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Hepatoprotective effect of *Syringae vulgaris flos* ethanolic extracts in streptozotocin-induced diabetes in rats

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Abstract

Taking account of increasing world population life expectancy, health services will face with a large number of elderly people with chronic age-related diseases. It has been established that chronic diseases are usually accompanied by oxidative stress induced by the overproduction of reactive oxygen species damaging cellular constituents, under conditions of weakening antioxidant defense systems. The balance between free radicals and antioxidant endogenous systems has a defining role in preventing the damage of macromolecules. In addition to the enzymatic (catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase) and non-enzymatic (vitamins A, C, E) endogenous systems, a good source of natural antioxidants are medicinal herbs products or phytochemical compounds. The aim of this study is to evaluate the hepatoprotective effect of *Syringae vulgaris flos* ethanolic extracts in a rat model of streptozotocin-induced diabetes.

Keywords: *Syringa vulgaris*, hepatoprotective effect, diabetes mellitus, streptozotocin.

Introduction

The increasing world population life expectancy, alongside the genetic predisposition and environmental factors such as excess food and lack of physical activity, will cause the augmentation of chronic age-related diseases.

Clinical and experimental studies revealed the major role that oxidative stress plays in the appearance or development of various pathological conditions. Oxidative stress, due mainly to an excessive production of reactive oxygen species (ROS) and impairing body antioxidant scavenger systems, is involved also in diabetes mellitus pathogenesis and its long-term complications (atherosclerosis, nephropathy, neuropathy or retinopathy). Based on body exposure to hyperglycemia, a consequence of insufficient insulin secretion or tissue resistance to it, diabetes is training complex metabolic changes, including hyperlipidemia, thus creating favorable conditions to the emergence of free radicals overproduction and advanced glycation end-products (AGE) formation and further damage of cells constituents [1, 2]. Many studies showed that the liver is affected in diabetes, which can increase the risk of nonalcoholic fatty liver disease (NAFLD) or hepatocellular carcinoma (HCC) appearance [3–5]. Therefore, therapeutic means must be found to prevent the excessive production of highly unstable ROS (superoxide anion radical, hydrogen peroxide), which is leading to damage of proteins, lipids, nucleotides or disulfide bonds altering cell membrane structure and disturbing its normal functionality [3–5]. Another strategy to prevent this cascade of events is improving either enzymatic (catalase, superoxide dismutase – SOD, glutathione peroxidase – GPx, glutathione reductase – GR) and non-enzymatic (vitamins A, C, E) antioxidant body defense systems. Thus, could be avoided oxidative damages of macromolecules which lead to necrosis and cell death or induce mutagenic conditions [1, 2, 6].

Researches were focused on finding natural antioxidants that improve tissues sensitivity to insulin action and also protect against damages caused by oxidative stress. It should be noted that there are numerous studies on plants and natural products thereof, which have the ability to lower blood sugar levels, improve lipid profile and prevent liver injury thereby maintaining body homeostasis [6–9]. Phytochemical constituents (flavonoids, polyphenolic acids, anthocyanins, tannins), which are found in all parts of the plant, could represent a natural source of antioxidants to support harmful free radicals scavenger or anti-glycation activities.

Syringa vulgaris (*Oleaceae*), worldwide known as lilac and recognized for its immunomodulatory virtues, is used as a traditional remedy in relieving pain cused by osteoarthropathy or gout arthritis [10, 11]. Studies regarding the beneficial role of verbascosides from *S. vulgaris* in experimental animal models of inflammation (colitis, spinal cord trauma, and periodontitis) [12–14] provide a scientific basis for its traditional use.

In this study, we aimed to identify polyphenolic compounds from two ethanolic extracts of *Syringae vulgaris flos* f. *álba* and f. *violácea* and assess their

hepatoprotective effect during progression of streptozotocin-induced diabetes in rats.

Materials and Methods

Chemicals

All of analytical grade chemicals used for this study were purchased from commercial approved sources. Streptozotocin (STZ), diphenylboriloxyethylamine (DFBOA), polyethylenglycol 400 (PEG 400) and solvents were obtained from Sigma-Aldrich and substances for histological staining from Bio-Optica. Blood glucose and cholesterol levels were measured using a gluco- and cholesterol-meter (Accu-Chek® Instant Plus) from Roche Diagnostics.

