Protective Role of Bovine Liver and Tomato Powder Against Negative Effects of Flummox Antibiotic in Rats

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ABSTRACT

This research sought to determine whether interactions between rat diet and the biological and metabolic responses could mitigate the side effects of the antibiotic Flummox. The addition of tomato powder that had been cooked, dried, and ground, along with 10% of lyophilized bovine liver. Out of 44 mature male rats, four main categories were developed. As a negative control, the first set only received regular meals. The third group received lyophilized bovine liver, the fourth group received tomato powder, and the second group received a fundamental diet together with Flumox as a positive control. In comparison to the Flumox group, the liver and tomato-treated groups displayed significantly lower levels of liver enzymes (ALT, AST, and ALP), kidney functions, lymphocytes, leukocytes, and oxidative stress, as well as significantly higher levels of red blood cells hemoglobin and glutathione (GSH) in the hepatic and renal tissues. One can conclude that combining some nutrients (liver, tomato) can reduce the amount of excess weight in rats and treat the many physiological and hematological aberrations caused by oxidative stress diseases connected with antibiotics.

Keywords: Flummox, Animal liver, tomatoes, adiposity.
INTRODUCTION

Drug, food, and supplement interactions can lead to difficult and complex issues. In other cases, the interactions can be employed to enhance drug absorption or lessen side effects; therefore, they are not always negative to therapy (Anadón et al., 2021). Innovation in technology, industrial expansion, and the widespread use of artificial substances to boost agricultural and livestock animal productivity and treat a wide range of illnesses have become serious threats to the environment and the public’s health (Hong and collaborators, 2018; Ngangom and others, 2019). Additionally, Clark and others (2016) noted that livestock farmers frequently provide multiple antibiotics in varying dosages to livestock to treat a variety of illnesses. Other studies that have been published (Hussain et al., 2022; Shahid et al., 2021) have shown that these methods not only cause some health problems in both humans and livestock, but also cause leftover antibiotics to pass through the food chain for human beings through food sources derived from animals, especially dairy products, meat, and poultry. Antibiotics serve a significant role in managing pathogenic infections for an extended time, even though Otlewska et al. (2020) underlined that they are often viewed as safe and well-accepted but associated with a wide range of negative consequences (Xi and coworkers, 2016). Exposure to such antibiotics produces mutations in typical biological activities and fertility problems in both targeted and non-target mammals, claim Andleeb and collaborators, 2020; Grenni et al., 2018. Amoxicillin and flucloxacillin, two bactericidal penicillins, are combined to form Flumox. According to Konari and Jacob (2015), gram-positive and gram-negative bacteria are killed by this mixture. Amoxicillin is frequently used to treat a wide range of illnesses in humans as well as animals because of its broad-spectrum activity, low prices, excellent pharmacokinetics, and antibacterial effectiveness. Amoxicillin is also used for preoperative preventive antibiotics in animal healthcare, according to Uddin et al. (2021); Djebala and coworkers (2019).

Tomatoes, which are technically a fruit and not a vegetable, are very beneficial to health (Lal, 2021). Consuming tomatoes that contain carotenoid
and lycopene molecules can lessen the risk of several ailments, including cancer, Type 2 diabetes, and cardiovascular diseases, according to population studies (Kumar and others, 2020; Perveen et al., 2015). The metabolism of the body is impacted by these compounds, which also control the detoxification process. You may protect yourself against lifestyle diseases and the return of cancer by doing this (Ghadage et al., 2019).

The dehydrated, fat-free beef liver can be utilized as a dietary supplement due to its substantial amount of protein, minimal calorie count, and full of nutrient composition (Kang and collaborators, 2017). Cow liver is reported to be high in many vitamins (A, E, B), EPA, DHA (omega-3 fatty acids), zinc, copper, and iron, according to research from Duizer and associates in 2017 and Fayemi et al. in 2018. In addition to helping to maintain the myelin layer that shields nerves, vitamin folate (B9) is the production of DNA and RNA. Pernicious anemia, tiredness, and peripheral nerve degeneration are symptoms of B9 deficiency (Dalens and Prikhnenko, 2015). A diet of traditional foods (eggs, liver, etc.) can readily provide significantly more choline per day than the estimated average intake for humans (Wallace et al., 2018). The most significant source of endogenous choline production is the liver as mentioned by May and others in 2023. Therefore, this investigation aimed to ascertain if nutrients may mitigate the negative effects of the antibiotic Flumox (1g) by interacting with them in the biological and metabolic processes of the rats.

