Original Article

The Effect Of Dietary Nano-Selenium And Cannabis Seeds On Liver Tissues And Functions In Male Karadi Lambs

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Abstract

This study was done to look into the impact of nano-selenium, cannabis seeds in powdered form and a combination of nano-selenium with cannabis seeds on the liver tissue and liver functions and enzymes in Karadi male lambs. The lambs were divided into four groups; each group included five lambs. The first group was considered as a control group. The second group was orally administrated 0.5 mg/kg of fed of nano-selenium, and the third group was orally administrated 250 mg/kg fed of powdered cannabis seeds. The fourth group used both elements (0.5 mg/kg body weight and 250 mg/kg fed) of nano-selenium and powdered cannabis seeds, respectively. The treatment groups were administrated daily orally for 60 days. To determine the impact of nano-selenium and cannabis seeds on liver tissue and function, tests for alkaline phosphatase, glutamic oxaloacetic transaminase, and glutamic pyruvic transaminase (ALP, GOT, and GPT), serum lipid profile includes (cholesterol, triglyceride, LDL, HDL and VLDL), serum total protein, albumin, and globulin, as well as liver histopathology, were conducted. The level of serum total protein and albumin were not significantly changed; however, the level of globulin was altered significantly ($p<0.05$) in the nano-selenium group compared to the control group. Cannabis seeds and nano-selenium with cannabis seeds had significant alteration effects on serum total protein and globulin levels ($p<0.01$) and ($p<0.05$), respectively. Cannabis seeds showed a significantly increasing effect on the albumin level ($p<0.05$). The cholesterol level decreased significantly in the nano-selenium group compared to the control group ($p<0.05$). The level of cholesterol, Triglyceride, LDL and VLDL was significantly decreased in the cannabis seeds group ($p<0.01$), but was no statistically changed in HDL level. Serum lipid profile was significantly decreased ($p<0.01$) in nano-selenium with cannabis seeds groups compared to the control group. ALP was decreased significantly in all treated groups compared to the control group ($p<0.01$). Liver tissue was shown a protective effect of cannabis seeds as there was no evidence of necrosis, apoptosis and hepatic architectural change; however, the effect of nano-selenium showed no sign of fibrosis, necrosis, neoplasia, or dysplasia, but apoptosis was shown. In contrast, AST was decreased significantly in the nano-selenium group ($p<0.05$) and in the cannabis seeds treated group ($p<0.01$), but the level of ALT was not changed significantly in any treated groups. This finding can conclude that the size and dosage of nano-selenium used in the present study are safe for liver tissue as well as have a good impact on increasing the immune system as globulin was increased. Whole cannabis seeds in powdered form have a remarkable impact on liver tissue protection, decreasing lipid profile, increasing protein metabolism by increasing total protein and albumin and boosting the immune system via alteration of globulin.

Keywords: Nano-selenium, Cannabis seeds, liver enzymes, Liver functions and liver histology.

INTRODUCTION:

A recent study evaluated the effect of using nano-selenium and cannabis seeds orally administrated on liver function and hepatocytes in male lambs because, in biology, the element selenium (Se) and cannabis are crucial [1, 2]. Cannabis seeds also have called hempseeds. Due to its high nutritional characteristics and less psychoactive components, hempseed has traditionally been recognized as one of the most nutritionally complete meals [3]. It can be distributed as a whole, a shelled seed, or an unshelled seed in addition to its processed goods like canvas, flour, and protein grease paint (hempseed kernel) [4]. Despite the fact that numerous studies have noted excessive variability in hempseed composition, it typically contains 20–25 lipids with completely distinct and fatty acid (FA) compositions, 20–25 proteins that are smooth to condensation and fat in essential amino acids, and 20–30 carbohydrates, of which a sizeable portion may be made up of nutritive fiber, especially unavoidable, as well as vitamins and minerals [5]. The majority of the phenolic compounds isolated from hempseed were found to have high levels of radical scavenging activity [6]. In addition, the lignan amides 3, 3′-dimethyl-grossamide and 3, 3′-demethylheliotropamide were also able to inhibit the demonstrating properties similar to those of the medications used to treat moderate to mild Alzheimer's [7]. The biological effect of hemp seeds and hemp seeds derivatives (especially hemp seeds oil) is the main subject of different studies. Inordinate amounts of saturated-fatty acids (SFAs) and polyunsaturated fatty acids (PUFAs) are two characteristics of hempseed oil [8]. According to genotype and environmental factors, hempseed oil can contain up to 90 unsaturated fatty acids, of which 70 to over 80 are polyunsaturated fatty acids (PUFAs). Monounsaturated fatty acid from abecedaria (MUFA) [8, 9]. All of the essential amino acids (EAAs) needed by humans are present in hempseed proteins, and glutamic acid (3.74–4.58 of the overall seed) and arginine are the two most prevalent amino acids (2.28–3.10) [10]. In actuality, arginine plays a significant function in maintaining cardiovascular health since it is a dietary precursor for the production of nitric oxide (NO), a crucial mediator of vascular tone. Particularly, arginine and NO have been connected to muscle shape and the most suited susceptible properties [11]. Some of these compounds' anti-inflammatory and antioxidant actions on microglia cells, which may be immune cells of the essential fearful system and can be concerned with regulating the immune responses within the brain, have been shown to be particularly important in the neuroprotective effects of various hempseed phytochemicals. Microglia cells play a crucial role in brain infection and inflammation [12, 13]. Selenium is naturally found in two different forms: inorganic (selenite and selenate) and organic (Selen methionine and selenocysteine) [14]. In nature, selenium can be found in both crystalline and amorphous polymorphism forms [15]. It is possible to create nano-selenium chemically, physically, or biologically by using plant or microbe extracts (so-called green synthesis) [16, 17]. The rapid development of nanotechnology holds great promise for application in medicinal and nutritional science because nano-materials have been found to exhibit novel properties different to those at micro-scale and bulk materials. For both humans and animals, selenium (Se) is an essential vitamin, but too much of it can be detrimental [18]. Smaller selenium nanoparticles (N.P.s) have higher biological activity, which is influenced by their size. Particle size has an impact on how well N.P.s are absorbed by cells. For instance, the in vitro absorption of 0.1 mm particles was 2.5 and 6 times higher than that of 1 and 10 mm particles [19, 20]. Besides the advantages of selenium in bulk and Nano-form in fisheries and cattle, high dietary selenium levels (> 20–30 ppm) are harmful to most animals, including livestock and fish. Chronic selenium poisoning was discovered in the fish population, with symptoms such as gill telangiectasia (swelling), low hematocrit and hemoglobin levels, corneal cataracts, pathological changes in major organs, reproductive failure, and teratogenic abnormalities [21]. Different sizes and doses of nano-selenium have shown variations in their impacts on cellular protein contents and enzyme activities of intracellular sodium-potassium adenosine triphosphatase, lactate dehydrogenase, glutathione peroxidase, and superoxide dismutase in primary cultured intestinal epithelial cells [22]. In this study, the impact of nano-selenium and cannabis seeds on hepatocytes and liver functions in specific dosages was demonstrated in male lambs to the possible use of the contribution of nano-selenium and cannabis seeds as dietary supplementation in livestock. The aim of this study is to the possible use of whole cannabis seeds and nano-selenium together as dietary supplementation in livestock and evaluation the effect of used specific dosage on liver tissue and functions in a specific period of time.

MATERIAL AND METHODS:

The study used 20 Karadi male lambs three months old with an average LBW of 20.75 ± 0.63 kg. The lambs were put into four groups of five (5) animals randomly. The first group served as a control group and was not given cannabis or nano-selenium supplements. At the same time, other groups received daily for 60 days orally the doses of Nano-selenium 0.5 mg/kg fed and powdered cannabis seed 250 mg/kg fed. The mixed Nano-selenium with cannabis seed was 0.5 mg + 250 mg/kg fed, respectively. These groups the first (Treatment 1, T1), second (Treatment 2, T2), and third (Treatment 3, T3) groups were compared to the control group. Each lamb was kept in its corral throughout the experiment (1.5 x 2 m). The focus diet consisted of wheat, rye, yellow maize, soybean meal, salt, and minerals, whereas the baseline diet consisted of wheat straw. To encourage maintenance and daily benefit, food was provided at 9:00 a.m. in portions estimated to be 3% of live body weight (LBW). Every week, the animals' weights were taken.