Vegetal materials

White and violet lilac flowers (*Syringae vulgaris flos* f. *álba* and f. *violácea*) and bilberry leaves (*Myrtilli folium*) were collected from the "Alexandru Buia" Botanical Garden of the University of Craiova, Romania, and identified at the Department of Pharmacognosy and Phytotherapy, Faculty of Pharmacy, University of Medicine and Pharmacy of Craiova, in whose Herbarium voucher specimens are preserved. *Vaccinium myrtillus* (*Ericaceae*), worldwide known as bilberry, is a traditional medicine recognized for its antidiabetic virtues [15]. Collected plant materials were shade air dried at room temperature and stored. Powdered dried vegetal material was obtained using an electric grinder. According the X^{th} edition of the Romanian Pharmacopoeia, ethanolic extracts were obtained by simple percolation using ethanol 70^0 as solvent and then were stored into closed brown glass bottles in the refrigerator.

Thin-layer chromatography (TLC) analysis

Flavonoid heterosides and phenol carboxylic acids were identified using silica gel G F_{254} Merck TLC plates as the stationary phase, ethyl acetate–water–formic acid–acetic acid (72:14:7:7, *v/v*) as mobile phase. Analyzed samples were 20% ethanolic extracts of *Syringae vulgaris flos* f. *álba* and f. *violácea* and *Myrtilli folium*. 0.1% methanolic solutions of rutoside, hyperoside, apigenin-7-neohesperidoside, quercitroside, luteolin-7-glucoside, apigenin-7-glucoside, caffeic acid and chlorogenic acid were used as standards. 20 µL of the test samples and 10 µL of reference solutions were applied to the start line, the bands having 1 cm width with 1.5 cm between them. The plates were developed over a path of 7.5 cm and 12 cm. Revelation was done by spraying NEU/PEG solution (1% methanolic solution of DFBOA and 5% methanolic solution of PEG 400). The plates were then examined in UV light (λ 365 nm) using a CAMAG Reprostar 3 apparatus.

Animals and experimental model

Experiments were performed on 25 adult male Wistar rats of similar age and weight, maintained in optimal conditions of temperature, light and humidity, on standard food and water *ad libitum*. All the experiments were conducted in accordance to the ethical norms regarding the scientific research approved by our institution as respects the maintenance and use of animals following European standards on the protection of vertebrate animals.

Toxicity study

The toxicity of *Syringae vulgaris flos* f. *álba* and f. *violácea* was evaluated in an early stage after oral administration of elevated concentrations of ethanolic extracts and detailed observation of animals for any signs of behavioral changes and/or mortality for the following 48 hours after a single dose (acute toxicity) or for the following three weeks of daily administration. Based on these toxicity tests, we did not note any toxic or lethal effects for the ethanolic extracts and therefore these could be classified as non-toxic.

Study design

Streptozotocin (STZ) was intraperitoneally injected in a single dose of 70 mg/kg body weight (b.w.), to overnight-fasted rats. Glycemia was monitored with the glucometer by tail bleed procedure. Rats were considered diabetics when fasting glucose levels where higher than 150 mg/dL at least three days after STZ injection, following established protocols described previously [16].

Rats were divided into five groups, as follows: G1 – untreated healthy rats serving as normal control; G2 – diabetic untreated rats serving as diabetic control; G3 – diabetic rats treated daily with 150 mg/kg b.w. from a 20% ethanolic extract of *Syringae vulgaris flos* f. *violácea* (SV); G4 – diabetic rats treated daily with 150 mg/kg b.w. from a 20% ethanolic extract of *Syringae vulgaris flos* f. *álba* (SA); G5 – diabetic rats treated daily with 150 mg/kg b.w. from a 20% ethanolic extract of *Myrtilli folium* (MF). Rats from G3, G4 and G5 were orally treated for six weeks. Blood glucose and cholesterol levels and body weight were measured every morning at the same hour.

Histology

After six weeks, the animals were sacrificed and the liver was harvested and fixed for 48 hours in 10% buffered formalin. Liver fragments were processed for paraffin embedding (dehydrated in graded ethanol, cleared in xylene and embedded in paraffin). From the paraffin blocks were then cut sections of 4 µm thickness used for histological staining with Hematoxylin and Eosin (HE). Slides were observed and registered with a Nikon Eclipse microscope coupled to a digital camera for photomicroscopic observations on the liver architecture of the control and treated rats. Images taken were finally processed using the Microsoft Office Picture Manager.

