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Nano-Selenium-Mediated Alterations in Lipid Profile, Liver and Renal Functions, and Protein Parameters in Male Lambs: An Experimental Study

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Abstract. The impact of nano-selenium supplementation on lipid metabolism, liver enzymes, renal function, and protein levels in male lambs was investigated in this study during a 60-day period. Lambs were divided into two groups — control and experimental, which were administered nanoselenium orally at a dosage of 0.5 mg/kg of feed once a day, daily. Each group consisted of five male lambs. According to the findings, cholesterol levels dropped significantly at days 45 ($P < 0.01$) and 60 ($P < 0.05$), whereas triglyceride levels significantly rose at days 15 and 30 ($P < 0.01$). Throughout the trial, the levels of uric acid and creatinine were constant. At days 15 and 60, aspartate aminotransferase (AST) levels were significantly decreased ($P < 0.05$), while at days 45 and 60, alkaline phosphatase (ALP) levels declined ($P < 0.01$ and $P < 0.05$, respectively). At days 15 and 30, albumin levels dropped significantly (0.05), but day 60 saw an increase in globulin levels significantly ($P < 0.05$). The drop in AST and ALP levels might be a sign that liver function has possibly improved. However, the drop in albumin levels and rise in globulin levels raise the possibility that taking nano-selenium supplements may affect how proteins are metabolized. The findings of this study concluded that the effects of nano-selenium supplementation on lipid metabolism, liver enzymes, renal function, and protein levels in male lambs were time-dependent. In addition to possible improvements in liver function based on decreased AST and ALP levels and changes in albumin and globulin levels, the study also noticed an initial transient increase in triglyceride levels that was followed by a decline in cholesterol levels. To determine the time-dependent changes in liver function, liver enzymes, as well as renal function in response to nano-selenium supplementation in male lambs was objective of this study.

Key words: lipid metabolism, liver enzymes, protein levels, aspartate aminotransferase, alkaline phosphatase

Conflict of Interests. Authors declare that there is no conflict of interest.

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Introduction

In recent years, interest in nano-selenium, a type of selenium with smaller particle size, has grown due to its possible health advantages. The vital trace mineral selenium is needed for several physiological processes, including liver and kidney function. Scientific study and examination have been conducted on the impact of utilizing nano-selenium on renal function, liver enzyme levels, and function [1]. Supplementing with nano-selenium might benefit liver health. Glutathione peroxidase, an antioxidant enzyme that aids in preventing oxidative stress and damage to liver cells, is largely composed of selenium. According to studies, nano-selenium increases the activity of glutathione peroxidase, which may aid in lowering the levels of liver enzymes like alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST), which are indicators of liver damage. Furthermore, it has been demonstrated that nano-selenium possesses anti-inflammatory effects, which could help lessen liver inflammation and enhance liver function [2]. The possible impact of nano-selenium on renal function has also been researched. Selenium is known to play a role in the control of kidney function, and nano-selenium has been found to have protective effects on the kidneys. Various animal models have demonstrated that supplementing with nano-selenium may assist enhance renal function by lowering oxidative stress and inflammation in the kidneys, enhancing antioxidant status, and minimizing kidney damage [3]. A form of fat called cholesterol is necessary for many physiological functions, but too much of it can be detrimental, especially when the level of low-density lipoprotein (LDL), commonly referred to as “bad” cholesterol, is high. High-density lipoprotein (HDL), also known as “good” cholesterol, has an anti-heart disease protective effect. Another form of fat that can be increased in the blood and increase the risk of cardiovascular disease is triglycerides. Triglycerides are carried in blood by a kind of lipoprotein called very low-density lipoprotein (VLDL) [4]. Supplementing with nanoselenium may help to improve lipid profiles. Nano-selenium supplementation has been shown to increase HDL cholesterol levels while decreasing total cholesterol, LDL cholesterol, and triglyceride levels in both animal and human studies. It has been proposed that nano-selenium has antioxidant properties that can aid in the prevention of lipid peroxidation, a process that harms lipids and aids in the emergence of cardiovascular diseases. Additionally, it has been demonstrated that nano-selenium alters the expression of genes involved in lipid metabolism, which may strengthen the lipid-lowering effects of the substance [5]. The evidence for nano-selenium's effects on protein metabolism is sparse and ambiguous. Supplementing with nanoselenium may improve levels of proteins, especially albumin. The liver produces albumin, which is the most prevalent protein in blood. It is essential for maintaining oncotic pressure and moving different substances through the blood, such as hormones and medications. An improvement in liver function and protein synthesis may be indicated by an increase in albumin levels after taking nano-selenium supplements. On the other hand, it is less certain how nano-selenium affects globulins, a different class of blood proteins. Immune function involves the use of globulins [6]. The effects of nano-selenium on renal function and liver function and enzyme levels, however, may differ depending on the dosage, length of supplementation, and individual characteristics like age, health status, and underlying medical disorders. To completely comprehend the mechanisms and recommended dosages of nano-selenium for the health of the liver and kidneys, more research is required. The study aimed to investigate the impact of nano-selenium on liver and renal function in different period of time.