Lambs were fed for ten days without receiving any additive or supplements before the experiments started to let them adjust to their new surroundings. Each lamb receiving BULITEL injection (Ivemectin and Closantel Sodium 1%+10%, Chongqing BULL Animal Pharmaceutical, Rongchang, China) received 1ml subcutaneously for both internal and exterior parasites. After 30 days, the second dose of 1ml was administered subcutaneously. For Entrotoxia-blackleg, the purified vaccination SYMTEROVAC (Biopharma, Morocco) was employed. In this investigation, whole cannabis seeds were used. The cannabis seeds were donated by the PK Cannabel company in Moscow, Russia. A sophisticated grinder was used to ground the cannabis seeds into a powder. The Wuhan Dongxin Mill Imp and Exp Trade Co, Ltd. produces Nano-selenium particles sourced from China. It is a 99.99% pure black powder that is kept in a closed, dry environment. In order to check a sample of Nano-selenium particles for close to 2-3 gm from the material, an X-Ray Diffraction was utilized in the central lab at Bagdad University/College of Education for pure science/Ibn Alhaitham. The prevailing trend's average nano-selenium crystal size was computed as (101) The average crystal size, as determined by the Debye-Scherer equation, is about (15nm):

Dav= $K\ \lambda / \beta\ COS$ Ө _____________ (1)
Dav: the average crystal size
K: constant = 0.9 spherical volume
λ: The wavelength of the X-rays falling on the target (1.54.5 Â)
β: Curved width at the centre of the peak (FWHM).
Ө: Brac diffraction angle

Liver function parameters such as liver enzymes level, total protein, albumin, globulin, Triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) and cholesterol levels were estimated in sera using Cobas e 311 (Roche, Germany). The reagents used to estimate liver functions were obtained by

Roche/ Hitachi company. At the end of the study, tissues from the liver were collected for histological examination. Livers were collected and cut in 1 cm^2. The samples were put in 10% formalin. The tissues were processed using LEICA TP 1020 automatic Tissue Processor (Heidelberger, Nussloch, Germany 2018). Rotary microtome (Accu-Cut SRMTM 200, Sakura Finetek Europe B.V.) was used for sectioning. 5 µm thick sections were cut from a paraffin block and mounted on a microscope slide. Tissues were stained using hematoxylin and eosin (H&E). The mean and standard deviation of the groups were presented as the statistically analyzed data, and the means were compared using IBM SPSS 26 software. The differences were statistically significant at P<0.01 and P<0.05.

RESULTS:

Using male lambs as test subjects, the results demonstrated the effect of nano-selenium and cannabis seeds on various biochemical or medical markers. Table 1 showed the effect of nano-selenium shows no significant effect on serum total protein and albumin; however, its effect appeared only on globulin level, which was significantly increased (P<0.05). Cannabis seeds showed significant alteration in the level of total protein (P<0.01), albumin and globulin (P<0.05). Serum total protein and globulin in nano-selenium with cannabis seeds group were significant altered (P<0.01) (P<0.05), respectively, while serum albumin was not significantly changed. In Table 2, the level of serum cholesterol showed a significant (P<0.05) decrease in the nano-selenium-treated group when compared with the control group. In contrast, Triglyceride, HDL, LDL and VLDL showed no significantly changed (P<0.05). Serum HDL level in the cannabis seeds group was not significant compared to the control group (P<0.05). Cannabis seeds have decreased effect on the level of serum cholesterol, Triglyceride, LDL and VLDL (P<0.01). The lipid profile was decreased using nano-selenium with cannabis seeds (P<0.01). Table 3 shows the effect of cannabis seeds and nano-selenium on liver enzymes, the level of serum ALP in treated groups was decreased significantly (P<0.01), AST level was decreased significantly in the nano-selenium group (P<0.05), and also in cannabis seeds group (P<0.01), but there was no any significant change shown in nano-selenium with cannabis seeds group. Serum ALT levels were not significant changed in any treated groups compared to the control group.

Table 1. liver function tests (total protein, albumin and globulin) for male lambs treated with Nano-selenium, Cannabis and Nano-selenium with Cannabis compared with the control group. The table shows mean ± standard deviation in (g/dl).

