Hypoglycemic and Antihyperglycemic Effects of Different Extracts and Combinations of *Withania coagulans* Dunal and *Acacia arabica* Lamk in Normal and Alloxan Induced Diabetic Rats

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**ABSTRACT:** Introduction: *Acacia arabica* and *Withania coagulans* commonly known as babool and paneer phool respectively are used in traditional Indian medicine for treatment of diabetes mellitus. Methods: The hypoglycemic effect of aqueous extracts (hot and cold water) and hydroalcoholic extract of *Acacia arabica* and *Withania coagulans* was investigated. Oral administration of a cold water extract of *Acacia arabica* bark, and a hydroalcoholic extract of *Withania coagulans* to diabetic and normal rats at a dose of 400 mg/kg body weight resulted in a significant reduction of blood glucose, cholesterol and triglycerides in alloxan induced diabetic rats. Results: Phytochemical investigation found that saponins, flavonoids, alkaloids and tannins were present in the *Withania coagulans* extracts, and phenolic compounds were present in *Acacia arabica* extracts. The hydroalcoholic extract of *Withania coagulans*, and the cold water extract of *Acacia arabica* were found to reduce the blood-glucose level to normal level within seven days. Promising effects were observed when a combination of both extracts was administered to the test animals. Histological studies of the β-cells indicate that the extracts affect pancreatic cells. Conclusions: The hydroalcoholic extract of *Withania coagulans* and the cold aqueous extract of *Withania coagulans* exhibited antihyperglycemic activities in alloxan-induced diabetic rats. Combination of both plants in the ratio 400+400 mg/kg shows higher antihyperglycemic activities. Regereneration of β-cells, is seen in combinations of extract of both the plants.


**INTRODUCTION**

Diabetes mellitus is caused by an absolute or relative lack of insulin that, among other consequences, leads to an increase in plasma glucose concentration. In type I insulin-dependent diabetes mellitus (TIDM), previously called juvenile diabetes, there is an absolute lack of insulin. The condition is caused by a lesion in the beta cells of the pancreas. As the number of people with diabetes multiplies worldwide continues to increase, it is projected to become one of the world's main disasters. The regions with greatest potential for increased rates of DM are Asia and Africa, where DM rates could rise to two to three-fold the present rates.

Apart from currently available therapeutic options, many herbal medicines have been recommended for the treatment of diabetes. Traditional plant medicines are used throughout the world for a range of diabetic presentations. The phytochemical investigation in this study showed the presence of saponins, alkaloids, flavonoids and tannins. Hyperglycemia results in the generation of free radicals, leading to the disruption of cellular functions, oxidative damage to membranes and enhanced susceptibility to lipid peroxidation. Flavonoids are one of the most numerous and widespread groups of phenolics in higher plants. Some of them, due to their phenolic structure, are known to be involved in the healing process of free radical-mediated diseases, including diabetes. Some are reported to be hypoglycemic in some literature.

*Withania coagulans* Dunal belongs to the family Solanaceae and is reported to have a number of pharmacological activities, i.e. hepatoprotective and anti-inflammatory activity, antifungal and antibacterial activity and hypoglycemic activity. *Withania coagulans* is rich in steroidal lactones, which are known as withanolides. One of the characteristic features of the plants that produce withanolides...
is their extraordinary ability to introduce oxygen functions at almost every position of the carbocyclic skeleton and side chain. Previous phytochemical examination of the whole plant resulted in the isolation of 25 compounds, including 24 withanolides and one dimeric lignan, bispercopodophyllin glucoside.[12]

*Acacia arabica* belongs to family Mimosaceae and is reported for *In vitro* antibacterial activity[13] antimiobacterial and immunomodulatory activities. Previous studies of the bark of *Acacia nilotica* show the presence of following chemical constituent’s gallic acid, catechin 5-O-gallate, galloylated derivatives of catechin 5-O-gallate, the pentacyclics acanilol A (C_{19}H_{16}O_{7}) and B (C_{18}H_{14}O_{6}) diterpene nilotican (C_{20}H_{30}O_{2}), new compound gallocatechin 5-O-gallate in addition to methyl gallate, gallic acid, catechin, catechin 5-O-gallate, 1-O-galloyl-β-D-glucose 1,6-di-O-galloyl-β-D-glucose and digallic acid.

