*ejpmr, 2018,5(11), 87-92*

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

# THE IMPACT OF THE DRUG RUTAN ON IMMUNOLOGICAL PARAMETERS IN NAFLD MODEL IN RATS

**Ruzibakieva M. R.*[1], Ariphojaeva F. Z.[2], Aripova T. U.[3], Abdulajanova N.G[4], Batyrbekov A. A.[5], Grigoriants K. E.[6], Ashurova F. K.[7] and Lubentsova O. V.[8]**

[1]Senior Researcher, MD, PhD., Institute of Immunology and Human Genomic AS RUz.
[2]Researcher, Institute of Immunology and Human Genomic AS RUz, Tashkent Pediatric Medical Institute.
[3]Institute of Immunology and Human Genomic AS RUz.
[4]Institute of Bioorganic Chemistry AS RUz.
[5]Prof., Institute of Immunology and Human Genomic AS RUz.
[6,7]Researcher, Institute of Immunology and Human Genomic AS RUz.
[8]Researcher, Tashkent Medical Academy.

***Corresponding Author: Ruzibakieva M. R.**
Senior Researcher, MD, PhD., Institute of Immunology and Human Genomic AS RUz.

**Article Received on 05/09/2018** **Article Revised on 26/09/2018** **Article Accepted on 16/10/2018**

## ABSTRACT

Non-alcoholic fatty liver disease includes is a liver pathologies which range from simple steatosis to non-alcoholic steatohepatitis. Polyphenols are members of a family of plant-derived compounds that can have beneficial effects on human health, and thus their study has become an increasingly important area of human nutrition research. The aim of the present study is to find the role of Rutan on immunological immunological parameters in NASH model in rats. The results reported clearly show that In liver pathology, the number of antibody-forming cells in the spleen, starting from 90 days, is significantly reduced; the total number of cells in the spleen decreases from 120 days. Under the influence of Rutan, the number of ABPC on the 30th day insignificantly increases by 21%, and on the 90-120s the stimulating activity of the drug is lost. The titer of antibodies to sheep erythrocytes in the peripheral blood of rats with liver pathology on the 90th day is significantly reduced by 22%, and on the 90th day - by 30%. Rutan does not have a significant impact on this indicator. In liver pathology, the number of cells in the thymus on the 30th day decreases by 6% ($p > 0.05$), on the 90th day - by 12% ($p > 0.05$), on the 120th day - on 16% ($p > 0.05$). The number of cells in the lymph nodes on the 30th day decreases by 2% ($p > 0.05$), on the 90th day - by 13% ($p > 0.05$), on the 120th day - by 15% ($p > 0.05$). Rutan does not affect the number of cells in the thymus and lymph nodes of rats with liver pathologies. Rutan does not affect the red and white sprout of blood formation in rats with liver pathology.

**KEYWORDS:** Animal model, High-fat diet, Non-alcoholic fatty liver disease, Non-alcoholic steatohepatitis.

## INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is one of the common problems for the Public health all over the world. It is a liver manifestation of metabolic syndrome, and it also may be a cause of liver diseases including cirrhosis and hepatocellular carcinoma (HCC).[1] After inflammatory and fibrotic responses are activated in the liver, NAFLD progresses to non-alcoholic steatohepatitis (NASH).[2] There are a number of studies, showing that there is a theory of a "multiple-parallel-hit" in which unregulated lipid metabolism and insulin resistance are the first hit to the liver, followed by a "second-hit" or "multi-hits" such as oxidative stress, inflammation, apoptosis and fibrosis.[3] The most important question for now is how to prevent NAFLD and for the first, how to decrease an inflammation. As well known, for today, good results in NASH treatment shows extracts of coffee[4], garlic[5,6], green tea[7], milk thistle[8] and resveratrol.[9] Rutan is polyphenol combination The Institute of Bioorganic Chemistry AS RUz has developed drugs with proven antiviral and immunostimulating action. The developed drug "Rutan" is obtained from the ground part of Rhus coriária and is supposed to be used as an antiviral agent. Drugs are low-toxic compounds. The mechanisms of action of Rutana are associated with high interferon-inducing activity and direct virucidal action. However, to date there are no data on the anti-inflammatory effect of these drugs. So we decided to check the immune activity in the model of NAFLD, which we have optimized and described before.[10]

## MATERIALS AND METHODS

The study was conducted on rats. All animals on a high-fat diet in the high-calorie feed was added Rutan at the rate of 25 mg / kg of body weight.

