glucose levels in diabetes and non-diabetic rats. (Mean ± SE) (n=5)
Table 2: Effect of the extracts of Olive leaves and Morus alba leaves on serum albumin levels in diabetes and non-diabetic rats. (Mean ± SE) (n=5)

<table>
<thead>
<tr>
<th>No.</th>
<th>Parameters Group</th>
<th>Serum Albumin Level (mg/dl)</th>
<th>1st day</th>
<th>1st week</th>
<th>2nd week</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>G1 (Control given 0.2ml normal saline)</td>
<td>4 ± 0.141</td>
<td>4 ± 0.141</td>
<td>4 ± 0.141</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>G2 (Diabetic by 45 mg/kg b. wt. STZ)</td>
<td>5.2 ± 0.240</td>
<td>5.2 ± 0.240</td>
<td>5.2 ± 0.240</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>G3 (Diabetic treated with Cidophage at 500 mg/kg b. wt.)</td>
<td>3.8 ± 0.170</td>
<td>4.2 ± 0.114</td>
<td>3.9 ± 0.044</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>G4 (Diabetic treated with alcoholic extract of Olive leaves at 500 mg/kg b. wt.)</td>
<td>3.74 ± 0.087</td>
<td>4.12 ± 0.06</td>
<td>4.132 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>G5 (Diabetic treated with alcoholic extract of Morus alba leaves) at 600 mg/kg b. wt.)</td>
<td>3.84 ± 0.176</td>
<td>4.16 ± 0.05</td>
<td>3.84 ± 0.097</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2: Effect of the extracts of Olive leaves and Morus alba leaves on serum albumin levels in diabetic and control rats. (Mean ± SE) (n=5)

Table 3: Effect of the extracts of Olive leaves and Morus alba leaves on serum total protein levels in diabetes and control rats. (Mean ± SE) (n=5)

<table>
<thead>
<tr>
<th>No.</th>
<th>Parameters Group</th>
<th>Serum Total Protein Level (mg/dl)</th>
<th>1st day</th>
<th>1st week</th>
<th>2nd week</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>G1 (Control given 0.2ml normal saline)</td>
<td>7.44 ± 0.294</td>
<td>7.44 ± 0.294</td>
<td>7.44 ± 0.294</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>G2 (Diabetic by 45 mg/kg b. wt. STZ)</td>
<td>6.88 ± 0.058</td>
<td>6.88 ± 0.058</td>
<td>6.88 ± 0.058</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>G3 (Diabetic treated with Cidophage at 500 mg/kg b. wt.)</td>
<td>7.02 ± 0.066</td>
<td>7.2 ± 0.130</td>
<td>7.12 ± 0.124</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>G4 (Diabetic treated with alcoholic extract of Olive leaves at 500 mg/kg b. wt.)</td>
<td>6.96 ± 0.067</td>
<td>7.23 ± 0.111</td>
<td>7.46 ± 0.206</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>G5 (Diabetic treated with alcoholic extract of Morus alba leaves) at 600 mg/kg b. wt.)</td>
<td>7.6 ± 0.170</td>
<td>7.66 ± 0.092</td>
<td>7.54 ± 0.304</td>
<td></td>
</tr>
</tbody>
</table>
Figure 3: Effect of the extracts of Olive leaves and Morus alba leaves on serum total protein levels in diabetic and control rats. (Mean ± SE) (n=5)

![serum Total protein level](image)

1 = 1st day after 30 day from treatment
2 = 1st week after 30 day from treatment
3 = 2nd week after 30 day from treatment

Table 4: Effect of the extracts of Olive leaves and Morus alba leaves on serum creatinine levels in diabetic and control rats (Mean ± SE) (n=5)

<table>
<thead>
<tr>
<th>No.</th>
<th>Parameters</th>
<th>Group</th>
<th>Serum Creatinine Level (mg/dl)</th>
<th>1st day</th>
<th>1st week</th>
<th>2nd week</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-</td>
<td>G1</td>
<td>(Control given 0.2ml normal saline)</td>
<td>0.728 ± 0.025 d</td>
<td>0.728 ± 0.025 c</td>
<td>0.728 ± 0.025 c</td>
<td></td>
</tr>
<tr>
<td>2-</td>
<td>G2</td>
<td>(Diabetic by 45 mg/kg b. wt. STZ)</td>
<td>0.906 ± 0.027 a</td>
<td>0.906 ± 0.027 a</td>
<td>0.906 ± 0.027 a</td>
<td></td>
</tr>
<tr>
<td>3-</td>
<td>G3</td>
<td>(Diabetic treated with Cidophage at 500 mg/kg b. wt.)</td>
<td>0.804 ± 0.021 b</td>
<td>0.73 ± 0.013 b</td>
<td>0.766 ± 0.026 b</td>
<td></td>
</tr>
<tr>
<td>4-</td>
<td>G4</td>
<td>(Diabetic treated with alcoholic extract of Olive leaves at 500 mg/kg b. wt.)</td>
<td>0.810 ± 0.010 c</td>
<td>0.766 ± 0.020 b</td>
<td>0.742 ± 0.038 bc</td>
<td></td>
</tr>
<tr>
<td>5-</td>
<td>G5</td>
<td>(Diabetic treated with alcoholic extract of Morus alba leaves) at 600 mg/kg b. wt.)</td>
<td>0.786 ± 0.015 c</td>
<td>0.752 ± 0.021 b</td>
<td>0.722 ± 0.034 b</td>
<td></td>
</tr>
</tbody>
</table>