Flavonoids, sterols/triterpenoids, alkaloids and phenolics are known to be bioactive antidiabetic principles.[14,15] Flavonoids are known to regenerate the damaged beta cells in the alloxan induced diabetic rats.[16] Phenolics have been found to be effective antihyperglycemic agents.[17] In the present study, hypoglycemic activity of different extracts of *Withania coagulans* and *Acacia arabica* and combinations of both were observed in alloxan induced diabetic albino rats.

**MATERIAL AND METHOD**

**Plant material**

*Acacia arabica* bark was collected from the village Garhpehra near Sagar (M.P.), India. Dried *Withania coagulans* fruits were purchased from a local market and identified by chief botanist of Dr H.S.Gour University Sagar (Dr. Pradeep Tiwari). A voucher specimen was deposited in the Herbarium, Botany Department, University Sagar. Accession numbers of the herbs are *Withania coagulans*: Bot/H/3362, *Acacia arabica*: Bot/H/2698

**Preparation of extracts**

**Hydroalcoholic extract**

The dried fruits of *Withania coagulans* and *Acacia arabica* bark were finely powdered and extracted by hot percolation method using Soxhlet apparatus. The solvent used was 50% methanol. After extraction the extract was dried in a water bath at a temp 35-40 °c (yield 25.54% w/w).

**Hot water extract**

The dried fruits *Withania coagulans* and *Acacia arabica* were finely powdered and extracted by boiling with water for 2 hr. After extraction the extract was dried in a water bath at a temp 35-40 °c (yield 9.926% w/w).

**Cold water extract**

The dried fruits *Withania coagulans* and *Acacia arabica* were finely powdered and extracted by macerating with water for five days. After extraction the extract was dried in a desiccator. (Yield 14.028% w/w).

**Animals**

Albino Wistar rats (120-150 g) 60 days old of either sex were obtained from CRC BMCP Mandsaur (M.P). Before and during the experiment rats were fed with standard diet (Lipton, India Ltd). After randomization into various groups, the rats were acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity, and dark/light cycle. Animals described as fasting were deprived of food and water for 16 h ad libitum.

**Sample collection**

Blood samples were collected from tail vein and the blood glucose content was estimated using electronic glucometer (Smart Care, Taiwan).

**Preliminary oral LD50 determination**

Preliminary oral LD50 dose of *Withania coagulance* hydroalcoholic extract (WCHAE), *Withania coagulance* Hot water extract extract (WCHWE), *Withania coagulance* cold water extract extract (WCCWE), *Acacia arabica* hydroalcoholic extract (AAHAE), *Acacia arabica* Hot water extract extract (AAHWE), *Acacia arabica* cold water extract extract (AACWE) in rats were determine according to the OECD guideline.

**Experimental design**

All the animals were divided into the six groups with five animals in each group:

- **Group I**: Normal control.
- **Group II**: Diabetic control (alloxan treated without other treatment).
- **Group III**: Diabetic rat treated with Glibenclamide.
- **Group IV**: Treated with WCHAE (with and without treated alloxan).
- **Group V**: Treated with WCHWE (with and without treated alloxan).
- **Group VI**: Treated with WCCWE (with and without treated alloxan).
- **Group VII**: Treated with AAHAE (with and without treated alloxan).
- **Group VIII**: Treated with AAHWE (with and without treated alloxan).
- **Group IX**: Treated with AACWE (with and without treated alloxan).
- **Group X**: Treated with WCHAE+AACWE (400 mg/kg + 400 mg/kg)
Assessment of extracts and combinations on glucose level of normal animals
Rats were divided in different groups and their normal glucose levels were determined with the help of glucometer. Different doses of the extracts were administered with the help of oral feeding tube and the glucose level was determined at different time intervals i.e. 60, 120, 150, 180 mins post treatment.

Assessment of extracts and combinations on alloxan-induced diabetic animals
Rats were induced to become diabetic by a single intraperitoneal injection of alloxan monohydrate (150 mg/kg).