Results

The chromatograms developed with NEU/PEG and visualized in UV light (365 nm) are presented in Figures 1 and 2. The chromatograms of *Syringae vulgaris flos* f. *violácea* and *Syringae vulgaris flos* f. *álba* ethanolic extracts revealed the presence of bands with the same fluorescence and R_f values near those for some standards. In all samples, we noted the presence of flavonoids and polyphenol carboxylic acids. In both extracts of *Syringae vulgaris flos* f. *violácea* and *Syringae vulgaris flos* f. *álba*, we identified rutoside and caffeic acid. *Syringae vulgaris flos* f. *violácea* extract contains other three flavonoids, one maybe quercitroside, and two other polyphenol

carboxylic acids. In the ethanolic extract of *Syringae vulgaris flos* f. *álba*, we observed the presence of other three flavonoids, two of which may be hyperoside and apigenin-7-neohesperidoside, and another polyphenol carboxylic acid. In comparison with the extracts of *Syringae vulgaris flos* that of *Myrtilli folium*, a natural product recognized for its antidiabetic properties, contains also chlorogenic acid and maybe apigenin-7-glucoside.

Histological sections obtained from untreated normal animals showed the normal architecture of the liver lobules with hepatocyte cords radiating from the centrilobular vein (Figure 3).

Histological examination of liver cross-sections from diabetic untreated animals (G2) stands out obvious alteration of hepatic structures compared to normal control group. One can observe the degeneration of the liver parenchyma, and dilatation of the centrilobular vein. There was extensive infiltration with lymphocytes in the portal space and hepatocellular degenerescence with loss of cellular boundaries and various degree of lipid vacuolization (Figures 4 and 5). Many hepatocytes appeared binucleated or displayed nuclear pyknosis (Figure 4). However, in animals treated with all ethanolic extracts, the severity of hepatic damage was decreased when compared with the hepatic damage observed in diabetic untreated rats. Liver sections obtained from diabetic rats treated with *Myrtilli folium* extract (G5) showed that the liver lobule relatively preserved its normal architecture with hepatocyte cords radiating from the centrilobular vein and only mild sinusoidal dilatation (Figure 6).

Diabetic rats treated with ethanolic extracts of *Syringae vulgaris flos* (G3 – SV and G4 – SA) showed increased cellularity, near normal radiating cords of hepatocytes and mild sinusoidal dilatation around the centrilobular vein or the portal space (Figures 7 and 8). Supplementation of ethanolic extracts reduced the hypertrophy of hepatocytes and lymphocyte infiltration in the central vein and portal space sustaining their hepatoprotective effect.

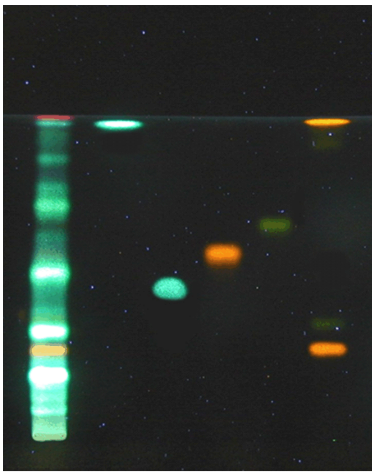

Figure 1 – ***Thin-layer chromatogram of Syringae vulgaris flos f. violácea ethanolic extract and standards, in UV light, at 365 nm. 1 – Syringae vulgaris flos f. violácea; 2 – Caffeic acid; 3 – Chlorogenic acid; 4 – Luteolin-7-glucoside; 5 – Apigenin-7-glucoside; 6 – Rutoside, hyperoside, apigenin-7-neohesperidoside, quercitroside (bottom up).***