**MATERIALS AND METHODS**

**Materials**

Beef liver and tomatoes were purchased from the local market. Flumox (1g) capsules, an antibiotic, were purchased from a nearby pharmacy in Cairo, Egypt. 44 wholesome adult male "Sprague Dawley strain" albino rats from the VACSERA Helwan Farm in Cairo, Egypt was used as test subjects. Diets were created using Reeves et al., (1993) recommendations.

**Methods**

**Diet Preparation:** Tomato powder had been dried in an oven after being cleansed of contaminants and washed with tap water. A freeze-drying procedure was used
to lyophilize bovine liver according to Deissroth and Dounce (1969).

**Tomato and Bovine Liver Chemical Analysis:**

The measurements of protein, ash, fat, and fiber were performed using the techniques of Sullivan and Carpenter (1993) and A.O.A.C. (2005). According to Wrolstad (2012), the differences were used to compute the total carbohydrates.

**Biological Experiment:**

Forty-four wholesome male albino rats of the "Sprague Dawley strain" weighing (140±10g) were given fresh water and a regular diet while they were housed in the fundamental conditions (12 h light: 12 h dark, 22±2 °C). The National Hepatology and Tropical Medicine Research Institute (NHTMRI) granted permission for the study (Protocol NO: A1-2023), and it was carried out in compliance with the Protocol for the Maintenance and Utilization of Lab Animals.

Rats were given a base food to help them adapt. After a week, they were split up into four sets (11 rats/ set), and each set was housed in a cage of wire as follows:

- **G1 (for 28 days)**
  - Rats were employed as healthy controls and simply given the basic diet.

- **G2 (for 28 days)**
  - As a positive control group, rats were given the antibiotic Flummox (1g) while merely receiving a basic diet.

- **G3 (for 28 days)**
  - Rats were given a standard meal adding 10% lyophilized beef liver (instead of starch) and administered antibiotic Flummox (1g).

- **G4 (for 28 days)**
  - Rats received normal nourishment with 10% tomato powder (in substitution for starch) and antibiotic flummox (1g).

The animals were slaughtered under sedation after the trial, blood samples were taken via a retro-orbital injection and placed in two sterile tubes, one containing EDTA for analysis within 24 hours and the other a dry centrifuge tube for serum, which was kept in sterile vials at - 20 °C up to analysis. Additionally, the liver and kidney were removed. To find oxidation biomarkers, a fraction of them were stored at -20°C.
**Biological Assessment**
Calculated feed intake (FI), body weight gains (BWG), and feed efficiency ratio (FER) assessments were used to evaluate the given diets biologically.

The amount of food left in the cage was subtracted from the daily amount of food supplied to every animal to determine FI (g) (Manjula and Krishna, 2016).

**Biochemical Examinations:**
The enzymatic colorimetric method of Young (2001) was used to measure the serum levels of uric acid, creatinine, urea, as well as the aminotransferases alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). Malondialdehyde (MDA) in liver and kidney tissues was detected using the calorimetric method (Uchiyama & Mihara, 1978). The hepatic and renal concentration of GSH was measured using the spectrophotometric method (Beutler and associates, 1963).

**Statistical inquiry:**
The results were presented as the mean using the standard deviation (SD). To compare across groups, a one-way analysis of variance (ANOVA) and post hoc least significant difference (LSD) investigations were performed using the statistical program for social science (SPSS) version 16. Daniel and Cross (2018) defined statistical significance as a value of $P$ value less than 0.05.

**RESULTS AND DISCUSSION**

**Macronutrients and Micronutrients Bovine Liver and Tomato Contents**
Micronutrients are vitamins and minerals, whereas macronutrients include foods like carbs, lipids, and proteins. Micronutrients are necessary substances that humans and other living beings need in various amounts throughout their lifetimes to control the physiological processes for maintaining good health. The required quantities of micronutrients are often below 100 milligrams per day for human nutrition, whereas the quantities of macronutrients are in grams per day (Godwill et al., 2020).