Alloxan was first weighed individually for each rat and then solubilized with 0.2 ml saline just prior to injection. Two days after alloxan injection, rats with plasma glucose levels of >140 mg/dl were included in the study. Treatment with plant extracts was started 48 h after alloxan injection. Fasting blood glucose estimation and body weight measurement were recorded on day 1 and 7 of the study. On day 7, blood was collected by cardiac puncture under mild ether anesthesia from overnight fasted rats and fasting blood sugar was estimated.

Serum was separated and analyzed for serum cholesterol, serum triglycerides and serum creatinine by enzymatic DHBS method. The whole pancreas from each animal was removed after sacrificing the animal and was collected in 10% formalin solution and processed for histological examination.

Statistical analysis
All the values of fasting blood sugar and biochemical estimations were expressed as mean ± standard error of means (S.E.M.) and analyzed by ANOVA and post hoc Dunnett’s t-test. Differences between groups were considered significant at P < 0.05 levels.

RESULTS
Acute toxicity
The LD_{50} dose of all the extracts was found to be 400 mg/kg, where as in case of AAHAE the LD_{50} dose was found to be 196 mg/kg due to some toxicity the detail study in this relation is under progress.

Acacia arabica and Withania coagulans extract studies
Alloxan is cytotoxic to the pancreatic β-cells thus it is an effective diabetes-induction agent. It has previously been widely used to induce diabetes mellitus in experimental animal models, allowing investigation of hypoglycemic agents in the treatment of diabetes.

Alloxan injection consistently produced symptoms characteristic of diabetes mellitus including hyperglycemia, decreased insulin levels, polyuria and weight loss. In our approach, we demonstrated the efficacy of Alloxan through the glibenclamide studies in diabetic rats as well as in normal hyperglycemic rats.

In the present study, the hypoglycemic activity of cold water extracts, hot water extracts and hydroalcoholic extracts from Acacia arabica bark and Withania coagulans were evaluated in normal and alloxan-induced diabetic rats. A single oral administration with a combination of all the three extracts from Acacia arabica bark and Withania coagulans caused a significant decrease in serum glucose levels in normal rats with dose 400 mg/kg b.w for AACWE and AAHWE whereas in AAHAE dose is 196 mg/kg b.w.

(Table 1). Moreover, these doses of the extract from Acacia arabica bark and Withania coagulans produced the maximum glucose lowering in diabetic rats serum (Table 2). A significant time-dependent hypoglycemic effect was shown throughout.

Table 1: Effect of different extracts of Withania coagulans and Acacia arabica on blood glucose level of normal rats

<table>
<thead>
<tr>
<th>Group No</th>
<th>Treatments</th>
<th>Dose mg/kg p.o</th>
<th>0 min</th>
<th>60 min</th>
<th>120 min</th>
<th>180 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>5 ml</td>
<td>84.00 ± 2.14</td>
<td>83.80 ± 1.96</td>
<td>83.00 ± 1.76</td>
<td>82.20 ± 1.68</td>
</tr>
<tr>
<td>II</td>
<td>Standard drug</td>
<td>10</td>
<td>88.00 ± 0.71</td>
<td>72.40 ± 0.92**</td>
<td>65.80 ± 1.28**</td>
<td>67.21 ± 0.86**</td>
</tr>
<tr>
<td>IV</td>
<td>WCHAE.</td>
<td>400</td>
<td>84.40 ± 1.50</td>
<td>74.00 ± 0.70**</td>
<td>71.20 ± 1.39**</td>
<td>67.40 ± 1.53**</td>
</tr>
<tr>
<td>V</td>
<td>WCHWE.</td>
<td>400</td>
<td>82.6 ± 2.21**</td>
<td>83.2 ± 2.17**</td>
<td>81.4 ± 3.50**</td>
<td>78.6 ± 3.45**</td>
</tr>
<tr>
<td>VI</td>
<td>WOCWE.</td>
<td>400</td>
<td>84.60 ± 1.83**</td>
<td>86.40 ± 1.69**</td>
<td>85.6 ± 1.03**</td>
<td>83.00 ± 1.14**</td>
</tr>
<tr>
<td>VII</td>
<td>AAHAE.</td>
<td>196</td>
<td>88.6 ± 0.88</td>
<td>82.61 ± 1.54**</td>
<td>79.0 ± 2.08**</td>
<td>80 ± 0.54**</td>
</tr>
<tr>
<td>VIII</td>
<td>AAHWE.</td>
<td>400</td>
<td>87.12 ± 1.52</td>
<td>78.33 ± 0.33**</td>
<td>65.67 ± 0.88</td>
<td>66.0 ± 0.57**</td>
</tr>
<tr>
<td>IX</td>
<td>AACWE.</td>
<td>400</td>
<td>87.00 ± 1.14</td>
<td>66.81 ± 2.70**</td>
<td>60.60 ± 2.54**</td>
<td>61.20 ± 1.81**</td>
</tr>
<tr>
<td>X</td>
<td>Com 1</td>
<td>400 + 400</td>
<td>86.1 ± 1.67</td>
<td>66.7 ± 2.88**</td>
<td>54.8 ± 1.85**</td>
<td>45 ± 2.96**</td>
</tr>
<tr>
<td>XI</td>
<td>Com 2</td>
<td>400 + 200</td>
<td>84.4 ± 1.69</td>
<td>69.0 ± 3.63**</td>
<td>56.61 ± 2.82**</td>
<td>48.2 ± 2.51**</td>
</tr>
<tr>
<td>XII</td>
<td>Com 3</td>
<td>200 + 400</td>
<td>83.79 ± 1.24</td>
<td>71 ± 2.86**</td>
<td>57.80 ± 2.57**</td>
<td>54.8 ± 2.83**</td>
</tr>
</tbody>
</table>