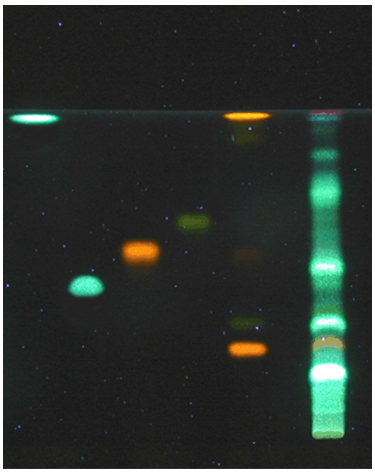

Figure 2 – ***Thin-layer chromatogram of Syringae vulgaris flos f. álba ethanolic extract and standards, in UV light, at 365 nm. 1 – Caffeic acid; 2 – Chlorogenic acid; 3 – Luteolin-7-glucoside; 4 – Apigenin-7-glucoside; 5 – Rutoside, hyperoside, apigenin-7-neohesperidoside, quercitroside; 6 – Syringae vulgaris flos f. álba (bottom up).***

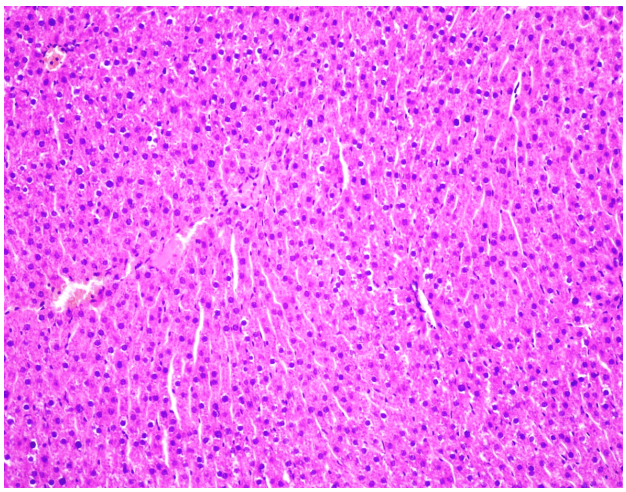

Figure 3 – ***Liver cross-section from an untreated normal control (G1) rat showing cords of hepatocytes radiating from the centrilobular vein (HE staining, ×100).***

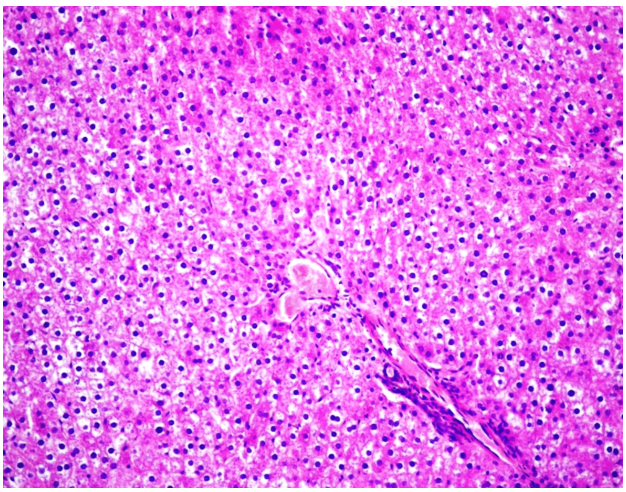

Figure 4 – ***Liver cross-section from a diabetic untreated (G2) rat showing a disorganized architecture of the liver parenchyma, the dilatation of the central vein and the inflammatory infiltration of the portal space. Noted many binucleated hepatocytes and pyknosis (HE staining, ×100).***

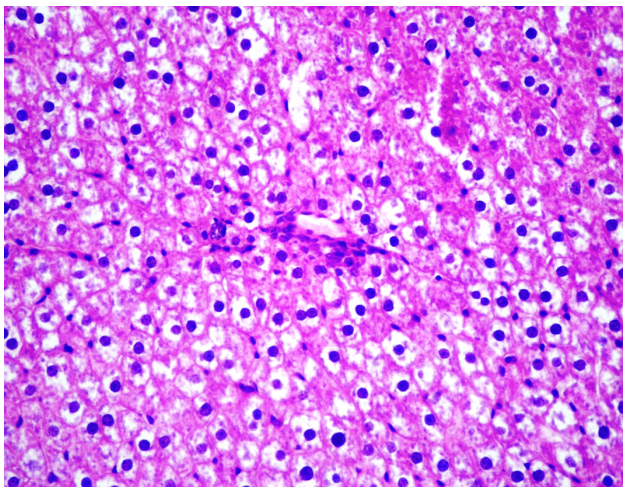

Figure 5 – ***Liver cross-section from a diabetic untreated (G2) rat showing hepatocytes degeneration and lipid vacuolization (HE staining, ×200).***

