BIOCHEMICAL AND HISTOLOGICAL STUDIES ON ADVERSE EFFECTS OF DIETARY WEIGHT LOSS SUPPLEMENT ON FEMALE RATS: ADIOS

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ABSTRACT:
Herbal and dietary supplements are commonly used throughout the world; these unlicensed supplements are not recommended due to concerns about their clinical efficacy and safety. Adios are marketed for weight loss in Iraq and many other countries and are commonly used by women. The safety of these weight loss aids is unknown. This study aimed to evaluate adios adverse effects by conducting biochemical, hematological, and histological examinations on the liver and kidney. Rats were orally administrated for 30 days with different concentrations of adios supplements (0.001g/100g), (0.002g/100g), and (0.003g/100g) as low (LD) medium (MD) and high (HD) concentrations, respectively. The results showed no weight loss in rats over 30 days across experiments in all the treated groups (LD, MD, and HD) compared to the control. Adios showed significant differences between the control and the treated concentrations in the following hematological parameters: white blood cells (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), lymphocyte (LYM), and red cell distribution width (RDW) in a dose-dependent pattern. Furthermore, there were various alteration in the histopathological examination of the kidney and liver. From the current data, it can be concluded that adios has zero efficacy in weight loss and is not recommended for use because it has adverse effects on many hematological and biochemical parameters and with obvious pathological changes in both the kidney and liver.

KEYWORDS: Adios, Supplements, Weight loss, Adverse effect, Kidney, Liver

1. INTRODUCTION
Herbal and dietary supplements are widely used. Weight loss supplements are popular and can be purchased easily from online shopping websites, pharmacies and local markets. Unfortunately, 80% of overweight individuals seeking weight loss depend on nonprescription herb products without the help of healthcare professionals (Blanck, et al.,2001). These extensively marketed weight loss products promise a significant weight loss achievement, and consumers believe that herbal drugs and supplements are very safe and free from adverse effects, but researchers have found inconclusive evidence for the safety and effectiveness of many of the ingredients in such products (Johnston et al.,2016; Haaz et al., 2006 Saper et al.,2004; Allison et al.,2001). In addition many studies reported the potential toxicity of different herbal and plant extracts due to their harmful ingredients (Connolly et al., 1997; Eliason et al.,1994). The harmful ingredients can affect the vital organ tissue and induce toxicity to many organs such as the liver (Nigatu et al., 2017), kidney (Yang et al., 2018; Lai et al., 2009), heart (Eliason et al.,2012), spleen (Nigatu et al., 2017), and reproductive organs (Alraei, 2010). In addition, some weight loss supplements can lead to severe toxicity and even death (Reytmann et al., 2014; Eliason et al.,2012).

A variety of over-the-counter weight loss supplements are widely available which include caffeine, chromium, ephedra, green tea, fucoxanthin guar gum, chitosan, conjugated linoleic acid, ginseng, glucosamine, hydroxy citric acid, L-carnitine, psyllium, and pyruvate (Saper et al.,2004; Lenz & Hamilton,2004).

Adios Grase, the herbal diet supplements are marketed as weight loss in Iraq, the UK, and some other countries (Relton et al.; 2014, Bello et al.; 2012 Rajashekar., 2009) for many years and are formally licensed as medicines with an authorized NHS numbers (LLRAPC, 2023; Bowdler, 2010; Rajashekar, 2009). Adios capsules contain the following ingredients: Fucus Dry Extract, Boldo Dry Extract, and Dandelion Root (Bowdler, 2010). Clinically, Fucus along with other chemicals is recommended as a nutritional treatment to lose weight among the overweight people (Vodouhè et al., 2022). In addition, Bolo and dandelion are widely used in folk medicine and are recognized as a medicinal herb in pharmacopoeia (Schütz, et al., 2006; Muñoz & Wilkomirska 2001).

Due to the lack of studies regarding the efficacy and side effects of these supplements, we conducted a study to explore the anti-obesity effects of adios supplements and also their effect on some of the biochemical, hematological and histological parameters of different organs: liver and kidney.