The macronutrients in tomato and cow liver were examined. On a dry weight basis, figure 1 findings revealed that the liver had the highest protein content, with 19.5 g/100 g, parallel to the tomato’s 1.1 g/100 g. In contrast to 0 and 75 g/100g
observed in bovine liver, tomatoes provided 0.6 and 98 g/100g of fibers and carbs, respectively. Data in Figure 1 (c and d) demonstrate tomatoes and livestock liver content of micronutrients. The highest content of iron was discovered to be present in the bovine liver. However, it had the lowest amount of vitamin C, whereas tomatoes had the highest amount but the lowest amount of iron.

Due to its abundance of protein, low energy content, and highly nutritious profile, the dehydrated, fat-free cow liver can be used as a dietary supplement (Kang and collaborators, 2017). Several vitamins (A, E, B), EPA, DHA (omega-3 fatty acids), zinc, copper, and iron are said to be abundant in cow liver, according to studies from Duizer and associates in 2017; Fayemi et al. in 2018. According to observations made on rats recorded by Wu (2016), beef kidney and liver proteins, in particular, have more nutritional value than beef muscle proteins. 40% of the recommended vitamin C daily intake is found in tomatoes (Lal, 2021). Beta-carotene, which the body converts into vitamin A and has antioxidant properties, also vitamin E and vitamin C are all present in tomatoes (Çolak and others, 2020). These findings were also supported by Kumar and colleagues (2020); Wako and Muleta (2022), who stated that tomatoes are considered to be "the most popular vegetable fruit" due to their relative abundance of minerals (iron, calcium, and phosphorus) and vitamins (vitamin C).

Feeding nutrients (Tomato and Bovine Liver) and an antibiotic (Flummox 1g) have an impact on obesity measures

Adiposity has been a serious public health concern in recent years, and it is now being acknowledged as a true pandemic (Del Fiol et al., 2018; Lovic and others, 2020). Obesity not only makes it harder to move around, but it also comes with serious medical issues (diabetes, hypertension, and dyslipidemia) (Utkirzhonovna, 2022).

The animals were weighed once a week. Figures 2a, 2b& 2c display BWG (g), FI (g/day) & FER in several experimental groups. There was no difference in the FER values across any one of the groups, using a one-way ANOVA analysis (Fig. 2c).
Although we saw a greater rate of BWG and FI for G2 (Flumox set) compared to the healthy control group (G1), this finding was statistically immensely significant ($p < 0.0001$). According to several laboratory models, antibiotic-induced weight gain is mediated by the drug's effects on the microbiota (Scott et al., 2016). These may be the result of the antibiotic altering the microbiota (dysbiosis), which causes an increase in dietary energy consumption and, ultimately, obesity (Del Fiol and coordinators, 2018). These findings were supported by research from D’Alessandro and coworkers (2022); Marciano et al., (2017); Principi and Esposito (2016).

When compared to the control group (G2), the bovine liver (G3) and tomato (G4) treated groups' FI and BWG were significantly lower ($P < 0.0001$) (Fig. 2). According to Shang et al., (2018), the bovine liver offers a highly nutritious source of protein while enhancing vitality, metabolism, and digestion. Increased protein intake improves blood levels of the amino acid arginine, which enhances the responsiveness to insulin, promotes the burning of fatty acids and glucose in muscular tissue, promotes metabolic rate, and reduces the mass of white fat in overweight individuals (Kamei and workmates, 2020). This may help explain why eating more protein benefits weight loss.

Furthermore, according to Leidy and colleagues (2015), adequate dietary protein consumption in free-living subjects can have a satisfaction effect along with as a result, decrease calories or food intake by hindering the secretion of ghrelin, an appetite-promoting polypeptide, and stimulating the release of peptide YY and glucagon-like peptide 1, which are appetite-suppressing polypeptides (Liu and collaborators, 2022). Both Wu (2016) and Wyness (2016) concurred with these findings.