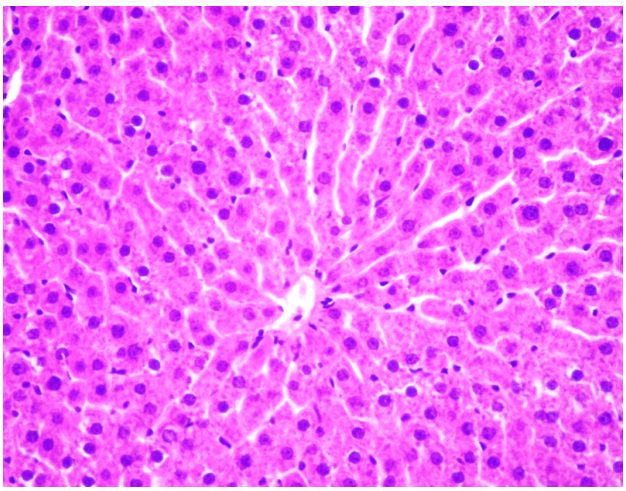

Figure 6 – ***Liver cross-section from a diabetic rat treated with MF extract (G5). Liver lobule with relatively normal aspect, mild sinusoidal dilatation (HE staining, ×200).***

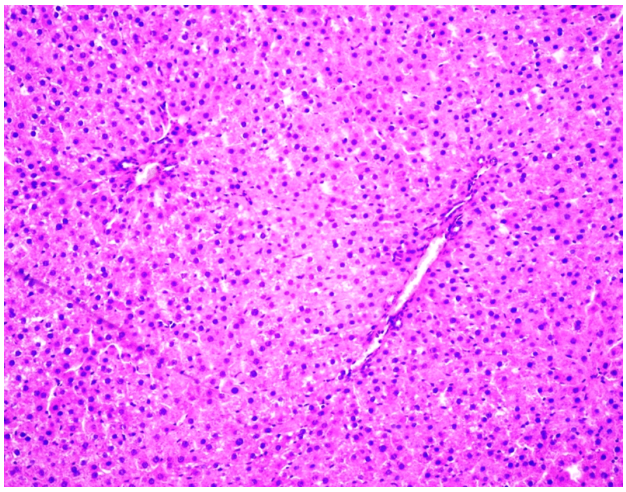

Figure 7 – ***Liver cross-section from a diabetic rat treated with SV extract (G3) showing increased cellularity and diminished inflammatory infiltration of the portal spaces (HE staining, ×100).***

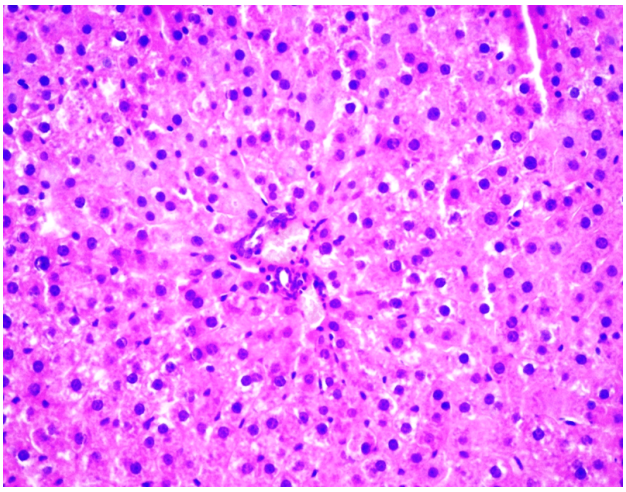

Figure 8 – ***Liver cross-section from a diabetic rat treated with SA extract (G4) showing near normal hepatocytes, mild sinusoidal dilatation around the portal space (HE staining, ×200).***

Discussion

Diabetes, the most common metabolic disorder is characterized by decreased insulin secretion or increased insulin resistance of tissues, thereby causing alteration of glucose uptake and impairment of its use to obtain the energy needed for cellular activity.