2. Materials and Methods

2.1 Animals of the study
Twenty-four healthy female albino rats (Rattus norvegicus) were used for the study. Animals were acquired from the animal house of the Biology Department, College of Science, University of Duhok. The animals were randomly divided into 4 groups: Control (T), low dose (LD), moderate dose (MD), and high dose (HD) groups. The animals were placed in ventilated cages made of polycarbonate; five rats were kept in each cage under normal standard ambient conditions of temperature between 24-31 °C, humidity between 50%-55%, and a photoperiod of 12 h natural light and 12 h dark was maintained. The rats had access to a standard diet and water under ethical and standard laboratory conditions for 30 days (Maliklak & Wanwimolruk, 2001).

2.2 Weighting the rats
The weighing of rats was done using a weighing balance. Their weights ranged between 180-200 g. Weight recording was done every 5 days throughout the study.

2.3 Dosage, route, and duration of administration of Adios supplements.
Adios coated tablet (Aeropak-manufactured) was dissolved in distilled water and administrated to rats daily at three different concentrations (0.001g/100g), (0.002g/100g) and (0.003g/100g) as low (LD), medium (MD), and high (HD) concentrations, respectively. The control group was given only distilled water; each rat received 1ml of the dissolved concentration orally by gavage. Rats were weighed to adjust the dose administration for weight changes. The dose was administered according to the dosages recommended by the manufacturer to the consumer (1 tablet 3 or 4 times a day). This experimental process lasted 30 days (Shin et al., 2021 & Maeda et al., 2005).

2.4 Hematological, biochemical, and hormonal analyses.

On day 31, animals were anesthetized with diethyl ether. For hematological parameters, blood samples were obtained directly by heart puncture with 2 ml of blood collected in heparinized tubes and analyzed an automated hematological analyzer. The hematological parameters including the total count of red blood cells (RBC), white blood cell count (WBC), hemoglobin concentration (HGB), hematocrit (HCT), red cell distribution width (RDW), platelet count (PLT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) were calculated. For biochemical and hormonal estimation, 5ml of blood was placed in a sterile gel tube and allowed to clot for 30 minutes before centrifugation at 4000 rpm for 15 minutes. Liver biochemical parameters included total protein, alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST). Kidney biochemical parameters included Albumin (ALB), Urea (URE), and Creatinine (Cr). All tests were performed by Cobas 600(C501), an automated chemistry analyzer, and measured according to their kit procedures depending on the absorbance photometry (enzymes, substrates, and specific proteins).

2.5 Histopathology Processing

After dissecting the animals, the liver and kidney were fixed in a 10% neutral buffered formalin solution. The samples were processed using routine histological processing method according to (Suvarna et al., 2018), thin sections (5 µM) were prepared for light microscopy studies and stained with hematoxylin (H) and eosin (E).

2.6 Statistical analysis

All statistical tests were performed with the statistical program SPSS 16.0. The results were expressed as mean ± standard errors. The one-way analysis of variance (ANOVA) was calculated using the software GenStat 18th (Lawes Agricultural Trust, VSN International Ltd, Oxford, UK). Tukey’s multiple range test, all groups were compared with the control group (pointed as *) (*P< 0.05), **= (P< 0.01), ***= (P< 0.001).

3. RESULTS

3.1 - The Effect of Adios Supplements on the Body Weight of Rats.

The body weight of each animal was recorded before the start of the adios administration; during the adios administration, animal weights were recorded every 5 days. Oral administration of adios with different concentrations and over 30 days showed no weight changes compared to the control group. Unexpectedly, a little weight gain was observed in the rats’ weights over time (within 1-15 days). After 15 days, the weight of rats was stable in all the treated groups with no changes. Furthermore, all the treated groups (LD, MD, and HD) showed similar effects on rat body weight loss as shown in Figure (1).

![Figure 1: The effect of Adios supplements on rat weight loss; each value point represents Mean±SEM. body weight; n = 6](image)

3.2 - The Effect of Adios Supplements on Hematological Parameters.