Figure 4: Effect of the extracts of Olive leaves and Morus alba leaves on serum creatinine levels mg/dl in diabetic and control rats. (Mean ± SE) (n=5)

![serum Creatinine level](image)

1 = 1st day after 30 day from treatment
2 = 1st week after 30 day from treatment
3 = 2nd week after 30 day from treatment
Figure 5: Pancreas showing apparently normal endocrine and exocrine cellular structure, H&E original magnification x130 (control group)

Figure 6: Pancreas showing severe pancreatitis represented by congested blood vessels, besides fat necrosis and hemorrhage with fibrous tissue proliferation, which replaced portions of exocrine and endocrine portions of pancreas H&E, original magnification X52 (STZ group)

Figure 7: Pancreas showing mild vacuolation islets of Langerhans cells with necrotic aciner epithelial cells H&E, original magnification X52 (1st day after end of treatment) (Cidophage group)

Figure 8: Pancreas showing apparently normal islets of Langerhans except for mild degenerative changes H&E, original magnification x520 (2nd week after end of treatment) (Cidophage group)

Figure 9: Pancreas showing edema, vacuolation and focal necrosis of cells in the islets of Langerhans H&E, original magnification x520 (1st day after end of treatment) (Olive leave group)

Figure 10: Pancreas showing more healthy architecture of islets of Langerhans and aciner cells with eosinophilic material in the interlobular duct in comparison with pancreas of group 1st day H&E, original magnification x520 (2nd week after end of treatment) (Olive leave group)
In current study diabetes mellitus is induced in all rats recorded significant decrease in serum glucose levels of all effects of Olive leaves and Morus alba leaves extracts on some biochemical parameters (Glucose, Albumin, Total protein and Creatinine) and histopathology of pancreas:

4. Discussion

Effects of Olive leaves and Morus alba leaves extracts on some biochemical parameters (Glucose, Albumin, Total protein and Creatinine) and histopathology of pancreas:

In current study diabetes mellitus is induced in all rats (except negative control) by injection of streptozotocin (STZ) at dose 45 mg /kg b.w. i.p. single dose. The result recorded significant decrease in serum glucose levels of all treated groups in comparing with STZ untreated negative control. The present study recorded a hypoglycemic effect induced by Olive leave extract, this finding is supported with the data obtained by Gonzalez et al., (1992) who indicated that Olea europaea (Oleuropein, the active principle of olives) possessed a hypoglycemic effect by two mechanisms, the first is by potentiation of glucose induced insulin release and the second is by increasing peripheral uptake of glucose. Furthermore Ivora(1988) observed that olive extract enhances the insulin release from destroyed pancreatic beta cells, either by regenerating the partially destroyed pancreatic beta cells or by the release of insulin stored in the granules.

Administration of hydro alcholic extracts Morus alba leaves also induced a significant decrease in serum glucose levels as compared to diabetic control rats. Andallu and Varadacharyulu(2003) reported that mulberry administration remarkably decreased blood glucose concentrations in diabetic rats. This effect may be due to the presence of the N-containing sugars which inhibit the functions of α-glucosiadase, α-mannosidase and β-galactosidase (Asano, et al., 1994), and fagomine which potentiates the glucose induced insulin release similar to the action of glibenclamide (Kimura, et al., 1995) as well as increasing the tissue uptake of glucose (Chen, et al., 1995). These results were agreement to the study of Singab, et al., (2005) and Muhammedi, et al., (2008).

Treatment of diabetic rats with Olive leaves or Morus alba extracts significantly decreased the levels of albumin and significantly increase the total protein levels in their serum, compared with the diabetic group. This finding is in accordance with Al-Attar and Shawush (2014) who stated that, the levels of albumin, glucose and high density lipoprotein cholesterol were significantly decreased in serum of olive leaves extract treated rats. The effect of olive oil on liver synthesis of total proteins which are used as markers for liver damage; a rise in these proteins is associated with the development of a nonalcoholic liver pathology, such as fatty liver, linked to metabolic syndrome (Clark and Diehl, 2003). Covas (2007) stated that olive oil raised the levels of total protein and A/G ratio in treated rats.

Removal of metabolite wastes such as urea, uric acid and creatinine by the kidneys maintains optimum chemical composition of body fluids. In the current study, the increased levels of creatinine indicate kidney dysfunction in diabetic rats. Renal dysfunction indicated by elevation of renal markers in diabetic rats has been proved through previous studies (Jaraldetal, 2008 and Chandramohan, et al., 2011). Elevation of the renal markers may be due to metabolic disturbance in diabetic animals reflected in high activities of xanthine oxidase, lipid peroxidation, and increased triacylglycerol and cholesterol levels (Madinov, et al., 2000).

The administration of Olive leave and Morus alba extracts to diabetic rats caused a modulation in the regeneration of β-cells of pancreas, compared with the diabetic group. The obtained result is supported by the results recorded by Jamshid and Prakash (2012) and Al-Jashamy, et al (2011). The authors conclude that, the histopathological studies undertaken on the pancreas of diabetic rats demonstrated the recovery of damage islets and an improvement in the number of β-cells after treatment with both leaves extracts of the plants.

References


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