Oxidative stress is often cited as underpinning diabetes and its complications. Because of normal metabolic processes and interactions between body and environment, the body continuously produces oxygen free radicals. Oxidative stress results from an imbalance between the production of free radicals and decrease of the antioxidant defense or both. Hyperglycemia, main feature of diabetes, underlies ROS overproduction and enhances AGE formation, which in turn becomes a major source of reactive species. ROS overproduction in diabetes and their ineffective scavenging are key elements in development of tissue lesions [1, 2].

Streptozotocin is a chemotherapeutic drug used for insulinoma treatment. For its selective pancreatic β-cells cytotoxicity, it is recognized as diabetes inducer in experimental animal models [16–18]. Histological analysis performed on liver sections from STZ-diabetic rats, revealed moderate inflammation in liver parenchyma. Previous experimental studies have shown that STZ-induced diabetes is characterized by increased oxidative stress associated with lower levels of antioxidant enzymes activities (SOD, GPx, GR) and intensive lipid peroxidation [16–19], able to cause direct damage to hepatocytes.

Human body cells, including hepatocytes, are constantly exposed to the direct action of various harmful compounds and their metabolites, which induce the increase of free radicals production and lipid peroxidation with consecutive oxidative injuries of cell membranes. In liver, an organ with a central role in metabolism, storage and detoxification, oxidative stress is able to change not only its normal functionality of cells membranes and intracellular organelles but also induces abnormal signaling and cellular responses that can affect the whole body homeostasis. It should be mentioned that for the liver reduced glutathione (GSH) and glutathione related enzymes (GPx, GR and glutathione S-transferase) are very important. In diabetic liver, a lower level of GSH is in accordance

with decreased GPx and GR activities and large amounts of ROS and AGE. The liver plays also a vital role in glucose homeostasis; in case of insulin resistance, when cellular glucose disposal is impaired, lipolysis will be promoted to meet energy cells demand with enhanced release in circulation of free fatty acids. These processes lead to enhanced liver uptake of very-low-density lipoproteins (VLDL) and triglycerides synthesis [1, 2, 20–24]. Therefore, lipid accumulation observed in the liver may be due to dysregulation of uptake, output, release and oxidation of free fatty acids.

So far, were not discovered drugs that stop the progression of liver damage without any adverse effect. In this regard, herbal remedies can be considered as a source of compounds able to prevent or improve liver conditions. Phenolic compounds (flavonoids, polyphenolic acids, lignin and lignans, anthocyanins) are thought to play an important role against many diseases because they proved many biological activities. Flavonoids and phenol carboxylic acids are polyphenolic compounds markedly found in medicinal plants that play an important role in detoxification of free radicals. Glycosidic flavonoids are much more readily absorbed by humans.

Beneficial effects of *Syringae vulgaris flos* extracts may be attributed to the content of antioxidants able to scavenge ROS and protect liver tissue from oxidative damages. The current knowledge on the phytochemistry of *Syringa vulgaris* comprise the verbascoside with antioxidant and anti-inflammatory activities [12–14], but in light of recent research on the antioxidant potential [11, 16] must be analyzed the types and distribution of phenolic compounds taking into account regional climatic conditions and harvest time.

Major polyphenolic compounds identified in *Syringae vulgaris flos* extracts were rutoside and caffeic acid. Recent researches on various experimental models proved the ability for hepatoprotection both for rutoside and caffeic acid [25, 26]. For example, a previous study on animal model of carbon tetrachloride toxicity reported the hepatoprotective effect of rutoside [25]. Other researchers sustain the beneficial effect of caffeic acid against liver toxicity induced by *tert*-butyl hydroperoxide [26]. Hepatoprotective mechanisms are not yet entirely understood but protection against cellular injuries induced by ROS overproduction, inflammatory processes or direct cytotoxicity might be due to complex actions given by the multitude of bioactive compounds.

Conclusions

Since the association between diabetes and chronic nonalcoholic liver disease has been certified, but a temporal relationship is not clearly established, it could be therapeutically useful an augmentation of protective cellular pathways against ROS overproduction-induced injuries. Treatment with *Syringae vulgaris flos* extracts will provide a better defense against oxidative stress and could be a source of natural antioxidants able to prevent free radicals diabetes-induced tissue damages.

Conflict of interests

The authors declare that they have no conflict of interests.

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