The effect of 30 days’ oral treatment with adios supplements in different concentrations (LD, MD and HD) on the hematological parameters of the rat is shown in Table 1. As indicated, oral treatment with MD and HD of the adios supplement showed a significant (P <0.05) decrease in WBC counts as compared to the control group. In contrast, a significant (P <0.05) increase in MCV(FI) was observed with all the adios treated groups (LD, MD and HD) as compared to the control. Moreover, a significant (P <0.05) decrease in the LYM count was found in both the MD and HD groups. In contrast, the LD and HD treatment induced marginal significant increase in RDW-SD as compared to the control. Contrary to the above effects, there was no difference between the values of RBC, HGB, MPV and PCT% count of all the tested groups and the control group.

| Table 1: The effect of the oral administration of adios supplements with different concentrations on some hematological parameters in rats. |
3.3-The effect of Adios supplements on Liver and Kidney parameters.

The effects of 30 days’ oral treatment with adios supplements on the serum biochemical parameters of rats are shown in Table 2. All the measured parameters were not significantly different. Similarly, there was no significant difference in kidney serum biochemical parameters at LD, MD, and HD. Serum ALB, ALP, ALT, and AST total protein levels appeared to decrease after treatment with adios in a dose-dependent manner, though non-significantly. Similarly, all the kidney serum parameters measured (Cr, URE, and UA) were not significantly different.

Table 2: Effect of oral administration of different concentrations of adios supplements on some Liver and Kidney parameters in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control (T)</th>
<th>Low Dose (LD)</th>
<th>Medium Dose (MD)</th>
<th>High Dose (HD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALB (g/dl)</td>
<td>4.16±0.62</td>
<td>4.14±0.09</td>
<td>4.15±0.12</td>
<td>4.09±0.9</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>198.20±2.43</td>
<td>169.60±3.8</td>
<td>169.00±3.2</td>
<td>188.40±2.71</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>55.80±1.34</td>
<td>56.40±1.27</td>
<td>42.00±2.78</td>
<td>46.80±0.93</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>180.60±7.33</td>
<td>168.80±9.28</td>
<td>163.80±4.3</td>
<td>159.00±9.09</td>
</tr>
<tr>
<td>Cr (mg/dl)</td>
<td>0.37±0.16</td>
<td>0.39±0.02</td>
<td>0.37±0.13</td>
<td>0.42±0.18</td>
</tr>
<tr>
<td>URE (mg/dl)</td>
<td>40.00±2.22</td>
<td>41.80±1.39</td>
<td>43.00±0.03</td>
<td>38.00±0.08</td>
</tr>
</tbody>
</table>

Values are mean ± SE, *= (P< 0.05), **= (P< 0.01), ***= (P< 0.001) for comparison all groups with the control group.

3.4- Effect of adios treated groups on Kidney and liver tissues.

Kidney

The rat kidneys in the control group showed a normal histological structure of the kidney. The renal cortex which is composed of renal corpuscles and renal tubules (RT), can be seen in Figure 2 A. Bowman’s capsule shows a normal histological structure with glomerular space, parietal layer, visceral layer, podocytes, and glomerulus capillaries (Figure 2 B).

The kidney sections revealed abnormal histological features in the LD group, with an increase in the periglomerular space, necrosis of glomeruli, disruption of the brush border of normal tubule cells, and an increase in the lumen of these tubules. There was also hyperplasia of the glomerulus, narrowing of the lumen of some renal tubules (Figure 3A & B). This group also showed destruction of some renal tubules and congested blood vessels with markedly thick wall and the presence of inflammation cells around the blood vessel (Figure 3 C). Destruction of the epithelial cells lining and condensation of the epithelial cells nuclei that line the renal tubules was observed (Figure 3 D&E). In the MD group, kidney damage was detected as degeneration of renal tubules, glomerular hyperplasia, obliterating of periglomeruli space, and hemorrhage among glomeruli (Figure 4A). The destruction of the epithelial lining of renal tubules and increased inflammatory cell infiltration within the renal stroma were also detected within this group (Figure 4B). Renal tubules in this group showed narrowing of the lumen due to the obvious swelling of their epithelial lining; moreover, some degeneration of some tubules was observed (Figure 4C & D).