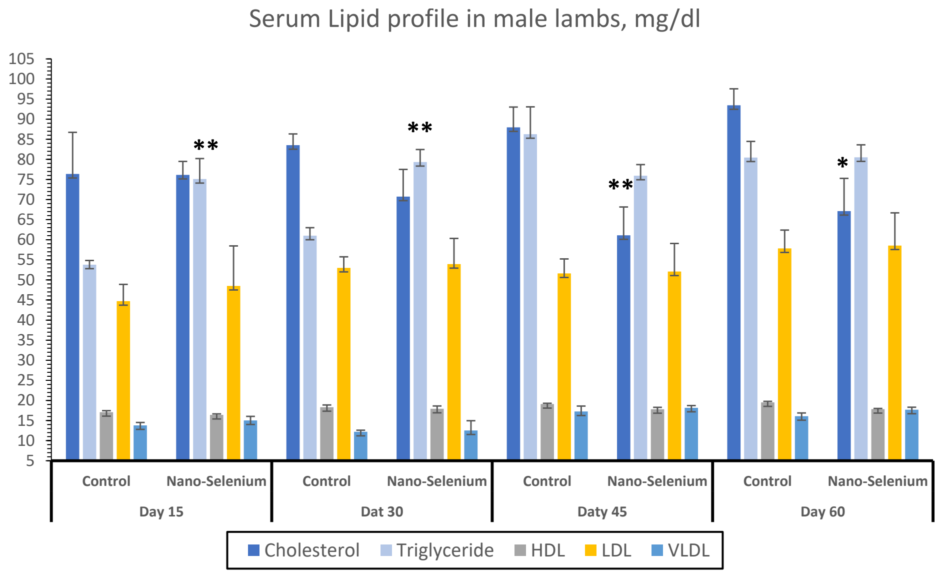
Materials and Methods

Twenty 3- to 4-month-old male Karadi lambs were divided into two groups. Each lamb had its own cage and was fed separately. The broadcast was made in a special facility in Sulaymaniyah, in the Iraqi Kurdistan area. The second group received a capsule containing 0.5 mg of nano-selenium, whereas the first group acted as the control. To encourage maintenance and daily benefit, food was provided once per day at 9:00 a.m. in portions estimated to be 3% of live body weight (LBW). Every day in the morning, after eating, the capsules were administered through gavage tube within two hours. The China-based Wuhan Dongxin Mill Imp and Exp Trade Co, Ltd was the source of the Nano-selenium particles employed in this investigation. These particles were kept in a dry, contained space and have a purity level of 99.99%. They are a black powder. At the central lab of Bagdad University's College of Education for Pure Science/Ibn Alhaitham, a sample of the Nano-selenium particles, weighing roughly 2–3 gm, was examined using X-Ray Diffraction to guarantee their quality. The crystal structure and the relative intensities of each peak were revealed by the X-Ray Diffraction pattern, which was acquired in the angular range of $2 = 10°–140°$. To verify the accuracy of the study outcomes, this data was used for hypothetical calculations and comparison with global parameters. Every 15 days, blood samples were taken from the jugular vein using a disposable needle. About 6 cc of blood were collected and put in a special gel tube to make serum. Using cobas e 311 (Roche, Germany), biomedical parameters like liver enzyme levels, total protein, albumin, globulins, triglycerides, and cholesterol were assessed in serum. P-nitrophenyl phosphate is broken down by the enzyme phosphatase into two different forms of phosphoric acid, p-nitrophenol, in the presence of magnesium and zinc ions.