**The study of immunological parameters**
Rats were intraperitoneally immunized with sheep erythrocytes (ShE) at a dose of $5 \times 10^6$.

Rats were killed and the number of antibody-forming cells (AFC) in the spleens was determined by the method of Jerne and Nordin (1963). The number of KLA was calculated on the whole organ and on 106 spleen cells. The total number of nucleated cells of the spleens (NCS) was counted.

The following immunological and hematological parameters were studied:
The number of KLA on the entire spleen (absolute value); 2. The number of KLA per 106 cells of the spleen (relative ratio); 3. The total number of nucleated cells of the spleen (JKSX); 4. The titer of antibodies to ShE in the serum; 5. The total number of cells in the thymus; 6. The total number of cells in the mesenteric lymph nodes; 7. The number of red blood cells in the peripheral blood; 8. The number of leukocytes in the peripheral blood.

Antigen. The sheep erythrocytes (ShE), which were taken from animals from the jugular vein into sterile vials with glass beads, were used as antigen. Before immunization, ShEs were washed 2-3 times in medium 199 for 10 minutes at 1000 rpm. DL in a dose of 2´108 was administered once intraperitoneally.

Complement. A guinea pig weighing 400 g was used as a source of complement. After the decapitation of the guinea pig, serum was obtained, which was stored until the experiment was set at -200 C. Before the experiment, the serum was diluted with saline in a ratio of 1:20.

Isolation of cells from lymphoid organs. After slaughter, spleens, mesenteric lymph nodes and thymus were removed from animals. Thymus, lymph nodes and spleen were cleaned of adipose tissue and homogenized in a glass homogenizer in medium 199. Then the cell suspensions were passed through a three-layer nylon filter.

In all cellular suspensions of the organs of the immune system, the number of nucleated cells in Goryaev's cell was counted and recalculated for the whole organ. In this way, the total number of cells in the central (thymus) and peripheral (lymph nodes, spleen) organs of immunity was determined.

Determination of antibody-producing cells (ABPC) in rat spleen. The number of KLA in the spleen was determined on the 4th day after immunization of animals by the method of local hemolysis in agarose according to N. Jerne and A. Nordin (1963). For the formulation of the reaction, a 0.6% solution of agarose (Serva) was prepared in a single Hanks solution at a temperature of + 49°C. Then, 0.1 ml of a suspension of spleen cells, 0.03 ml of a 20% ShE solution and 1 ml of agarose solution at + 49ºC were poured into plastic Petri dishes for tissue culture (diameter 40 mm). With intensive movements, the mixture of agarose, ShE and spleen cells were evenly distributed over the bottom of the dish and placed for 1.5 hours in a thermostat at +37°C. After that, the cups were removed from the thermostat, poured into them with 1 ml of the guinea pig complement diluted in saline at a ratio of 1:20 and again placed in a thermostat for 1 hour. Then the complement was poured and hemolysis zones ("plaques") were counted on the cups and the number of ABPC was recalculated for the whole spleen and 1 million spleen cells.

Determination of antibody titer to sheep erythrocytes in peripheral blood of rats. 96 well plates were placed in 50 µl saline each. Then, 50 µl of the serum of the immunized rat was placed in the first well, mixed thoroughly and titrated by successive transfer to the next well to the last but one in a row. In the last hole was only physical. solution (control). Then, 50 µl of a 1% solution of ShE was added to all wells. Tablets for 1 hour were placed in a thermostat at + 370C. After that, the antibody titer in the hemagglutination reaction was determined. The antibody titer was taken as the last dilution of serum, which gave a reaction with ShE (the presence of an "umbrella" in the well). The results were expressed in logarithms with base 2 (log2).