Perveen et al., (2015) demonstrated that tomato extract containing lycopene inhibited gaining weight. Interestingly, tomato lycopene-containing supplements reduced body weight gain even more; in the groups treated with tomato powder, substantial ameliorations were observed in BWG and feed efficiency. The result might have happened because tomatoes and
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Tomato byproducts are excellent providers of micronutrients, including antioxidants, ascorbic acid, vitamin E, colors like lycopene and beta-carotene, and amino acids, particularly lysine. The conclusions presented here are in agreement with those made by Elkomy and colleagues (2016); Elwan and others (2019).

Hepatic Influence of Feeding Nutrients (Tomato and Bovine Liver) and Antibiotic (Flumox 1g) Interaction

Figure 3 depicts the hepatic implications of feeding nutrients (tomato and beef liver) and an antibiotic (Flumox 1g) on liver function tests (ALT, AST, and ALP) in all groups.

As shown in Fig. 3a, 3b, and 3c the mean AST, ALT, and ALP of the Flumox control group (G2) were 69, 138, and 193 U/L, respectively, as opposed to 30, 27, and 31 U/L in the control group (G1). The quantity of liver intracellular enzymes that have entered the circulation is determined by serum enzymes known as aminotransferases, like ALT and AST. These are the signs of hepatocellular damage, claims (Nageen, 2022).

According to Ermaya and Prasetyo (2023), elevated blood ALP levels are related to liver illness due to intra- or extrahepatic cholestasis along with some hepatic cell membrane damage. Olaniyan and Adepoju (2019) state that the serum ALT and AST values are regularly examined to detect liver disease, evaluate liver health, and look for any potential hepatocellular damage from toxic substances such as medicines and viral infections. Similar findings showing a substantial increase in ALT, AST, and ALP values in rats treated with amoxicillin have previously been described in published literature (El-Kholy and collaborators, 2019; Nasir and Taher, 2021). Hepatitis, liver injury, and hepatotoxicity brought on by amoxicillin can all result in elevated ALT and AST plasma levels (Hussain et al., 2022).

When compared to the group receiving only Flumox (G2), the group feeding bovine liver lowered ALT, AST, and ALP by 18%, 34%, and 48%, respectively. The tissues of adults, including the brain, heart, kidneys, liver, and gut, require a sufficient diet of superior protein from animal sources, such as meat and liver, according to Wu (2016; 2020). The quantity in the liver of
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The effects of the interaction between the antibiotic Flumox 1g and the nutritional feeding (tomatoes and beef liver) on renal function (uric acid, urea, and creatinine) are depicted in Figure 4. Figures 4a, b4& 4c show that after antibiotic supply (G2), there was a highly significant increase (P < 0.0001) in urea, creatinine, and uric acid (in comparison to the control group (G1). Antibiotic medication impacts the target pathogen in addition to the beneficial bacteria that are normally present in the gut flora, which might have adverse effects like tendinopathy, eye damage, and renal toxicity. (Baggio and Ananda-Rajah, 2021; Güller and others, 2020). Akhavan et al., (2022); Olaniyan and Fowowe, (2020) reported comparable results of significantly increased kidney functions in the past and suggested
that nephrotoxicity was caused by an amoxicillin overdose, an unusual pharmacological reaction. The drug crystallizing within the tubular lumen, renal venules, or interstitium; direct intracellular toxicity; vasoconstriction; or an acute hypersensitive reaction are a few of the postulated mechanisms of the renal problems linked to amoxicillin.

When compared to the Flumox control group (G2), the tomato-fortified group's creatinine, urea, and uric acid collapsed by 27%, 30%, and 14%, respectively (Figure 4). According to Udensi and Tchounwou's (2017) research, tomatoes are high in threonine, lysine, K, Mg, Na, Ca, and other nutrients that are essential for kidney function. Another study contends that dietary K$^+$ intake can alert the kidney to ion concentrations through a splanchnic mechanism for sensing (Oh et al., 2011; Palmer and Clegg, 2019).