The amount of p-nitrophenol produced varies in direct proportion to ALP's catalytic activity. Use the increased absorbance to calculate. The kit was a Gundersen ALP2S one. Alanine aminotransferase (ALT) in serum and plasma is quantitatively evaluated in vitro. ALT catalyzes the reaction between L-alanine and 2-oxoglutarate. In a process facilitated by lactate dehydrogenase (LDH), NADH lowers the pyruvate to L-lactate and NAD+. The behavior of the catalytic ALT is inversely correlated with the rate of NADH oxidation. The calculation is based on the decrease in absorbance [7]. Divalent copper can form a purple-colored biuret complex with protein peptide bonds in an alkaline solution for the colorimetric measurement of total protein, albumin, and globulin, respectively. Potassium iodide inhibits copper auto-reduction while sodium potassium tartrate inhibits the production of copper hydroxide. It is possible to measure photometrically how closely the protein concentration correlates with the biuret complex's color intensity. Albumin's cationic nature at pH 4.1 enables it to combine with the anionic dye bromcresol green (BCG), resulting in the formation of a blue-green complex. Albumin levels can be determined photometrically by measuring the intensity of the blue-green color, which is directly proportional to the amount of albumin in the sample [8]. The enzyme cholesterol esterase breaks down cholesterol esters to produce free cholesterol and fatty acids. The subsequent oxidation of cholesterol to cholest-4-en-3-one and hydrogen peroxide is catalyzed by cholesterol oxidase. A red quinone-imine dye is created when phenol and 4 aminophenazone undergo an oxidative coupling in the presence of peroxidase. The relationship between the cholesterol concentration and the dye's color intensity is direct. The rise in absorbance is measured to ascertain it. Triglycerides are three long-chain fatty acid esters of the trihydric alcohol glycerol. They are partially produced in the liver and partially taken in from meals. Triglyceride measurements are used to diagnose and treat individuals with diabetes mellitus, nephrosis, liver obstruction, problems of lipid metabolism, and a variety of other endocrine diseases. The Eggstein and Kreutz enzymatic triglycerides assay still required saponification with potassium hydroxide. Thereafter, lipase was used in numerous attempts to replace alkaline saponification by enzymatic hydrolysis. Wahlefeld employed an esterase from the liver in conjunction with a very efficient lipase from *Rhizopus arrhizus* for hydrolysis. Bucolo and David investigated a lipase/protease cocktail [9].

Results and Discussions

Orally administered nano-selenium had no discernible impact on serum cholesterol levels at days 15 and 30, however the level was lower in the nano-selenium groups (fig. 1). While cholesterol levels considerably dropped at days 45 and 60 ($p < 0.01$), respectively, this indicates that the effects of nano-selenium on cholesterol take time to manifest. The results of this investigation were in agreement with those of several other studies in terms of the nano-selenium lipid profile (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 [10]. However, on days 15 and 30 of this trial, triglyceride levels dramatically increased ($p < 0.01$). Triglyceride levels may rise as a result of using the gut's and intestine's functions to enhance or increase food intake and diet digestion [11].



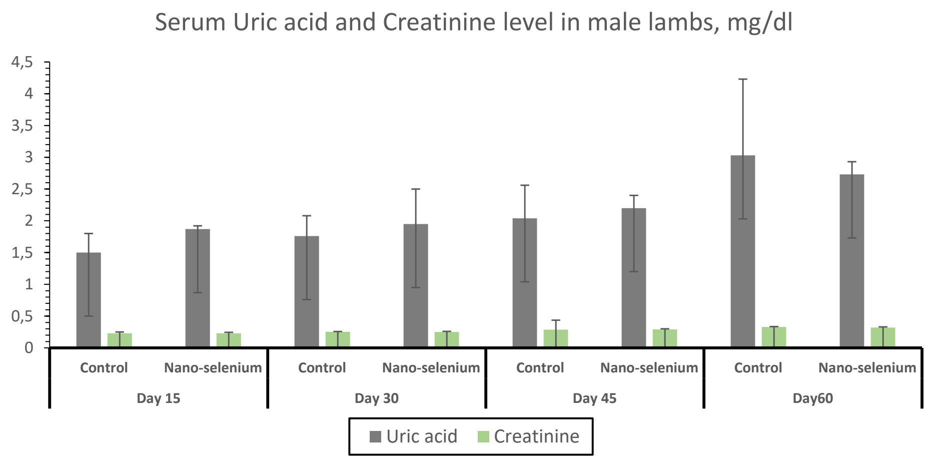
Note: *P < 0.05, **P < 0.01. Indicates significant differences with the control group.