Groups	Total protein	Albumin	Globulin
Control	6.13±0.18	4.13±0.39	2.00±0.57
Nano-selenium	7.00±0.47$^{a, b}$	3.26±0.65$^{a, b}$	3.73±0.22*
Cannabis seeds	8.72±0.15**	5.28±0.36*	3.44±0.47*
Nano-Selenium and Cannabis seeds	8.22±0.44**	4.84±0.16^{NS}	3.37±0.28*

NS: No significant difference between control and treatment groups. * P<0.05, ** P<0.01. Indicates significant differences with the control group. a, b, c indicates significant differences between groups in the same column.

Table 2. Compared with the control group, the lipid profile for male lambs treated with Nano-selenium, Cannabis and Nano-selenium with Cannabis. The table shows mean ± standard deviation in (mg/dl).

Groups	Cholesterol	Triglyceride	HDL	LDL	VLDL
Control	93.46±4.10	80.46±4.01	19.53±0.25	57.84±4.56	16.09±0.80
Nano-Selenium	67.13±8.14*	80.50±3.12^{NS}	17.86±0.15^{NS}	58.56±8.13$^{a, b}$	17.70±0.62$^{a, b}$
Cannabis seeds	60.76±4.99**	55.00±2.26$^{**a, b}$	19.03±0.30^{a}	30.73±5.42**	11.00±0.45**
Nano- Selenium and Cannabis seeds	60.86±7.0**	50.96±3.27**	18.70±0.17**	31.97±6.65**	10.19±0.65**

NS: No significant difference between control and treatment groups. * P<0.05, ** P<0.01. Indicates significant differences with the control group. a, b, c indicates significant differences between groups in the same column.

Table 3. liver enzyme tests (ALP, ALT and AST) for male lambs treated with Nano-selenium, Cannabis and Nano-selenium with Cannabis compared with the control group. The table shows mean ± standard deviation in (U/L).

Groups	ALP	ALT	AST
Control	613±21.65	22.6±0.87	200.6±6.81
Nano-selenium	116.6±15.5$^{**a, b}$	16.46±4.9^{NS}	168.7±5.35*
Cannabis seeds	47.33±11.3**	16.9±6.32^{NS}	162.16±9.2**
Nano-Selenium and Cannabis seeds	43±7**	17.26±6.21^{NS}	186±12.34^{c}

NS: No significant difference between control and treatment groups. * P<0.05, ** P<0.01: Indicates significant differences with the control group. a, b, c indicates significant difference between groups in the same column.

The histological study was obtained to investigate the effect of cannabis seeds and nano-selenium on the liver tissue. Figure 1 shows the liver tissue of the control group, at 400x, revealed no signs of fibrosis, necrosis, neoplasia, or dysplasia, as well as pan-hepatic cytoplasmic swelling (white arrow), preservation of hepatic architecture, preservation of the portal tract (red arrow), and lymphocytic infiltration (black arrow). In the nano-selenium group, at 400x, there was no sign of fibrosis, necrosis, neoplasia, or dysplasia. Instead, there was pan-hepatic cytoplasmic swelling (white arrow), a moderate deformation of the hepatic architecture, and a region of lymphoid aggregates (black arrow) with apoptosis (Councilman body-green arrow), as shown in figure 2. Cannabis seeds, at 400x, revealed no signs of fibrosis, necrosis, neoplasia, dysplasia, pan-hepatic cytoplasmic swelling (white arrow), preservation of hepatic architecture, and lymphocytic infiltration (black arrow), as shown in figure 3. The effect of nano-selenium and cannabis seeds, at 400x, there was no sign of fibrosis, necrosis, neoplasia, or dysplasia. Instead, there was pan-hepatic cytoplasmic swelling (white arrow), a moderate deformation of the hepatic architecture, and a region of lymphoid aggregates (black arrow) with apoptosis (Councilman body-green arrow), as shown in figure 4.