The statistical data processing was performed using the Excel 2007 application package.

**RESULTS**
We studied the immunological and hematological parameters in rats with liver pathology at 30, 90 and 120 days after the start of the experiment. To do this, 5 days before slaughter at the appropriate time (30.90 and 120 days), animals were intraperitoneally immunized with sheep erythrocytes (ShE) at a dose of 5×106. Rats were divided into 3 groups of 6 animals. The first group, control, received only ShE. The second group - rats with liver pathology received ShE. The third group - rats with liver pathology received ShE and Rutan.

On the 5th day after immunization, rats were sacrificed and the number of antibody-producing cells (ABPC) in the spleens was determined by the method of Jerne and Nordin (1963). The number of ABPC was calculated on the whole organ and on 106 spleen cells. The total number of nucleated cells of the spleens (NSC) was counted. In the thymus and mesenteric lymph nodes, the total number of nucleated cells was counted. In the peripheral blood of immunized rats, the titer of antibodies to ShE, the number of erythrocytes and leukocytes was determined.

The results of the dynamic studies on the number of ABPC and SNC are presented in Table 1. In the spleens of rats of the control group, 3,683.3×279.8 ABPC are formed. After 30 days in the process of development of steatosis, the number of ABPC in the spleen is not significantly reduced by 17% and amounts to 3150.0 ±

259.2. Under the influence of Rutan, the number of ABPC in the spleen compared with the previous group unreliable increases by 21%. Therefore, up to 30 days, Rutan to a certain extent contributes to an increase in the immune response to the antigenic stimulus (ShE). It should also be noted that there is no significant difference in the number of ABPC per spleen in the three groups compared. The number of SNC in rats with steatosis (30 days) and receiving Rutan slightly decreases, but still does not significantly differ from the control. The number of ABPC per 1 million splenocytes in rats with liver pathology decreases by 13% ($p > 0.05$), while in treated animals it increases by 23% ($p > 0.05$) in the treated animals. However, no significant difference between the 3 groups was found.

**Table 1: Dynamics of changes in immunological parameters during steatosis (30 days), steatohepatitis (90 days) and fibrosis (120 days), in rats treated with Rutan (M ± m).**

| Group | Quantity of SNC × $10^6$ | IR | Quantity of SNC | | | |
|---|---|---|---|---|---|---|
| | | | Spleen | IR | $10^6$ Spleen cells | IR |
| 30 days | | | | | | |
| 1. control (n=6) | 601,3± 48,6 | - | 3683,3 ±279,8 | - | 6,3±0,6 | - |
| 2. experiment (n=6) | 573,2± 37,1 | -1,05 | 3150,0 ±259,2 | -1,17 | 5,6±0,5 | -1,13 |
| 3.patology+Rutan (n=6) | 555,7± 22,8 | -1,08 | 3816,7 ±238,6 | +1,21 | 6,9±0,3 | +1,23 |
| 90 days | | | | | | |
| 1. control (n=6) | 708,3± 63,1 | - | 4766,7 ±292,9 | - | 7,0±0,7 | - |
| 2. experiment (n=6) | 642,1± 58,0 | -1,10 | 3733,3 ±243,1[a] | -1,28 | 6,0±0,6 | -1,17 |
| 3.patology +Rutan (n=6) | 646,5± 58,1 | +1,01 | 3833,3 ±243,1[a] | +1,03 | 6,1±0,6 | +1,02 |
| 120 days | | | | | | |
| 1. control (n=6) | 782,7± 34,8 | - | 5816,7 ±230,1 | - | 7,5±0,5 | - |
| 2. experiment (n=6) | 674,1± 30,0[a] | -1,16 | 4583,3 ±186,9[a] | -1,27 | 6,9±0,4 | -1,09 |
| 3.patyology +Rutan (n=6) | 680,4± 30,6 | +1,01 | 4683,3 ±186,9[a] | +1,02 | 7,0±0,4 | +1,01 |

Note: ABPC is the antibody-forming cells, NCS is the nucleated cells of the spleen, IR is the ratio to the control, and reliably to the control ($p < 0.05$), in brackets the number of rats.