Fig. 1. Serum lipid profile for male lambs orally administrated with Nano-selenium. The table representate mean ± standard deviation, mg/dl

Source: made by the authors

As demonstrated in figure 2, the effect of nano-selenium on the level of serum creatinine and uric acid was not substantially different from the control group, but there was a minor spike in both parameters. However, prior studies claimed that nano-selenium had no effect on serum biochemical markers in hens and rats, which is agreed with this study. There are also fewer studies about the effects of nano-selenium on lambs, but some studies show that dietary nano-selenium supplements led to a decrease in the level of serum uric acid in chicken, which is beneficial of positive effect on renal health [12]. In this investigation, there were no negative effects on the levels of serum uric acid and creatinine in male lambs, allowing researchers to conclude that nano-selenium at dosages of 0.5 mg/kg body weight is safe for renal health and can be utilized as a dietary supplement in ruminants.

The impact of nano-selenium on the liver enzymes of farm fish and rats has been adequately studied. According to studies done on fish, nano-selenium had no impact on the liver enzymes ALP, ALT, and AST. In additional experiments using mice, the effects of nano-selenium (Nano-Se) 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-Se, as seen by an increase in the serum level of the hepatotoxic marker. In the case of Nano-Se, after being treated with various forms of selenium, enzyme activity increased dramatically [13, 14]. In dairy cows, selenium supplementation, particularly nano selenium, significantly decreased blood alkaline phosphatase and aspartate amino transferase activity [15]. The results of this study were generally supported by studies; however, nano-selenium reduced the levels of AST and ALP in the serum on day 45 (P < 0.01 and P < 0.05, respectively). According to earlier studies, the ranges of the liver enzymes mentioned in the current study were within normal limits [16, 17]. Our findings suggest that nano-selenium has no effect on liver damage or a bone condition, whereas decreasing the level of ALP implies malnutrition or a zinc shortage (fig. 3). The lambs utilized in this investigation were healthy and in good shape; their teeth and bones did not show any signs of abnormality. The highest concentrations of AST are found in the liver and muscle, and they are mostly utilized to indicate whether the liver is functioning normally or whether it is ill or damaged [18].



*Note:**P < 0.05, **P < 0.01. Indicates significant differences with the control group.

Fig. 2. Renal functions tests (Uric acid and creatinine) for male lambs orally administrated with Nano-selenium. The table representate mean ± standard deviation, mg/dl

Source: made by the authors

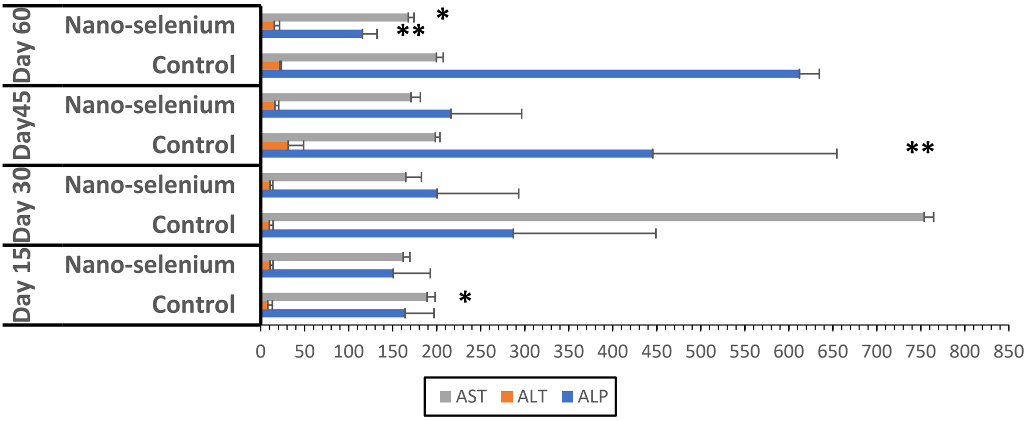
Numerous proteins disintegrate in the plasma. By examining the levels of these proteins, the doctor can gain knowledge about the health status in various organ systems. Serum, the liquid that remains after plasma clots, removing fibrinogen and the bulk of clotting factors, is used to evaluate protein levels. The amount of total protein in a sample can reveal some details about the general health of a patient, but fractionating the total protein results in more therapeutically relevant information [19].