Within the HD group, there was obvious dilation, destruction, degeneration, hydropic of renal tubules, and detachment of epithelial renal tubules from the basement membrane. Dilation of Bowman’s space, determination of the glomeruli from Bowman capsule, and degeneration of glomerular were detected. Congested blood vessel with markedly thick walls and the presence of inflammatory cells around the blood vessels with obvious fatty degeneration interstitial spaces between renal tubules was seen (Figure 5 A-F).
Figure 2: A. Transvers sections through the kidney of an adult rat in the Control group showing normal histological structure of the kidney. Renal cortex which composed renal glomerular (RG) and renal tubules (RT). (B) shows proximal tubules (PT), glomerular space (Blur arrow), parietal layer (White arrow), Visceral layer (Red arrow), podocytes (arrow), glomerulus capillaries (arrow star), (A 10x), (B 40x), H&E stain.
Figures 3: and 3. (high magnification of part of figure (A) Transverse section through the kidney of adult rat in the LD group showing increase in the periglomerular space (IGS) and necrosis of glomeruli (NG), disruption of the brush border of normal tubule cells associated (DTC) with an increase in the lumen of these tubules, and obliterating the periglomerular space (OPS), narrowing the lumen of some renal tubules (NRT). H&E stain. (C) Transverse section through the kidney of adult rat in the LD group showing destruction of some renal tubules (DRT), and congested blood vessels with markedly thick wall and the presence of inflammation cells around the blood vessel (CBV). (D) showing a large increase in the lumen of the renal tubule associated with the destruction of epithelial cell lining (DPC) increase the periglomerular space (IGS) and necrosis of the glomerular(NG) (E) showing condensation epithelial cells nuclei that line the renal tubules (CEC) (A 10x), (B 40x) (C 10x) (D 40x) (E10x) H&E stain.
Figure 4: A. Transvers section through the kidney of adult rat in the MD group showing degeneration of renal tubules (DRT), glomerular obliteration of periglomerular space (HOG) hemorrhage among glomeruli (HG) periglomerular necrosis (GN) H&E stain. (B) showing idening and destruction of epithelial lining of renal tubules (WDE) increased inflammatory cells infiltration within the renal stroma (IRS). (C), showing degeneration of the glomerular associated with the vascular degeneration of content (DG) obliteration of periglomerular space narrowing the lumen of renal tubules (NRT) that due to the obvious swelling of the epithelial lining of some tubules also showed the closed lumen occludes (CLO). (D), showing degeneration of renal tubules (DRT) glomerular hyperplasia (GH), RBC and inflammatory cells infiltration (RCI). (A 40x), (B 10x), (C 40x) (D 20x), H&E stain.
Figure 5: (A) Transverse section through the kidney of adult rat in the HD group showing glomerular hyperplasia (GH), dilation of bowman’s space (DBS) detachment of renal tubules from basement membrane (DRT) dilation and destruction of renal tubules (DDR). Figure 5(B) shows glomerular hyperplasia (GH), dilation of bowman space (DBS) and (DRT), and hydropic degeneration of renal tubules (PDR). Figure 5 (C) shows a congested blood vessel with a markedly thick wall (CBV) and the presence of inflammatory cells around the blood vessel (IBV) degeneration and (DRT). Figure 5(D) shows fatty degeneration intestinal spaces between renal tubules (FDS) widening of renal tubules (WRT). Figure 5(E) shows fibroblast cell infiltration (FCI), and (DPT). Figure 5(F) shows RBC infiltrations (RCF) dilation of Bowman space (DBC), determination of the glomeruli from Bowman capsule (DGB), degeneration of glomerular (DOG), and (DRT),(A 10x), (B 10x), (C 10x), (D 40x), (E 40x), (F 10x), H&E stain.

4. LIVER

Liver sections of the control group (Figure 6 A & B) showed live normal architecture including the hepatocyte, central vein with flat endothelial lining sinusoids, Kupffer cells, and binucleated hepatocytes. On the contrary, the liver of rats in the LD group (Figure 7 A-H) showed various pathological alterations such as branching, destruction, congestion of the central vein, diffuse vacuolar degeneration of hepatocytes focal infiltration of inflammatory cells, dilatation of the sinusoid, vacuolar degeneration, and atrophy of hepatocytes. There was also an increase in the inflamed area of liver parenchyma with the presence of the fibroblast cells. The histopathological effects of the Liver in the MD group were more similar to those found in...
the LD group as shown in Figure (8 A-D). Unlike the MD, the HD group shows the following histopathological features: pyknotic and dead hepatocyte, macrovesicular steatosis with large lipid droplet in hepatocytes displaced the nucleus to the cell periphery (Figure 9 A & B). Some congested central veins containing RBC, dilated sinusoid, ballooning hepatocytes, hypertrophy, and vacuolated cytoplasm, destroyed margin and inflammatory cell infiltration in liver parenchyma was also detected (Figure 9 C-F).