Values are given in average body weight (g) ± SEM for groups of five animals each. Vehicle (TWEEN 80). Diabetic control 150mg/kg b.w. dose. (Alloxan)

*P < 0.05 as compared to vehicle control. **P<0.01 as compared to normal. WCHAE. Withania coagulans hydroalcoholic extract; WCHWE. Withania coagulans Hot water extract extract; WCCWE Withania coagulans cold water extract extract; AAHAE. Acacia arabica hydroalcoholic extract; AAHWE. Acacia arabica Hot water extract extract; AACWE. Acacia arabica cold water extract extract; Com 1. Withania coagulans hydroalcoholic extract and Acacia arabica cold water extract extract; Com 2 Withania coagulans hydroalcoholic extract and Acacia arabica cold water extract extract; Com 3 Withania coagulans hydroalcoholic extract and Acacia arabica cold water extract extract.

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the period studied (Table 3). Based on the hypoglycemic effect in normal and diabetic rats, these results reinforce the hypothesis that the hypoglycemic mechanism involves an insulin-like effect, possibly through peripheral glucose consumption.\[24,25,26\] Although the cold water extract from Acacia arabica bark and hydroalcoholic extract of Withania coagulans displayed a higher significant hypoglycemic effect in normal rats, the main mechanism by which Acacia arabica and Withania coagulans brings about its hypoglycemic action probably is by stimulating peripheral glucose consumption. Whereas it is assuming that the Acacia arabica cold water extract exert its action similar to glibenclamide. A number of other plants have also been reported to have hypoglycemic effects. From the studies with the tested plant extract, the chronic effect antihyperglycemic activity of AACWE and WCHAE was demonstrated at a dose of 400 mg/kg (Table 3). Consequently, this dosage was considered as a quantitative basis to study in severe alloxan-diabetic rats or in normal animals.

**DISCUSSION**

The pancreas is the primary organ involved in sensing the organism’s dietary and energetic states via glucose concentration in the blood and in response to elevated blood glucose.

**Table 2: Effect of different extracts of Withania coagulans and Acacia arabica on blood glucose level of diabetic rats**

