Research Article

Biochemical and Histological Evaluation of Three Selected Medicinal Plant Extracts of Sri Lankan Origin on Dyslipidemia and Oxidative Stress in Alloxan Induced Diabetic Rats

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The objective of the present study was to investigate the effect of refluxed aqueous extracts of Gmelina arborea, Spondias pinnata, and Coccinia grandis on atherogenicity and oxidative stress in rats with chemically induced type 1 diabetes mellitus. Alloxan monohydrate (150 mg/kg, intraperitoneal) was used to induce diabetes to Wistar rats. Thereafter, diabetic rats (n=6 per group) were treated with the three selected plant extracts at their optimum effective therapeutic doses and glibenclamide (0.50 mg/kg, positive control) for 30 days. Administration of the three extracts in diabetic rats exhibited antihyperglycemic, antiatherogenic, and antioxidant effects in diabetic rats on the 30th day of the study. The atherogenic and coronary risk indices were also reduced in support of the antiatherogenic effects. The results of the study revealed that the bark extracts of G. arborea, S. pinnata, and leaf extract of C. grandis exerted beneficial effects against dyslipidemia, atherogenicity, and oxidative stress in alloxan induced diabetic rats. The selected plant extracts would be beneficial for the development of food supplements targeting main complications associated with diabetes.

1. Introduction

Diabetes mellitus (DM) is a leading cause of morbidity and mortality worldwide and predicted to affect over 500 million people by 2030. Indeed, it is a major cause of disability and hospitalization, posing a significant public health burden during the last two decades [1, 2].

The chronic hyperglycemia of diabetes is associated with deleterious damage, dysfunction, and failure of various organs. Oxidative stress has been suggested as a main contributory factor in the pathogenesis of diabetes and its vascular complications leading to neuropathy, retinopathy, nephropathy, cardiovascular disease, etc. [3]. In addition, diabetes is closely associated with dyslipidemia which is mediated through derangements in a variety of regulatory processes, especially in a state of insulin deficiency, thereby rendering diabetic patients more prone to hypercholesterolemia and hypertriglyceridemia. Several studies have revealed the positive correlation between dyslipidemia and development of premature atherosclerosis, coronary insufficiency, and myocardial infarction in diabetic subjects [4–7]. However, the occurrence of vascular complications in patients with diabetes mellitus has not been met with a comparable expansion in therapeutic options [1]. Thus, effect of natural products in the management of diabetic complications has recently received considerable attention, highlighting the importance of medicinal plant extracts as regulators of metabolism of carbohydrate and lipids [8].

It is being appreciated that traditional systems of medicine can offer some effective therapies from their treatise to be useful in diabetic complications. The remarkable chemical diversity encompassed by the medicinal plant extracts continues to be of relevance to the discovery of new antidiabetic agents with fewer side effects [9]. A number of
medicinal plant extracts in the form of polyherbal mixtures have been widely used in Ayurvedic medicine in Sri Lanka targeting diabetes complications [10]. However, most of the plant extracts have not been subjected to scientific evaluation and proper scrutinization using appropriate models of diabetes. Therefore, it is worth assessing the efficacy against hyperglycemia, dyslipidemia, and atherogenicity in an animal model of type 1 diabetes. The medicinal plants/parts selected for the present investigation were bark of *Gmelina arborea* Roxb. (family: Verbenaceae), *Spondias pinnata* (Linn. f.) Kurz (family: Anacardiaceae), and leaves of *Coccinia grandis* (L.) Voigt (Cucurbitaceae). The above plants have been widely consumed by the general public as dietary adjuncts in the Southern region of Sri Lanka and more importantly they are widely used in Ayurvedic preparations used in the treatment of vascular complications in diabetes since ancient time [10, 11]. Selection of the plant part was based on the use of the particular part in Ayurvedic preparations in the management of diabetes mellitus. Extensive research has been done for the investigation on phytochemicals, antidiabetic mechanisms, and *in vitro* antioxidant potentials of the selected plant extracts by our research group [12–16]. In addition, the optimum effective antihyperglycemic doses of the bark extracts of *G. arborea* and *S. pinnata* and leaf extract of *C. grandis* were found to be 1.00, 1.00, and 0.75 g/kg in alloxan induced diabetic rats, respectively [12]. The evidence in wide use of the plant extracts in the management of vascular complications tempted us to speculate that apart from the published antidiabetic effects and mechanisms, and the selected medicinal plant extracts might exert antihyperlipidemic and antioxidant effects *in vivo*. The objective of the present study was to investigate the effect of refluxed aqueous extracts of *G. arborea*, *S. pinnata*, and *C. grandis* on atherogenicity and oxidative stress in rats with chemically induced type 1 diabetes mellitus.