Figure 6: A and 6 B. High magnification of part of figure (A) of female rat control group showing the normal architecture of hepatocytes (H), central vein (CV) with flat endothelial lining (EL) sinusoids (SI), kupffer cells (KC) and binucleated hepatocytes (BH). (A 10x), (B 40x) H&E stain
Figure 7: A. Transverse section through the liver of adult rats in the LD group showing diffuse vacuolar degeneration of the hepatocytes (VDH), focal infiltration of inflammatory cells (FIC), branching and destruction of the central vein (BCV), (B). showing congest central vein (CCV) containing (RBC), dilution of the sinusoid (DSI), and vascular degeneration of hepatocytes (VDH), (C) and (D) magnification of (C), showing the normal structure of the central vein (CV) but this figure indicates vacuoles degeneration of hepatocyte (VDH), (E) and (F) of (E), showing increase the size of central vein (ICV), dilation and inflamed of the sinusoid (DSI), notice the presence of fibroblast cells (FBC), (G) showing increase inflamed area of liver parenchyma notice the presence of the fibroblast cells (FBC), dilation of sinusoid (DSI), atrophy of hepatocyte cells (TRH), binucleated hepatocytes (BH), (H) showing diffuse vacuolar degeneration of the hepatocytes (VDH).
degeneration of the hepatocytes (VDH) and dilation of the sinusoid (DSI), (A 10x), (B 40x), (C 10x), (D 10x), (E 10x), (F 40x), (G 40x), (H 40x). H&E stain

Figures 8: (A) and (B) are magnifications of the part of figure (A) Transverse section through the liver of adult rat in the MD group showing an increase in the size of the central vein, dilation of the sinusoid (DSI), (C) showing the dilution of sinusoid (DSI) and fibroblast cells (FBC), (D) showing a congested central vein contain inflammatory cells (CCV), and dilation of the sinusoid (DSI) which is surrounded by the inflammatory cells. (A 10x), (B 40x), (C 40x), (D 10x) H&E stain
DISCUSSION

Herb extracts and dietary supplements for weight loss or obesity management are attractive alternatives to the traditional therapy due to their accessibility to general publication. Many types of weight loss supplements are available in the markets and online stores, but, unfortunately, there is no or little knowledge about their efficacy, side effects, and toxicity is available. In Iraq and other countries, the use of adios herb extract supplements exists for more than tens of years and is very trendy among women wishing to lose weight. Unfortunately, based on our knowledge, no study has been conducted investigating the efficacy and effectiveness of adios supplements. Accordingly,
testing those herb-based capsules and studying their effect was found to be necessary.

In the present study, the oral administration of (LD), (MD) and (HD) of the adios supplement has shown no efficacy in rat body weight loss during the 30 days of the experiment; this is inconsistent with some studies that used well-known fat-burning ingredients such as fucus extract, boldo, and dandelion for weight loss and obesity management (Vodouhè et al., 2023; Olas, 2022). It is well documented that blood is an indicator of pathological and physiological status in humans and animals (Etim, et al., 2014). Furthermore, in acute toxicological studies, changes in hematological and biochemical parameters are usually used as indicators of toxicities. Adios showed significant decreases in WBC count and lymphocyte during the oral administration of (MD) and (HD) adios supplements. Additionally, our data suggested that adios supplement might cause iron deficiency as significant changes of low MCV and MCHC and high RDW with different concentrations observed. This data contrast with other studies which showed the antioxidant, antioxidant, and anti-inflammatory role of focus, boldo, and dandelion (Olas, 2022; Shin et al., 2021; Catarino et al., 2018; Modaresi, & Resalatpour, 2012.). Our finding suggests that the adios herbs extract could have the immunosuppression side effect which causes leucopenia; this means that adios consumers could be more likely susceptible to infections and diseases with prolonged use.