Nearly half of the serum's total protein content is made up of albumin. Between 30 and 40 percent of the body's total albumin pool is present in the intravascular compartment. The remainder is extravascular and is primarily located in the skin and muscle interstices. A number of human tissue fluids, including saliva, tears, gastric juice, and bile, include albumin in traces [20, 21]. Albumin is produced in the liver. Less than half of the liver's typical capability is used to produce albumin. Albumin production is significantly influenced by dietary protein and amino acid intake, colloidal osmotic pressure, the action of certain hormones, and disease states. Fasting or a protein-poor diet reduce albumin production so long as the deficit situation persists [19, 20, 22]. The liver increases albumin synthesis after every protein-rich meal in response to the increased supply of amino acids brought by the portal blood. The globulin fraction contains hundreds of serum proteins, including carrier proteins, enzymes, complement, and immunoglobulins. With the exception of immunoglobulins, which are produced by plasma cells, the majority of them are produced in the liver. Using electrophoresis, globulins are divided into four groups [23, 22]. Although there may also be an increase in other proteins in pathological conditions with distinct electrophoretic patterns, increases in the globulin fraction are mostly caused by increases in immunoglobulins. Malnutrition and congenital immunodeficiency can lead to a decrease in total globulins due to decreased synthesis, while nephrotic syndrome can cause a decrease due to protein loss through the kidney[19, 21]. This study concentrated on the levels of serum total protein, albumin, and globulin for the estimate of liver function (fig. 4). On days 15 and 30, serum albumin levels in the nano-selenium groups declined significantly ($p < 0.05$), but on days 45 and 60, there was no significant decrease. The findings suggest that nano-selenium may affect how dietary amino acids are consumed or may have an immediate impact on liver function. With prolonged use, the body may attempt to adjust to nano-selenium. Serum globulin levels considerably rose ($p < 0.05$) on day 60, indicating that nano-selenium has a positive impact on immune system support, immunoglobulin synthesis, and complement system function. On day 60, the serum's total protein level increased, but not significantly ($p < 0.05$). Our findings were corroborated by previous investigations showing that food or nano-selenium supplementation increases total serum protein and globulin levels [24, 25].

Overall, the effect of every day administrated nano-selenium for 60 days had positive impact on the male lamb's health and improved the immune system through elevating the amount of globulin in serum. In addition, there was no negative effect on liver and renal functions.