2. Materials and Methods

2.1. Chemicals and Instruments. Alloxan monohydrate, D-glucose, and glibenclamide were purchased from Sigma-Aldrich Company (St. Louis, MO, United States). No any purification was done on purchased chemicals. A UV visible spectrophotometer (Gallenkamp PLC, UK) and microplate reader (Mindray, China) were used for spectrophotometric and enzyme linked immunosorbent assay (ELISA) measurements, respectively. Olympus CX 21(Japan) microscope was used in the assessment of histopathology of the liver tissues.

2.2. Plant Material. The selected plant parts as stem bark of *G. arborea*, *S. pinnata*, and leaves of *C. grandis* were collected during May–June 2013 from the Southern region of Sri Lanka.

The botanical authentication of the selected medicinal plants was confirmed by comparing with the samples at the National Herbarium, Royal Botanical Gardens, Peradeniya, Sri Lanka. Voucher specimens were deposited at the Department of Biochemistry, Faculty of Medicine, University of Ruhuna, Sri Lanka (FM/09/Attanayake/1-3).

2.3. Preparation of the Hot Water Plant Extracts. The water extract was used for the study to simulate the form that is used in traditional medicinal preparations. The dried powdered plant material (50.00 g) of the three selected plant species was dissolved in 400.0 mL of distilled water and refluxed for 4 h separately. The mixture was strained and the final volume was adjusted to 50.0 mL. A single dose of the bark extract of *G. arborea* (1.00 g/kg) and *S. pinnata* (1.00 g/kg) and the leaf extract of *C. grandis* (0.75 g/kg) was administered orally to alloxan induced diabetic rats.

2.4. Laboratory Animals. Healthy adult male rats of Wistar strain (200 ± 25 g body weight) were purchased from the Medical Research Institute (MRI), Sri Lanka, and were used to carry out the experiments. They were housed in standard environmental conditions at the animal house of Faculty of Medicine, University of Ruhuna, Sri Lanka (temp 25 ± 2 °C, relative humidity 55–65% and 12 ± 1 h light/dark cycle). Rats were fed with a standard diet (MRI rat formulae, Sri Lanka) with free access to water before and during the experiment. The rats were randomized into various groups and allowed to acclimatize for a period of seven days under standard environmental conditions before the commencement of the experiments. The animals described as fasting were deprived of food and water for 12 h *ad libitum*. All protocols used in this study were approved by the Ethical Review Committee of Faculty of Medicine, University of Ruhuna, Sri Lanka, guided by the Council for International Organization of Medical Sciences (CIOMS) international guiding principles of biomedical research involving animals.

2.5. Induction of Diabetes Mellitus in Wistar Rats. Alloxan monohydrate dissolved in sterile saline at a dose of 150 mg/kg was administered intraperitoneally to fasted rats [17]. Rats with fasting blood glucose concentration of 12.0 mmol/L or above were considered as hyperglycemic and used for the experiments.

2.6. Experimental Group Design. The rats were divided into 6 groups and each group consisted of 6 rats.

- **Group 1**: Healthy control rats treated with distilled water for 30 days
- **Group 2**: Diabetic control rats treated with distilled water for 30 days
- **Group 3**: Diabetic rats treated with bark extract of *G. arborea* (1.00 g/kg) for 30 days
- **Group 4**: Diabetic rats treated with bark extract of *S. pinnata* (1.00 g/kg) for 30 days
- **Group 5**: Diabetic rats treated with leaf extract of *C. grandis* (0.75 g/kg) for 30 days
- **Group 6**: Diabetic rats treated with glibenclamide (0.50 mg/kg) for 30 days

On the 30th day, animals were sacrificed and blood was collected by cardiac puncture and the liver was excised from sacrificed rats. Serum was separated from blood of all rats for
the estimation of biochemical parameters. Liver tissue was excised for the assessment of histopathology.