As shown in Figure 4 (a, b, and c), feeding the bovine liver to group G3 decreased renal functions (creatinine, urea, and uric acid) in contrast with group G2 which received Flumox alone. Beef liver contains various vitamins, minerals, and fatty acids (EPA and DHA), which may help explain this (Fayemi and others 2018, Sergin et al., 2022). Multiple research investigations have shown that supplementing with omega-3 fatty acids can increase creatinine clearance and decrease the risk of developing end-stage renal disease (ESRD) (Rund et al., 2020). The study by Heshmati and colleagues (2019) revealed that omega-3 fatty acids are associated with a lower prevalence of albuminuria and considerably reduce the risk of ESRD. These results corroborate those of Capelli and teammates (2019), who discovered a potential link between cardiovascular risk and the development of chronic renal disease and the breakdown of vitamin B12 and folic acid homeostasis. Animal liver is another typical source of choline (May and others, 2023). Betaine, a crucial osmolyte generated by choline oxidation in the renal glomerulus, aids in the reabsorption of water from the kidney tubule (Kansakar et al., 2023). According to Hasson et al. in 2022, choline therapy in murine sepsis-associated acute kidney injury (SA-AKI) improved renal function.

When compared to the positive-controlled group, the
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Tomato-based treatment lowered uric acid by 14% whereas the animal's liver set reduced uric acid by 33%. This means beef liver was more effective than tomatoes in diminishing uric acid. The relationship between tomatoes and serum urate, which supports the notion that tomatoes may affect either hepatic or renal uric acid metabolism, may help to explain this (Roman, 2019).

Hematological Research of Experimental Groups

White blood cells, leukocytes, and lymphocytes significantly increased, whereas red blood cells and hemoglobin dramatically decreased as compared to the control group (G1), according to an examination of hematological parameters of the control and experimental groups in Figure (5).

According to the hematological profile, there were more total leukocytes and lymphocytes in the G2 population. It is possible to link the increased population of these cells in treated rats to the various tissue damage caused by antibiotics. Similar outcomes in sheep (Elmajdoub et al., 2014) and rabbits (Hussain et al., 2022) treated with amoxicillin have also been reported in various earlier investigations. Additionally, the positive control group's RBC and Hb dramatically dropped as compared to the control group of healthy individuals. RBCs may have been destroyed or the rat's hemopoietic tissues have produced fewer RBCs than normal (Olaniyan and Oladega, 2018).

As a result, there is a reported drop in hemoglobin concentration as hemoglobin is a component of RBC (Hussain et al., 2022). This could be because, as described by D'Alessandro and colleagues in 2022, antibiotics can deplete nutrition, particularly micro-nutrients like vitamin K and B12.

Fig. 5 shows that compared to the Flumox group (G2), G4 and G3 had significantly greater levels of Hb and RBCs and lower levels of WBC and lymphocytes. According to Juárez and associates (2021), the bovine liver is a significant source of complete protein, which includes all essential amino acids, as well as highly accessible iron, zinc, selenium, and vitamin B12. According to Mohamed et al. (2020), vitamin B12 insufficiency causes pernicious anemia, weariness, and peripheral nerve degeneration. According to
Shubham et al. (2020), meals made from animal sources such as beef, liver, and fish are good sources of hem iron, which is more bioavailable. These findings supported those reported by Elkomy and associates (2016) who asserted that tomato waste increased the RBC levels in growing rabbits. Some blood physical features (RBCs, Hb, and WBCs) may have been improved due to the bioactive components in tomatoes, such as folate, phenolic, flavonoid, vitamin B12, copper, and iron, which are necessary for the normal development of cells and Hb (Elwan et al., 2019).

Nephrology and Hepatic Oxidative Exposure Status

Scirè et al., (2019) pointed out that GSH, which is essential for many cellular activities as cell defense against oxidative stress, xenobiotics, and radiation, is plentiful in the low intracellular thiol molecular weight. MDA, a hallmark of lipid peroxidation, is the major aldehyde product produced by the oxidation of the fatty acid content of LDL (Li and collaborators, 2022).