<table>
<thead>
<tr>
<th>Group No</th>
<th>Treatments</th>
<th>Dose mg/kg p.o</th>
<th>0 min</th>
<th>60 min</th>
<th>120 min</th>
<th>180 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>5 ml</td>
<td>84.00 ± 2.14</td>
<td>83.80 ± 1.96</td>
<td>83.00 ± 1.76</td>
<td>82.20 ± 1.68</td>
</tr>
<tr>
<td>II</td>
<td>Diabetic control</td>
<td>5 ml</td>
<td>215.20 ± 4.18</td>
<td>217.00 ± 3.61</td>
<td>216.80 ± 3.45</td>
<td>217.60 ± 4.54</td>
</tr>
<tr>
<td>III</td>
<td>Standard drug</td>
<td>10</td>
<td>280.41 ± 3.81</td>
<td>214.2 ± 4.9**</td>
<td>174.2 ± 4.41**</td>
<td>173.0 ± 3.22**</td>
</tr>
<tr>
<td>IV</td>
<td>WCHAE.</td>
<td>400</td>
<td>283.80 ± 1.59</td>
<td>242.00 ± 1.26**</td>
<td>233.07 ± 1.62**</td>
<td>231.40 ± 1.36**</td>
</tr>
<tr>
<td>V</td>
<td>WCHWE.</td>
<td>400</td>
<td>233.60 ± 7.5</td>
<td>231.00 ± 6.88**</td>
<td>227.80 ± 5.57**</td>
<td>226.00 ± 7.71</td>
</tr>
<tr>
<td>VI</td>
<td>WCCWE.</td>
<td>400</td>
<td>226.00 ± 7.5</td>
<td>224.80 ± 5.57**</td>
<td>228.60 ± 6.03**</td>
<td>216.61 ± 4.65**</td>
</tr>
<tr>
<td>VII</td>
<td>AAHAE</td>
<td>196</td>
<td>227. ± 5.41</td>
<td>205.81 ± 5.6**</td>
<td>187.40 ± 4.49**</td>
<td>185.6 ± 3.23**</td>
</tr>
<tr>
<td>VIII</td>
<td>AAHWE</td>
<td>400</td>
<td>224.6 ± 3.28</td>
<td>197.2 ± 6.93**</td>
<td>180.2 ± 5.39**</td>
<td>179 ± 5.76**</td>
</tr>
<tr>
<td>IX</td>
<td>AACWE.</td>
<td>400</td>
<td>218.8 ± 3.41</td>
<td>201.0 ± 5.9*</td>
<td>174.80 ± 2.87**</td>
<td>169.20 ± 4.22**</td>
</tr>
<tr>
<td>X</td>
<td>Com 1</td>
<td>400 + 400</td>
<td>282.2 ± 1.74</td>
<td>225.4 ± 9.17**</td>
<td>197.40 ± 3.04**</td>
<td>184.2 ± 5.64**</td>
</tr>
<tr>
<td>XI</td>
<td>Com 2</td>
<td>400 + 200</td>
<td>292 ± 1.59</td>
<td>232.61 ± 8.34**</td>
<td>205.8 ± 4.18**</td>
<td>193 ± 1.78**</td>
</tr>
<tr>
<td>XII</td>
<td>Com 3</td>
<td>200 + 200</td>
<td>283.4 ± 9.70</td>
<td>241.6 ± 8.81**</td>
<td>114.31 ± 2.48</td>
<td>208.4 ± 2.27**</td>
</tr>
</tbody>
</table>

*P < 0.05 as compared to vehicle control. **P<0.01 as compared to normal. WCHAE.

**Table 3: Effect of WCHAE, AACWE and COM 1 on blood glucose level of alloxan induced diabetic rats (chronic effect)**

<table>
<thead>
<tr>
<th>Group No</th>
<th>Treatments</th>
<th>Dose mg/kg p.o</th>
<th>1st Day</th>
<th>3rd Day</th>
<th>5th Day</th>
<th>7th Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>5 ml</td>
<td>84.00 ± 2.14</td>
<td>85.12 ± 2.16</td>
<td>83.10 ± 1.76</td>
<td>82.20 ± 1.68</td>
</tr>
<tr>
<td>II</td>
<td>Diabetic control</td>
<td>10</td>
<td>278.20 ± 4.18</td>
<td>280.15 ± 4.15</td>
<td>281.36 ± 2.56</td>
<td>280.14 ± 2.24</td>
</tr>
<tr>
<td>III</td>
<td>WCHAE.</td>
<td>400</td>
<td>283.80 ± 1.59</td>
<td>222.61 ± 1.48**</td>
<td>101.23 ± 2.16**</td>
<td>99.22 ± 1.35**</td>
</tr>
<tr>
<td>IV</td>
<td>AACWE.</td>
<td>400</td>
<td>225.75 ± 1.59</td>
<td>179.23 ± 1.82**</td>
<td>134.53 ± 1.39**</td>
<td>87 ± 1.56**</td>
</tr>
<tr>
<td>V</td>
<td>COM 1</td>
<td>400 + 400</td>
<td>233.60 ± 5.41</td>
<td>227. ± 5.41</td>
<td>187.40 ± 4.49**</td>
<td>185.6 ± 3.23**</td>
</tr>
</tbody>
</table>