2.7. Blood/Serum Glycemic Parameters. The percentage of glycated hemoglobin (HbA1C) was estimated in all rats using spectrophotometric enzyme assay kits [18]. Furthermore, the concentrations of serum insulin and C-peptide in all rats were estimated using enzyme linked immune-sorbent assay methods [19, 20].

2.8. Serum Lipid Parameters. The concentrations of serum total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), and triglyceride (TG) were estimated in all rats using spectrophotometric enzyme assay kits [21–23]. The concentrations of serum low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) were calculated using the Friedewald formulae [24]. Carotid protective index (CPI) was estimated in terms of HDL-C to LDL-C ratio, whereas atherogenic (AI) and coronary risk indices (CRI) were calculated by the following formulae [25]:

\[ \text{AAI} = \frac{\text{TC-HDL-C}}{\text{HDL-C}} \]

\[ \text{CRI} = \frac{\text{TC}}{\text{HDL-C}} \]

2.9. Antioxidant Markers. Fasting serum activities of liver enzymes, alanine aminotransferase (ALT, EC 2.6.1.2), aspartate aminotransferase (AST, EC 2.6.1.1), and alkaline phosphatase (ALP, EC 3.1.3.1) were estimated using spectrophotometric enzyme assay kits [26, 27]. The estimation of reduced glutathione (GSH), activities of glutathione reductase (GR, EC 1.6.4.2), glutathione peroxidase (GPx, EC 1.11.1.9), and glutathione S-transferase (GST, EC 2.5.1.18) in the liver homogenates were done using reported protocols [28–30]. Further, the extent of lipid peroxidation and total protein content were estimated in serum homogenates using the formation of malondialdehyde (MDA) by thiobarbituric acid and Lowry methods, respectively [31, 32].

2.10. Statistical Analysis. The replicates of each sample were used for statistical analysis and the values were expressed as mean ± standard deviation in the in vitro study. The data were analyzed using analysis of variance (ANOVA) and the mean values for each group were compared by Dunnett’s multiple comparison tests in the in vivo study. The level of significance was set at p<0.05.

3. Results

The results of biochemical parameters and calculated indices in all study groups are shown in Table 1. The reduction in the percentage of HbA1C was in the decreasing order of C. grandis (35%), G. arborea (31%), and S. pinnata (29%) in alloxan induced diabetic rats (p<0.05). The concentration of serum insulin and C-peptide were decreased significantly in a decreasing order of C. grandis (72%, 51%), G. arborea (44%, 44%), and S. pinnata (34%, 24%) in alloxan induced diabetic rats (p<0.05).

As mentioned in Table 1, alloxan induced diabetic rats treated with the extracts of G. arborea, S. pinnata, and C. grandis showed a reduction in the concentration of serum TC (33%, 21%, and 19%), LDL-C (46%, 28%, and 28%), VLDL-C (25%, 39%, and 17%), and TG (26%, 39%, and 18%) and a significant elevation in HDL-C (16%, 20%, and 17%) on the 30th day of study (p<0.05). The concentration of serum TC, LDL-C, VLDL-C, and TG were reduced by 36%, 43%, 40%, and 38% in glibenclamide treated alloxan induced diabetic rats respectively (p<0.05). In contrast, there was no significant change in the concentration of serum HDL-C with the treatment of glibenclamide (p > 0.05). Administration of the three plant extracts at their optimum effective therapeutic dose attenuated elevated levels of atherogenic index and coronary risk index to near normal while cardioprotective index was increased. In addition, the cardioprotective effect of the three selected plant extracts was better than the effect of glibenclamide in diabetic rats. There was an elevation in the serum activities of ALT (58%), AST (52%), and ALP (71%) and the concentration of MDA (164%) in alloxan induced diabetic rats when compared to untreated healthy control rats. In contrast, a reduction in the concentration of GSH (33%), GR (46%), GPx (44%), and GST (52%) was shown in alloxan induced diabetic rats (p<0.05). All three plant extracts showed significant antioxidant activities while the highest elevation was obtained by the extract of G. arborea treated alloxan induced diabetic rats, indicated through an increase in the concentration of GSH (29%), activities of GR (49%), GPx (23%), and GST (68%) and a reduction in the concentration of MDA (44%) (p<0.05). The reduction in the activities of liver enzymes and improvement on antioxidant enzymes were more prominent in plant extracts treated rats with compared to the glibenclamide treated rats which was further corroborated with the histopathological findings. Further, the plant extracts reduced the hepatic enzyme activities in the descending order of G. arborea, C. grandis, and S. pinnata (p<0.05).