On the 90th day, when steatohepatitis was formed in animals, the number of ABPC per spleen in control rats was 4,766.7 ± 292.9, and in experimental animals it decreased significantly by 28% (3,733.3 ± 243.1 ABPC). In rats that received Rutan for up to 90 days, the number of ABPC on the spleen does not change (3833.3 ± 243.1). Consequently, at the stage of steatohepatitis, immunological reactivity is significantly inhibited, but Rutan does not have a biological effect on the immune status. Significant changes in the index of SNC and the number of ABPC per 1 million between controls, untreated and treated rats for 90 days were not detected. Consequently, after 3 months of the formation of the pathology of the liver, the immune response to ShE is significantly weakened, and Rutan is not able to stimulate the immunological reactions of the rat organism.

On the 120th day (stage of fibrosis), as well as on the 90th day, there is a secondary immunodeficiency, as evidenced by the inhibition of the formation of ABPC in the spleen of rats. If in the control the number of ABPC is 5816.7 ± 230.1, then in experimental animals their number is 27% less (4583.3 ± 186.9 ABPC). Rutan only a 2% increase in the number of AFC in the spleen of rats immunized with ShE.

On the 120th day (stage of fibrosis), as well as on the 90th day, there is a secondary immunodeficiency, as evidenced by the inhibition of the formation of ABPC in the spleen of rats. If in the control the number of ABPC is 5816.7 ± 230.1, then in experimental animals their number is 27% less (4583.3 ± 186.9). Rutan only a 2% increase in the number of ABPC in the spleen of rats immunized with ShE.

In contrast to the previous observation periods (30 and 90 days), the number of NCS in rats with liver pathology was significantly reduced by 16% (control - 782.7 ± 34.8×$10^6$, experience - 674.1 ± 30.0×$10^6$). In the group of rats treated with Rutan, the number of NCS does not change. Significant changes in the number of ABPC per 1 million cells of the spleen was not detected.

In general, the following conclusion can be made. On the 30th day (steatosis) no significant changes in the immune

status occur, on the 90th (steatohepatitis) and 120th day (fibrosis) the rats' immune reactivity reliably weakens almost an 30%, but Rutan does not significantly affect the immunological reactivity.

Next, we conducted dynamic studies to determine the titer of antibodies to ShE in the peripheral blood of rats with liver pathologies who received Rutan (Table 2). In general, similar results were obtained as in the above-described studies for calculating the number of ABPC in the spleen of immunized rats. On the 30th day in experienced mice, the titer of antibodies to ShE in the blood decreases unreliable by 12%, and under the influence of Rutan, this indicator rises somewhat ($p > 0.05$).

**Table 2: Dynamics of antibody titer to erythrocytes of ram in serum with steatosis (30 days), steatohepatitis (90 days) and fibrosis (120 days) in rats treated with Rutan (M ± m).**

| Group | Antibody titer ($log_2$) | IR |
|---|---|---|
| 30 days | | |
| 1. control (n=6) | 4,7 ± 0,2 | - |
| 2. experiment (n=6) | 4,2 ± 0,2 | -1,12 |
| 3.patology+Rutan (n=6) | 4,5 ± 0,2 | +1,07 |
| 90 days | | |
| 1. . control (n=6) | 4,5 ± 0,2 | - |
| 2. experiment (n=6) | 3,7 ± 0,2[a] | -1,22 |
| 3. patology+Rutan (n=6) | 3,8 ± 0,2[a] | +1,03 |
| 120 days | | |
| 1. control (n=6) | 5,2 ± 0,3 | - |
| 2. experiment (n=6) | 4,0 ± 0,3[a] | -1,30 |
| 3.patology+Rutan (n=6) | 4,2 ± 0,3[a] | +1,05 |

Note: IR is the ratio index to the control, and reliably to the control ($p < 0.05$), in brackets the number of rats.

However, for 90 days the titer of antibodies to ShE in the blood significantly decreases by 22%, and on the 120th day, even more - by 30%. Rutan does not cause any changes in the titer of antibodies to ShE in the peripheral blood of immunized rats.