Conclusion

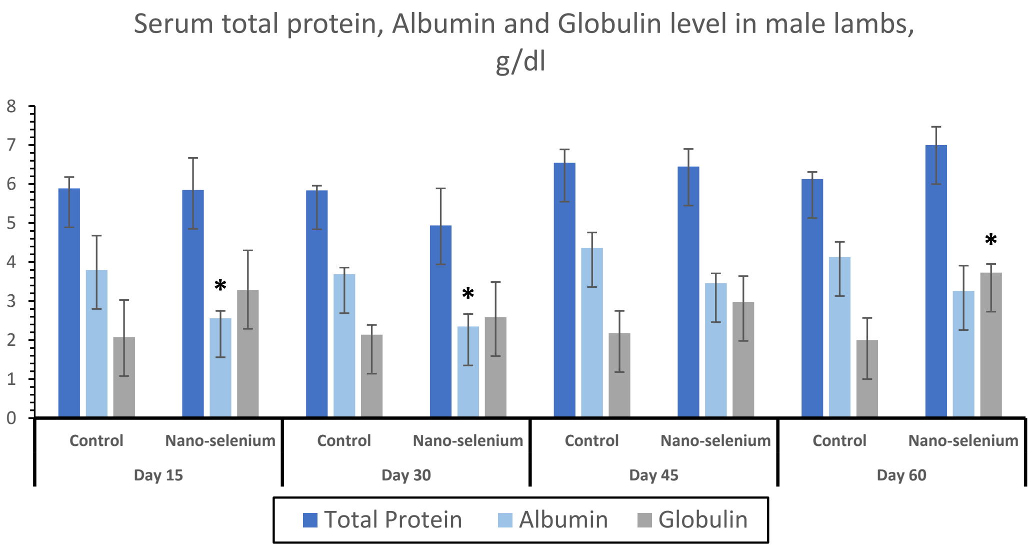
From our results, it could be concluded that Nano-selenium is effective in increasing different biochemical parameters, liver activities, and immune system in male lambs. Nano-selenium has increasing effect on triglyceride levels in the serum, its effect appears after 15 days of orally exposure 0.5 mg/kg of feed intake. There are no any adverse effects of nano-selenium on the renal functions, the level of uric acid and creatinine were no changed significantly. The effect of used nano-selenium on ALP and AST was estimated and the level of those enzymes were reduced significantly used 0.5 mg/kg of daily food intake. Nano-selenium has decreasing effect on serum albumin on days 15 and 30 but increasing impact on globulin that means nano-selenium has acute effect on liver function or metabolism and decreasing effect on albumin may led to blood or plasma lick out from blood vesicles but can support immune system by increasing globulin.



Note: *P < 0.05, **P < 0.01. Indicates significant differences with the control group.

Fig. 3. Liver enzyme tests (ALP, ALT and AST) for male lambs orally administrated with Nano-selenium. The table representate mean ± standard deviation, U/L

Source: made by the authors



Note: *P < 0.05, **P < 0.01. Indicates significant differences with the control group.

Fig. 4. Liver function tests (total protein, albumin and globulin) for male lambs orally administrated with Nano-selenium. The table representate mean ± standard deviation, g/dl

Source: made by the authors

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Влияние наноселена на изменения липидного профиля, функций печени и почек, белковых параметров у ягнят-самцов: экспериментальное исследование

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Аннотация. В исследовании на протяжении 60 дней изучалось влияние добавок наноселена на липидный обмен, ферменты печени, функцию почек и уровень белка у ягнят-самцов. Ягнят разделили на две группы — контрольную и экспериментальную, которой вводили наноселен перорально в дозировке 0,5 мг/кг корма один раз в день, ежедневно. Каждая группа состояла из пяти животных. Согласно полученным данным, уровень холестерина значительно снизился на 45-й ($P < 0,01$) и 60-й день ($P < 0,05$), тогда как уровень триглицеридов значительно повысился на 15-й и 30-й день ($P < 0,01$). На протяжении всего испытания уровень мочевой кислоты и креатинина был постоянным. На 15-й и 60-й дни исследования уровень аспартатаминотрансферазы (АСТ) был значительно снижен ($P < 0,05$), а на 45-й и 60-й день — уровень щелочной фосфатазы (ЩФ) ($P < 0,01$ и $P < 0,05$ соответственно). На 15-й и 30-й день уровень альбумина заметно снизился (0,05), но на 60-й день наблюдалось увеличение уровня глобулина ($p < 0,05$). Падение уровней АСТ и ЩФ может быть признаком того, что функция печени, возможно, улучшилась. Однако снижение альбумина и повышение глобулина увеличивает вероятность того, что прием добавок с наноселеном может повлиять на метаболизм белков. Результаты исследования показали, что влияние добавок наноселена на метаболизм липидов, ферменты печени, функцию почек и уровень белка у ягнят-самцов зависело от продолжительности приема. В дополнение к возможным улучшениям функции печени, основанным на снижении уровней АСТ и ЩФ и изменениях уровней альбумина и глобулина, исследование также выявило начальное временное повышение уровня триглицеридов, за которым последовало снижение уровня холестерина.

Ключевые слова: липидный обмен, ферменты печени, уровень белка, аспартатаминотрансфераза, щелочная фосфатаза

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