Microscopic observation of the H and E stained liver sections of the untreated alloxan induced diabetic rats showed very early microvesicular fatty change in the centrilobular areas of the liver tissue, mild congestion, moderate lymphocytic infiltrates mostly around portal tract, focal necrosis with inflammatory cell infiltrates, and focal fibrosis (Figure 1(b)). The appearance in the histopathology is corroborated with the biochemical values obtained in alloxan induced control rats. The liver tissues of the plant extracts treated diabetic rats showed a definitive reduction in microvesicular fatty changes with mild lymphocytic infiltrates and no necrosis (Figures 1(c)-1(e)).

4. Discussion

The present study was carried out to determine the effect of hot water extracts of G. arborea, S. pinnata, and C. grandis at their optimum therapeutic doses on glycemic parameters, lipid profile, and atherogenic indices and on selected oxidative stress parameters in alloxan induced diabetic rats [17]. Alloxan causes a destruction of α-cells of the islets of Langerhans in the pancreas resulting in a massive reduction in the synthesis and secretion of insulin, leading to hyperglycemia in Wistar rats [17, 33]. However, the
Table 1: Effect of plant extracts on selected biochemical parameters in alloxan induced diabetic rats after 30 days of treatment.

<table>
<thead>
<tr>
<th>Parameter tested in blood/serum</th>
<th>Healthy control rats</th>
<th>Diabetic control rats</th>
<th>Diabetic rats treated with G. arborea (1.00 g/kg)</th>
<th>Diabetic rats treated with S. pinnata (1.00 g/kg)</th>
<th>Diabetic rats treated with C. grandis (0.75 g/kg)</th>
<th>Diabetic rats treated with glibenclamide (0.50 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c (%)</td>
<td>4.8±0.2</td>
<td>9.8±0.1</td>
<td>6.7±0.1*</td>
<td>6.9±0.2*</td>
<td>6.3±0.1*</td>
<td>5.6±0.1*</td>
</tr>
<tr>
<td>Insulin (µIU/mL)</td>
<td>14.8±0.1</td>
<td>6.0±0.1</td>
<td>8.7±0.1*</td>
<td>8.0±0.1*</td>
<td>8.0±0.1*</td>
<td>10.4±0.1*</td>
</tr>
<tr>
<td>C-peptide (ng/mL)</td>
<td>9.4±0.1</td>
<td>5.3±0.1</td>
<td>7.7±0.1*</td>
<td>6.6±0.1*</td>
<td>8.1±0.1*</td>
<td>8.6±0.1*</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>3.8±0.2</td>
<td>6.0±0.0</td>
<td>4.0±0.3*</td>
<td>4.7±0.2*</td>
<td>4.8±0.1*</td>
<td>3.8±0.2*</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.1±0.1</td>
<td>1.0±0.1</td>
<td>1.3±0.1*</td>
<td>1.4±0.1*</td>
<td>1.4±0.1*</td>
<td>1.0±0.1</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>0.2±0.1</td>
<td>0.4±0.2</td>
<td>1.5±0.4*</td>
<td>1.6±0.1*</td>
<td>1.7±0.1*</td>
<td>1.2±0.2*</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>1.1±0.1</td>
<td>2.1±0.1</td>
<td>2.4±0.2*</td>
<td>3.2±0.1*</td>
<td>3.2±0.1*</td>