Further, dynamic studies were conducted to study the total number of cells in the thymus and lymph nodes of rats with liver pathology who received the immunomodulating drug Rutan (Table 3). On the 30th day, the number of cells in the thymus decreases by 6% ($p > 0.05$), on the 90th day - by 12% ($p > 0.05$), and on the 120th day - by 16% ($p > 0.05$). In the rats treated with Rutan, on the 30th day the number of thymocytes unreliable increased by 20%, and on the 90-120th day the stimulating effect of Rutan is lost.

**Table 3: The dynamics of changes in the number of cells in the thymus and lymph nodes in steatosis (30 days), steatohepatitis (90 days) and fibrosis (120 days) in rats receiving Rutan (M ± m).**

| Group | Thymus cells $\times 10^6$ | IR | Lymph node cells $\times 10^6$ | IR |
|---|---|---|---|---|
| 30 days | | | | |
| 1. control (n=6) | 331,7±30,5 | - | 97,5±8,4 | - |
| 2. experiment (n=6) | 312,6±27,2 | -1,06 | 93,9±5,9 | -1,02 |
| 3.patology+Rutan (n=6) | 374,6±32,7 | +1,20 | 107,5±6,8 | +1,04 |
| 90 days | | | | |
| 1. control (n=6) | 322,6±30,5 | - | 122,8±8,6 | - |
| 2. experiment (n=6) | 289,2±27,2 | -1,12 | 108,5±6,4 | -1,13 |
| 3.patology+Rutan (n=6) | 291,3±22,3 | +1,01 | 111,9±6,0 | +1,03 |
| 120 days | | | | |
| 1. control (n=6) | 340,7±17,1 | - | 138,4±8,6 | - |
| 2. experiment (n=6) | 293,3±14,7 | -1,16 | 120,3±7,5 | -1,15 |
| 3.patology+Rutan (n=6) | 298,0±15,6 | +1,02 | 122,4±7,9 | +1,02 |

Note: IR is a ratio to control, * - reliably to control, in brackets - the number of rats.

Analysis of the number of cells in the lymph nodes gave the following results. The number of cells in the lymph nodes during liver pathology on the 30th day does not change, on the 90th day it decreases by 13% ($p > 0.05$), and on the 120th day decreases by 15% ($p > 0.05$). Rutan has no effect on the number of cells in the lymph nodes of rats with liver pathology.

Thus, there were no significant changes in the number of cells in the central immunity organ - thymus and

peripheral immunity organs - lymph nodes in rats with liver pathology who received Rutan, were not detected.

In the next series, some hematological parameters were studied in rats with liver pathologies who received Rutan (Table 4).

**Table 4: Dynamics of changes in the number of erythrocytes and leukocytes in the blood during steatosis (30 days), steatohepatitis (90 days) and fibrosis (120 days) in rats receiving Rutan (M ± m).**

| Group | Red blood cells $\times 10^9$/мл | IR | White blood cells $\times 10^6$/мл | IR |
|---|---|---|---|---|
| 30 days | | | | |
| 1. control (n=6) | 7,2±0,8 | - | 9,4±0,4 | - |
| 2. experiment (n=6) | 7,0±0,7 | -1,03 | 9,2±0,3 | -1,02 |
| 3.patology+Rutan (n=6) | 7,4±0,6 | +1,06 | 9,6±0,2 | +1,04 |
| 90 days | | | | |
| 1. control (n=6) | 6,7±0,6 | - | 10,3±0,5 | - |
| 2. experiment (n=6) | 6,5±0,6 | -1,03 | 10,7±0,4 | +1,04 |
| 3.patology+Rutan (n=6) | 6,6±0,6 | +1,02 | 11,1±0,5 | +1,08 |
| 120 days | | | | |
| 1. control (n=6) | 6,8±0,6 | - | 11,1±0,3 | - |
| 2. experiment (n=6) | 6,3±0,5 | -1,08 | 10,6±0,2 | -1,05 |
| 3.patology+Rutan (n=6) | 6,5±0,5 | +1,03 | 10,8±0,2 | +1,02 |

Note: IR is the ratio index to control; in brackets is the number of rats

For all study periods (30th, 90th, and 120th days), no significant changes in the peripheral red blood cells of rats with liver pathology were found. Consequently, the red sprout of blood formation practically does not suffer in the studied pathology of the liver. Similar data were obtained when studying the number of leukocytes in the peripheral blood of rats.