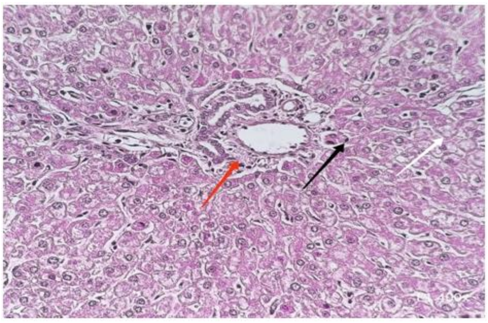

Figure 1. Liver tissue for control male lambs

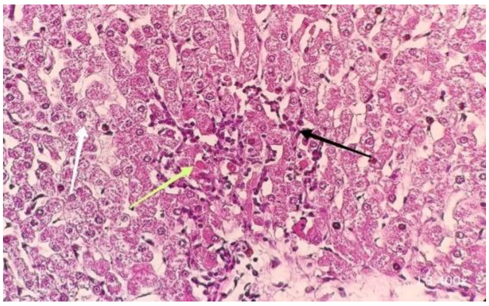

Figure 2. Liver tissue for group treated with 0.5mg/ kg fed of nano-selenium

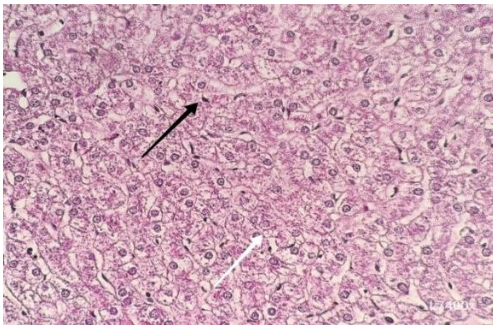

Figure 3. Liver tissue from male lambs orally

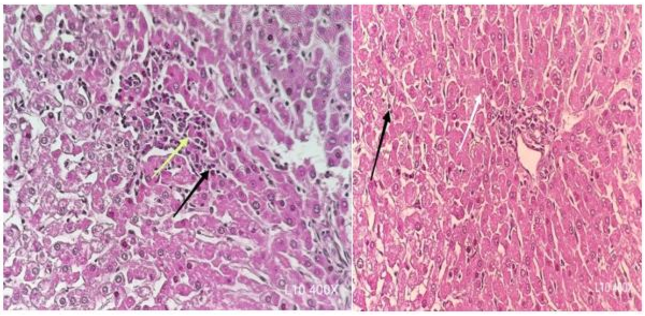

Figure 4. Liver tissue from male lambs orally administrated 0.5 mg/kg administrated 250 mg/kg of fed
fed of nano-selenium + 250 mg/kg body weight of cannabis
of cannabis seeds

DISCUSSION:

The metabolism, detoxification, storage, and excretion of xenobiotics or metabolites all depend on the sensitive liver [23]. The results of this investigation agreed with those of several other studies regarding the lipid profile of nano-selenium (cholesterol, high-density lipoprotein, low-density lipoprotein, and very low-density lipoprotein). According to reports, the amount of nano-Selenium supplementation had no appreciable impact on the blood biochemistry of sheep, including the amounts of cholesterol, HDL, and triglycerides [24]. This investigation looked at how cannabis seeds affected the body's lipid metabolism, which agreed with other studies. The effect of cannabis seeds was also seen in broiler chicken, where meals containing the seeds decreased levels of total blood cholesterol, low and very low-density lipoprotein, and T.G. In contrast, meals containing the seeds elevated levels of serum high-density lipoprotein (HDL) [25]. Lambs' lipid profiles are improved when cannabis seeds and nano-selenium are combined. However, the impact of cannabis seeds combined with nano-selenium on lipid profiles has not been studied. The impact of nano-selenium on the liver enzymes of livestock, fish, and rats has been adequately studied. According to studies on fish, nano-selenium has no impact on liver enzymes (ALP, ALT and AST) [26-28]. In additional mice experiments, the effects of nano-selenium on liver function markers were compared to those of inorganic and organic selenium. Abnormal liver function was more noticeable with selenite administration than with Nano-Selenium, as seen by an increase in the serum level of the hepatotoxic toxic marker. After being treated with various forms of selenium, in the case of Nano-Se, enzyme activity increased dramatically [29]. Dairy cows' serum alkaline phosphatase and aspartate aminotransferase activity were significantly decreased by selenium administration, especially nano selenium [30]. The findings did not line up with those of Antunovi, Zvonko et al. 2021, whose investigation employed hemp seeds cake in lamb diets. However, they discovered no appreciable impact on the activity of enzymes like AST, ALT, and ALP [31]. However, another study agreed with this as the AST and ALT levels decreased [32]. There isn't a single study on the impact of cannabis and nano-selenium on liver enzymes. Our research shows that nano-selenium in cannabis seeds has no detrimental effects on liver tissue, as the levels were within normal ranges and did not statically decrease. Albumin, globulin, and total protein levels were higher, which suggested that the powdered cannabis seeds encouraged protein synthesis and/or mobilization. The observed rise in globulin levels may be caused by the ability of cannabis seeds to produce antibodies. Albumin is the plasma protein with the highest level of concentration. It moves several substances all around the body [33]. It prevents blood from entering the tissue. This study agreed with most studies on mice, poultry, and livestock [33, 34]. By raising serum globulin levels, cannabis seeds can strengthen the immune system and immunoglobulins, have a favorable impact on osmotic pressure and hormone activity by raising serum albumin levels, and positively impact liver functions by raising total serum protein levels [25]. Significantly higher serum globulin levels suggest that nano-selenium positively impacts immune system support, immunoglobulin synthesis, and complement system function. Our findings were validated by several previous studies that showed total serum protein and globulin are increasing utilizing dietary or nano-selenium supplements, however not significantly [35]. Although there isn't a single study on the impact of cannabis seeds combined with nano-selenium, this combination's results positively affected liver function and support the immune system. The histological analysis of the controls' livers revealed typical hepatocyte architecture and structure. The majority of acute injury types result in cell swelling, which is an early shift that could signal the start of more profound alterations. In a damaged tissue, light microscopy frequently reveals larger cells as well as compression or displacement of neighboring structures. Frequently, the staining affinity is decreased, which usually causes the cells to seem light or fuzzy. Clear regions or vacuoles may form. These often indicate a dilated endoplasmic reticulum or a Golgi [36]. There has not been a single study on the impact of nano-selenium on lamb liver tissue. However, this study's findings indicate that the dosage and size of the nano-selenium utilized had no impact on male lambs' liver tissue damage. Using nano-selenium in broiler feeds, or drinking water caused more adverse alterations in the liver tissues, such as necrosis and inflammation, according to histopathological research on the substance's impact on liver tissue in chickens [37, 38]. In a similar vein, Benko et al. (2012) looked at the toxicity of Se sources in chicken. Researchers stated the effects caused by the various particle sizes used in nano-Se production. This implies that their use of larger nanoparticles may have greater negative effects than other groups' smaller ones [39]. Studies on the sizes of nano-Se particles have shown that the effects of nano-Se on mice depend on their size. Nano-Se may be a more effective chemo-preventive drug when it is in smaller nanoparticle sizes [40]. The impact of cannabis seeds on the liver tissue of lambs has not been investigated. However, a study was done on mice to determine the effects of cannabis extract. The histological results showed that cannabis extract, in particular CBD, did not seem to have an effect on the development of mouse liver necrosis [41]. The present study's findings were consistent with those of prior investigations, which showed that cannabis does not cause liver inflammation or hepatic cell necrosis. It is generally accepted that cytosolic enzymes are produced during both the reversible and irreversible phases of hepatocyte injury, indicating that their presence in the blood does not invariably denote cell death. It is also thought that reversible cell damage causes enzyme release even when there are no visible necrosis signals in the tissue.

CONCLUSION:

Selenium is a crucial necessary element that affects numerous physiological functions of the body by interfering with selenoproteins. Effectively preventing health issues from a selenium deficit can be accomplished by including an adequate supply of selenium in the diet. Especially in ruminants, where conventionally utilized selenium compounds demonstrate relatively low absorption in the digestive tract, selenium in its nanoform looks to be the most suitable for supplements due to its high bioavailability, low toxicity, and affordability. Orally administrated nano-selenium in male lambs with 0.5 mg/ kg of body weight for 60 days is safe for live tissue and function and may elevate the immune system by enhancing serum globulin. Using cannabis seeds in powdered form at a dose of 250 mg/kg of body weight for 60 days has a protective effect on liver tissue, supports liver function and protein metabolism, and can be used to boost the immune system and decrease the risk of cardiovascular problems. Nano-selenium and cannabis seeds influence protein metabolism, have a protective effect on hepatocytes and decrease cholesterol, LDL, VLDL and triglyceride, which means decreased cardiovascular problems.

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