<td>2.5±0.2*</td>
</tr>
<tr>
<td>VLDL-C (mmol/L)</td>
<td>2.4±0.1</td>
<td>4.5±0.1</td>
<td>0.3±0.3*</td>
<td>0.2±0.2*</td>
<td>0.3±0.2*</td>
<td>0.2±0.2*</td>
</tr>
<tr>
<td>AI</td>
<td>2.4</td>
<td>5.0</td>
<td>2.07</td>
<td>2.51</td>
<td>2.43</td>
<td>2.8</td>
</tr>
<tr>
<td>CRI</td>
<td>3.4</td>
<td>6.0</td>
<td>3.10</td>
<td>3.21</td>
<td>3.32</td>
<td>3.8</td>
</tr>
<tr>
<td>CPI</td>
<td>1.0</td>
<td>0.5</td>
<td>0.54</td>
<td>0.5</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>13.1±1.0</td>
<td>20.7±1.1</td>
<td>12.8±0.1*</td>
<td>16.0±0.1*</td>
<td>14.8±0.1*</td>
<td>18.3±0.2*</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>43.6±1.0</td>
<td>66.1±1.9</td>
<td>49.5±1.9*</td>
<td>60.1±1.2*</td>
<td>53.3±1.0*</td>
<td>63.0±1.8*</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>63.3±0.5</td>
<td>108.2±1.1</td>
<td>78.0±1.2*</td>
<td>73.1±2.8*</td>
<td>74.9±1.2*</td>
<td>104.7±1.0*</td>
</tr>
<tr>
<td>GSH (µg/g liver)</td>
<td>7128.8±7.4</td>
<td>474.8±8.7</td>
<td>612.3±26.8*</td>
<td>572.1±14.2*</td>
<td>5770.8±8.0*</td>
<td>5846.1±11.7*</td>
</tr>
<tr>
<td>GR (nmol/min/mg of protein)</td>
<td>9.0±1.6</td>
<td>4.9±0.4</td>
<td>7.3±1.2*</td>
<td>7.0±0.2*</td>
<td>7.1±0.3*</td>
<td>5.4±0.1*</td>
</tr>
<tr>
<td>GPx (nmol/min/mg of protein)</td>
<td>10.1±0.9</td>
<td>5.7±0.1</td>
<td>7.0±0.4*</td>
<td>6.2±0.9*</td>
<td>70.6</td>
<td>6.6±0.7*</td>
</tr>
<tr>
<td>GST (nmol/min/mg of protein)</td>
<td>10.5±1.4</td>
<td>5.0±0.1</td>
<td>8.5±1.0**</td>
<td>7.5±0.4**</td>
<td>7.4±0.9</td>
<td>5.4±0.1*</td>
</tr>
<tr>
<td>MDA (nmol/mg protein)</td>
<td>11.0±0.3</td>
<td>29.0±1.8</td>
<td>16.1±1.6*</td>
<td>21.3±2.1*</td>
<td>21.3±2.0*</td>
<td>17.5±1.5*</td>
</tr>
</tbody>
</table>

HbA1c: glycated haemoglobin, TC: total cholesterol, HDL-C: high density lipoprotein cholesterol, TG: triglyceride, LDL-C: low density lipoprotein cholesterol, VLDL-C: very low density lipoprotein cholesterol, ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, GSH: reduced glutathione, GR: glutathione reductase, GPx: glutathione peroxidase, GST: glutathione S-transferase, MDA: malondialdehyde, AI: atherogenic index, CRI: coronary risk index, and CPI: cardio protective index. The values are expressed as mean ± SEM (n=6/group). *Statistically significant from alloxan induced diabetic control rats at p<0.05 (ANOVA followed by Dunnett's test).
administration of the selected plant extracts significantly increased the concentrations of C-peptide and insulin indicating their potent antihyperglycemic activity in diabetic rats. The results are in accordance with our previously published data on investigation of detailed antidiabetic mechanisms of the selected extracts in streptozotocin induced diabetic rats [13, 15, 16, 34, 35].