Although the kidneys and liver have a cardinal role in metabolizing substances and excreting waste products like blood urea and creatinine, the serum biochemical measurement can also give an insight into the site of cellular tissue damage. However, adios showed some differences in their serum level (ALP, ALT, AST, urea, creatinine, and albumin), but it was not significant. In this study, hematoxylin and eosin-stained sections of the kidney in the treated groups (LD, MD, and HD) show damage in the glomerulus, renal kidney, and blood vessels; this involved: increasing the periglomerular space, necrosis of glomeruli, hyperplasia of glomerulus, increasing the periglomerular space, hemorrhage among glomeruli, narrowing the lumen of some renal tubules, destruction of some renal tubules, and congestion of the blood vessels with markedly thick wall, and the presence of inflammation cells around the blood vessel. Nevertheless, in disagreement with our findings, Zhang and his colleagues showed a nephroprotective role of focus in animal studies using models of kidney injury (Zhang et al., 2003; Milek et al., 2019). In addition, dandelion has nephroprotective features against kidney disorders (Karakuş et al., 2017; Lim et al., 2015).

Liver histology showed abnormal appearances such as degeneration of the hepatocytes, focal infiltration, branching, congestion, destruction of the central vein, presence of RBC, dilution of the sinusoid, vascular degeneration of hepatocytes, increase in the inflamed area of liver parenchyma, the presence of the fibroblast cells, dilation of the sinusoid, atrophy of hepatocyte cells pyknotic and dead hepatocytes, and steatosis with large lipid droplets are present in hepatocytes which displaced the nucleus to the cell periphery. Our findings disagree with those of Lim, Oles, Fernández, and their colleagues (Olas, 2022; Lim et al., 2015; Fernández et al., 2009), who described the protective changes induced by boldo, dandelion, and focus in liver pretreated with cytotoxic chemicals. However, our results can be supported by the number of reported cases of possible hepatotoxicity is increasing in patients consuming boldo as traditional medicine (Oliveria Sá, et al., 2020; Nortadas, & Barata, 2011 & Piscaglia et al., 2007).

The findings of the current study contradict most of those of the previous research studies. Focus, Dandelion, and Boldo were known to have many biological activities, such as anti-obesity (Vodouhè et al., 2022; Shin et al., 2021; Milek et al., 2019; Maeda et al., 2005; Zhang et al., 2003) anti-inflammatory, anti-oxidant capacity, anti-diabetics, anti-tumor, chemoprotective and organ protective as well (Olas, 2022; Karakuş et al., 2017; Lim et al., 2015; Fitton et al., 2015; Ramos-Peralonso, 2014; Modaresi & Resalatpour, 2012). This conflict in our results with other previous research could be due to the chemical composition used, all other studies have used only one component of adios herbs, with different concentrations. Best of our knowledge, this is the first in vivo study on rats to investigate the physiological and histological effect of adios supplements.

The adverse effect of adios in this study could be a result of the absorption, metabolism, and accumulation of these chemicals in the body (Olas, 2022; Shin et al. 2021; Catarino et al., 2018; Karakuş et al., 2017, Ramos-Peralonzo, 2014), or it might be a result of the incompatible herb-herb interaction (Che, 2013), since dandelion and boldo are recommended by scientists not to be mixed with other herbs (Armell et al., 2014). Moreover, some of the bioactive ingredients of dandelion and boldo contain toxic chemicals such as lectupirin, ascaridole, and boldine respectively, which could explain the adverse effect of adios in liver and kidney tissues (Gille, et al., 2010; Basharat et al., 2009 & Leung, 1980).

Finally, these observations could suggest that adios supplements have low efficacy for weight loss and possibly have risk of toxic effects on the immune system of the experimental rats at different concentrations. Overweight individuals often try to lose weight are doing so without the help of the health care. Therefore, if healthcare professionals become more knowledgeable about the products available for treating obesity or overweight, then obese patients can be managed more safely and appropriately.

Conclusion:
Based on findings in this study, it can be concluded that adios supplements are not safe to use by women; they do cause immunosuppression and have zero efficacy in weight loss even with high concentrations.

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