*P < 0.05 as compared to vehicle control. **P<0.01 as compared to normal. WCHAE. Withania coagulans hydroalcoholic extract; AACWE. Acacia arabica cold water extract extract; Com 1. Withania coagulans hydroalcoholic extract and Acacia arabica cold water extract extract; Com 2 Withania coagulans hydroalcoholic extract and Acacia arabica cold water extract extract; Com 3 Withania coagulans hydroalcoholic extract and Acacia arabica cold water extract extract.

**Combinations of active extract of Withania coagulans and Acacia arabica**

The hypoglycemic activity of different combinations of active extracts of Withania coagulans fruit and Acacia arabica bark (i.e. AACWE and WCHAE) was evaluated in normal and alloxan-induced diabetic rats. A single oral administration with all the three extracts caused a significant decrease in serum glucose levels in normal rats with dose 400mg/kg + 400mg/kg b.w all extracts studied (Table 1). Moreover, these doses of the extracts produced the maximum glucose lowering in diabetic rats serum (Table 2), a significant time-dependent hypoglycemic effect was shown throughout the period studied (Table 3). From the studies with the tested plant extract, the optimal antihypoglycemic activity was demonstrated at a dose of 400 +400 mg/kg.

In histological slides the β-cells are completely destroyed in the diabetic group animal (Figure 1). In contrast, the animals which are given with combination of extract have shown regeneration of the β-cells. No such regeneration was seen in the rats treated with single Withania coagulans or Acacia arabica extracts, no regeneration of the β-cells are seen.

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Insulin is secreted.[29] Alloxan (2,4,5,6-tetraoxypyrimidine; 2,4,5,6-pyrimidinetetraone) is an oxygenated pyrimidine derivative[30] and was originally isolated in 1818 by Brugnatelli and got its name in 1838 by Friedrich Wöhler and Justus Von Liebig. Alloxan is a toxic glucose analogue, which selectively destroys insulin-producing cells in the pancreas when administered to rodents and many other animal species. This causes an insulin-dependent diabetes mellitus (called “Alloxan Diabetes”) in these animals, with characteristics similar to type 1 diabetes in humans[31].

According to earlier studies, plant extracts cause antihyperglycemic effect by promoting regeneration of β-cells or by protecting these cells from destruction, by restricting glucose load as well as by promoting unrestricted endogenous insulin action. Antihyperglycemic effect may also be caused by the effect of plant extract on β-cells to release insulin or activate the insulin receptors to absorb the blood sugar and stimulate the peripheral glucose consumption[32-33]. In light of the results, our study indicates that Acacia Arabica and Withania coagulans extracts have good antidiabetic activity. The number of functionally intact beta-cells in the islet organ is of decisive importance for the development course and outcome of diabetes. The renewal of beta-cells in diabetes has been studied in several animal models. The total beta-cell mass reflects the balance between the renewal and loss of these cells. It was also suggested that regeneration of islet beta-cells following destruction by alloxan may be the primary cause of the recovery of alloxan-injected guinea pigs from the effects of the drug[34]. In our studies, the damage of pancreas in alloxan-treated diabetic control rats (Fig. 1a) and regeneration of beta-cells by Acacia arabica and combination of extracts (Figure 1c and 1d) was observed.

CONCLUSION

The hydroalcoholic extract of Withania coagulans and the cold aqueous extract of Withania coagulans exhibited antihyperglycemic activities in alloxan-induced diabetic rats. Combination of both plants in the ratio 400 + 400 mg/kg shows higher antihyperglycemic activities than for either extract alone.

ACKNOWLEDGEMENT

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