In the present study, alloxan induced diabetic rats had an alteration in the lipid profile at the end of the study period. Furthermore, a decrease in serum HDL-C concentration was also observed in diabetic rats, which reflects the low cholesterol transport by HDL particle in the blood from peripheral tissues to the liver for its metabolism [36]. The increase in the level of TC, TG, and LDL-C and a decrease level of HDL-C contribute to increase the risk for development of cardiovascular diseases in patients with diabetes mellitus [37]. In the present study, the *G. arborea*, *S. pinnata*, and *C. grandis* extracts significantly decreased the serum concentrations of TC, TG, and LDL-C and increased the serum concentration of HDL-C in diabetic rats as compared to diabetic control rats. The highest antihypercholesterolemic and antihypertriglyceridemic effects were demonstrated in the *G. arborea* extract treated group. The potential effects may be predominantly due to the inhibition of rate-limiting enzyme HMG-CoA reductase of cholesterol biosynthesis and improvement in the lipolysis by reducing the activity of hormone sensitive lipase respectively. The lipoproteins, especially LDL-C, are involved in the process of formation of atherosclerotic plaques [38]. The association between a low level of HDL-C and an increased risk of cardiovascular disease has been well established in diabetic patients through epidemiological and clinical studies [39]. In the present study, plant treatments led to a significant elevation of HDL-C, indicating its promising protective role against cardiovascular
disease via counteracting LDL oxidation, promoting reverse cholesterol transport pathway, inducing an efflux of excess accumulated cellular cholesterol, and preventing the generation of an oxidatively modified LDL particles [40]. In contrast, the administration of glibenclamide was not able to show any significant effect on the concentration of HDL-C in diabetic rats highlighting the importance of tested plant extracts in lowering the risk of cardiovascular diseases. The antihyperlipidemic potentials of the three extracts were further confirmed with the results of calculated indices including CPI, AI, and CRI. These indices are powerful indicators in evaluating the risk of cardiovascular diseases especially in diabetic subjects [38]. Higher values of AI and CRI imply higher risk of developing cardiovascular disease and vice versa [38].

The AI and CRI were higher while CPI was lower in diabetic control rats with compared to healthy control rats. The administration of the plant extracts of G. arborea, S. pinnata, and C. grandis were able to decrease the AI and CRI. In contrast, the CPI in terms of HDL-C/LDL-C ratio was increased with the plant treatments which further strengthen the potency of the medicinal plant extracts against atherogenicity in diabetic rats.

Oxidative stress is a key factor in the pathogenesis of diabetic complications [41]. Studies in animal models and clinical trials have established a relationship between hypercholesterolemia and lipid peroxidation [42, 43]. In agreement with the reported literature, our results demonstrated a significant increase in the concentration of MDA in the liver tissue of alloxan induced diabetic rats. On the other hand, treatment with the plant extracts of G. arborea, S. pinnata, and C. grandis caused a significant reduction in the concentrations of MDA in the liver tissue. A significant decrease in the activities of antioxidant enzymes, GR, GPx, and GST was observed and these are considered as parameters in antioxidant defense against oxidative injury. The antioxidant activities of the selected medicinal plant extracts may be linked to the presence of high content of different phytochemicals as polyphenol compounds, flavonoids, tannins, saponins, etc. [14, 44, 45].

5. Conclusions

The present study reveals that the bark extracts of G. arborea, S. pinnata, and leaf extract of C. grandis at their optimum effective therapeutic doses exerted beneficial effects against dyslipidemia, atherogenicity, and oxidative stress in alloxan induced diabetic rats. The highest antihyperlipidemic, antiatherogenic, antioxidative activities were obtained from the bark extract of G. arborea followed by S. pinnata and C. grandis in diabetic rats. Further studies are required to identify the active component(s) and mechanism(s) underlying the beneficial effects and to establish the therapeutic potential of the selected medicinal plant extracts. The results also scrutinize the therapeutic potentials of the selected plant extracts in the management of diabetic vascular complications. The selected plant extracts would be beneficial for the development of food supplement targeting main complications associated with diabetes.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

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