Based on the research, the following conclusions were made:

1. In liver pathology, the number of antibody-forming cells in the spleen, starting from 90 days, is significantly reduced; the total number of cells in the spleen decreases from 120 days.
2. Under the influence of Rutan, the number of ABPC on the 30th day insignificantly increases by 21%, and on the 90-120s the stimulating activity of the drug is lost.
3. The titer of antibodies to sheep erythrocytes in the peripheral blood of rats with liver pathology on the 90th day is significantly reduced by 22%, and on the 90th day - by 30%. Rutan does not have a significant impact on this indicator.
4. In liver pathology, the number of cells in the thymus on the 30th day decreases by 6% ($p > 0.05$), on the 90th day - by 12% ($p > 0.05$), on the 120th day - on 16% ($p > 0.05$). The number of cells in the lymph nodes on the 30th day decreases by 2% ($p > 0.05$), on the 90th day - by 13% ($p > 0.05$), on the 120th day - by 15% ($p > 0.05$). Rutan does not affect the number of cells in the thymus and lymph nodes of rats with liver pathologies.
5. Rutan does not affect the red and white sprout of blood formation in rats with liver pathology.

**REFERENCES**


1. Browning J. D. *et al.* Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. Hepatology, 2004; 40: 1387–1395.
2. Michelotti G. A., Machado M. V. & Diehl A. M. NAFLD, NASH and liver cancer. Nat. Rev. Gastroenterol. Hepatol, 2013; 10: 656–665.
3. Xiao J., Guo R., Fung M. L., Liong E. C. & Tipoe G. L. Therapeutic approaches to non-alcoholic fatty liver disease: past achievements and future challenges. Hepatobiliary Pancreat. Dis. Int., 2013; 12: 125–135.
4. Vitaglione P. *et al.* Coffee reduces liver damage in a rat model of steatohepatitis: the underlying mechanisms and the role of polyphenols and melanoidins. Hepatology, 2010; 52: 1652–1661.
5. Xiao J. *et al.* Garlic-derived S-allylmercaptocysteine is a hepato-protective agent in non-alcoholic fatty liver disease in vivo animal model. Eur. J. Nutr., 2013; 52: 179–191.
6. Xiao J. *et al.* Garlic-Derived S-Allylmercaptocysteine Ameliorates Nonalcoholic Fatty Liver Disease in a Rat Model through Inhibition of Apoptosis and Enhancing Autophagy. Evid. Based Complement. Alternat. Med., 2013; 2013: 642920.
7. Xiao J. *et al.* Epigallocatechin gallate attenuates fibrosis, oxidative stress, and inflammation in non-alcoholic fatty liver disease rat model through TGF/SMAD, PI3 K/Akt/FoxO1, and NF-kappa B pathways. Eur. J. Nutr. 10.1007/s00394-013-0516-8 (2013).

8. Cacciapuoti F., Scognamiglio A., Palumbo R., Forte R. & Cacciapuoti F. Silymarin in non alcoholic fatty liver disease. World J. Hepatol, 2013; 5: 109–113.
9. Xin P. *et al.* Alleviative effects of resveratrol on nonalcoholic fatty liver disease are associated with up regulation of hepatic low density lipoprotein receptor and scavenger receptor class B type I gene expressions in rats. Food Chem. Toxicol, 2013; 52: 12–18.
10. Ruzibakieva M. R., Ariphojaeva F.Z., Aripova T.U., Batyrbekov A.A., Grigoriants K.E., Ashurova F.K., Sharakhmedova M., Lubentsova O.V. The study of hystological parameters in the development of non-alcoholic fatty liver disease in rats. European journal of pharmaceutical and medical research, 2018; 5(8): 172-175.