Since the liver and kidneys are crucial for the detoxification of various metabolites, it is crucial to look into the effects of exogenous agents like antibiotics, particularly those that are often administrated, such as Flummox (an amoxicillin derivative). Figure 6 (a, b, c, and d) shows how a group (G2) receiving the antibiotic Flumox demonstrated noticeably higher levels of oxidative stress indicators like MDA concentrations and noticeably lower levels of GSH (P<0.0001) (in both hepatic and renal tissues) when compared to the untreated control group. Numerous earlier biochemical and enzymatic studies have shown a connection between antibiotic use and reactive oxygen species (ROS), which causes oxidative stress-related illnesses (Thomas et al., 2022).

These concurred with the findings of Güller et al., (2020), who found that amoxicillin raised MDA and lowered GSH levels. In truth, amoxicillin and its metabolite may interact with glutathione reductase (GR) as their principal effect after being metabolized in the liver. The kidney exhibits the same situation. A free radical chain reaction deteriorates the cell's structure and function (Khan and others, 2019). The higher MDA levels in this
study suggest that Flumox (1g)-induced oxidative stress in renal and hepatic tissues. Furthermore, Salah and colleagues (2020) published findings and these outcomes are comparable.

The mean G3 renal and hepatic MDA declined by 4% and 14%, whereas the renal and hepatic GSH had risen by 9% and 28%, respectively Fig. 6 (a and c). It may be due to the EPA and DHA (omega-3 fatty acids) presence in bovine liver (Fayemi et al., 2018; Sergin et al., 2022) and their capacity to scavenge free radicals (Soliman et al., 2020). These results supported the findings of Heshmati and colleagues (2019), who said that omega-3 fatty acids might be characterized as enhancing factors for antioxidant defense against ROS.

Figure (6) indicates that fortification of experimental animals with tomato powder (G4) significantly reduced MDA and elevated GSH compared with Flumox-treated animals (G2). Therefore, lycopene is present in commercial tomato products, which supports its antioxidant action (Blekkenhorst and collaborators, 2018). Tomato seeds include antioxidant compounds like phenols, flavonoids, condensed tannins, ascorbic acid (vitamin C), and carotenoids, according to Durante and others (2017); Rahaman and colleagues. (2023). Antioxidants interact with free radicals to exert their effects, which would otherwise injure the body's essential cells (Silva et al., 2019). These interactions include peroxide decomposition, radical scavenging, and binding to metal ions (Kumar et al., 2021)

**CONCLUSION**

Flumox causes oxidative stress, which damages internal tissues (such as the kidney and liver) when antioxidant enzymes are inefficient. An elevation of total white blood cells and a decrease in red blood cells with hemoglobin. In this study, an increase in the final body weight was seen, as well as the excess of liver enzymes (ALP, AST, and ALT) and renal functions (urea, creatinine, and uric acid).

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Fig. (1): Macronutrients and micronutrient content in Bovine Liver and Tomato. A & b) Macro-contents, c) & d) Micro-constitutes
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**Fig. (2):** Effect of Feeding nutrients (Tomato and Bovine Liver) and an antibiotic (Flumox 1g) impact on obesity measures (a-d) Represents the mean value ± S.D. (n=11 rats / group), Means that do not share a letter are significantly different using One-way ANOVA. (P <0.05)

**Fig (3):** Hepatic Influence of Feeding Nutrients (Tomato and Bovine Liver) and Antibiotic (Flumox 1g). (a-d) Represents the mean value ± S.D. (n=11 rats / group), Means that do not share a letter are significantly different using One-way ANOVA. (P <0.05)
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Fig (4): Nutritional Impact of Tomatoes and Bovine Liver with Antibiotic (Flumox 1g) on the Renal System
(a-d) Represents the mean value ± S.D. (n=11 rats / group), Means that do not share a letter are significantly different using One-way ANOVA. (P <0.05)
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**Fig (5):** Hematological Research of Experimental Groups
(a-d) Represents the mean value ± S.D. (n=11 rats / group), Means that do not share a letter are significantly different using One-way ANOVA. (P <0.05)
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Fig. (6): Nephrology and Hepatic Oxidative Exposure Status of experimental groups. a, b Hepatic status while c& d Nephrology status

(a-d) Represents the mean value ± S.D. (n=11 rats / group), Means that do not share a letter are significantly different using One-way ANOVA. (P <0.05)
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