World Academy of Science, Engineering and Technology
International Journal of Medical and Health Sciences
Vol:7, No:8, 2013

Pre-Clinical Studying of Antitumor Ramon Preparation: Chronic Toxicity

Raissa A. Muzychkina, Irina M. Korulkina, and Dmitriy Yu. Korulkin

***Abstract*—**In article the data of chronic toxicity for pre-clinical researches of Ramon preparation is described. Ramon effects to hormone system and gastrointestinal tract; local irritative effect, allergic, pyrogenic properties and reaction to the immune system were studied.

***Keyword*—**Cancer, toxicity, antitumor activity, pre-clinical testing, anthraquinones, phytopreparation, Ramon.

I. Introduction

CANCER is one of the most common devastating disease affecting millions of people per year. Cancer has been estimated as the second leading cause of death in humans. So there has been an intense search on various biological sources to develop a novel anti-cancer drug to combat this disease. Plants have proved to be an important natural source of anti-cancer therapy for several years. More than 70 plant derived compounds have been isolated so far and are currently under clinical trials. These anti-cancer compounds have been found to be clinically active against various types of cancer cells.

Among high-performance low-toxicity drugs, especially, preparations of selective action, an important place is occupied by the derivatives of anthraquinone. This is well proven in the literature on biological activity of anthracene-containing plants, natural anthraquinones, their synthetic analogues and phytopreparations, and it should be noted that synthetic analogues have wider spectrum of biological activity [1].

II. Results and Discussion

Study of Ramon chronic toxicity on being intraperitoneally injected was carried out with 290 rats-females and 300 rats-males. Ramon solution in water and 5%glucose was being injected daily for 10 days in the single doses 40, 45, 50mg/kg. The preparation in doses not exceeding maximal tolerable ones (40mg/kg if dissolved in water and 45mg/kg in glucose) didn't produce apparent toxic effect against intact animals as at the moment of injection as 30 days after. Calculation of indexes of peripheral blood taken from rats tail vein (number of erythrocytes, leukocytes, hemoglobin, leukocyte formulae) and general number of myelocariocytes in marrow of femoral bone (integral index of marrow hemogenesis) [2] brought out Ramon not to induce depression of blood and marrow hemogenesis. Number of nucleus-containing marrow cells remained normal. Absence of inhibiting effect towards lymphoid system and megacariocytes as well doesn't suppress thrombocytes formation, doesn't influence erythroid system, reticulocytosis and their intensive maturation to matured erythrocytes.

Effect produced by 2% water solution of Ramon when being injected daily for 10 days in doses 40 and 50mg/kg towards peripheral blood indexes was studied during therapeutical trials with animals having transplantated tumors (Pliss lymphosarcoma, Herene carcinoma). Blood was taken from the tail vein. Ramon appeared not to induce either oppressive effect to morphological composition of blood or substantial changes of spleen weigh coefficient of treated animals in comparison with control. Ramon intraperitoneal application in intolerable doses (50 mg/kg) induced side effect over intact rats and rats with transplanted tumors. Agitation expressed as anxiety, breathing acceleration appeared after 3 to 4 injections. After 5 to 6 ones it turned to oppression, strongly disheveled fleece with yellowish shade. Rats ruin in status of total depression happened after 4 to 7 injections. One could observe ascite agglomeration in abdominal cavity (5 to 20ml to the end of trial) and grayish-white coating of viscera. Rats when treated by Ramon tended to weight growth retarding (10%). 40 to 60% of animals died during intraperitoneal injection of Ramon and 10 days after it. The preparation on being dissolved in 5% glucose solution induced less ruin of rats (10 to 15%).

Blood of rats with Pliss lymphosarcoma after treatment with Ramon was analyzed concerning hormone factors. The course of treatment leads to lowering of sex hormones concentration (FSG and testosterone) and to the growth of the cortizole one (cortizole regulates immunic and flammatory cells activity) (see Table I).

TABLE I
Influence of Rats PlissLymphosarcoma Treatment by Ramon to Concentration of FSG, Testosterone and Cortizole in Blood

Reaction	Concentration of steroid hormones, mg/ml		Concentration of FSG in hypophysis	Tumor growth retarding
	cortizole	testosterone		
control	15.7±2.1	0.13±0.01	4.2±0.3	-
Ramon	19.3±1.8	0.02±0.01	2.8±0.1	80.6 to 94.3%

Raissa A. Muzychkina is with the Department Chemistry and Chemical Technology, al-Farabi Kazakh National University, Almaty, CO 050040 Kazakhstan (corresponding author to provide phone: 727-387-1751; fax: 727-292-3731; e-mail: rmuz@mail.ru).

Irina M. Korulkina is with the Center for Physical-Chemical Methods of Research and Analysis, al-Farabi Kazakh National University, Almaty, CO 050040 Kazakhstan (e-mail: i.r.k.a.k.i.m@list.ru).

Dmitriy Yu. Korulkin is with the Department Chemistry and Chemical Technology, al-Farabi Kazakh National University, Almaty, CO 050040 Kazakhstan (e-mail: Dmitriy.Korulkin@kaznu.kz).

Effect of Ramon on being variously injected to dogs', mice's, rats' guinea pigs' and rabbits' gastrointestinal tract were studied. Dogs: Ramon-substance was used perorally with food daily for 30 days in the dose 150mg/kg (the overall dose 4500mg/kg) on an empty stomach (10% water solution) and 180mg/kg (10% solution in 5% glucose). No substantial toxic effect was observed during reception itself and 45 days after. Viscera of dogs being slaughtered at the end of trial (2 males and 2 females) were without variations. Depressive influence to peripheral blood morphological composition was absent.

General condition of dogs was observed for 40 days Ramon being perorally used with food daily for 10 days in the dose 300mg/kg. Peripheral blood was also analyzed therewith. Noticeable toxic effect to the dog organism was not brought out. After the trial animals (males) pathologo-anatomically studied were slaughtered [3]. Substantial changes viewed from white and red blood were not displayed elements of peripheral blood being accounted. Biochemical analysis of blood serum exhibited slight lowering of general proteins and albumins to the end of trial but within confident bounds.

Mouses: water solution of Ramon was perorally injected daily for 28 days in doses 250, 500 and 750mg/kg. The autopsy brought out no pathological changes in gastrointestinal tract. Intestinal and stomach mucous membranes on being histologically studied demonstrated no deviations.

Rats: water solution of Ramon was perorally injected daily for 30 days in the dose 100mg/kg. The autopsy brought out no pathological changes in gastrointestinal tract. Intestinal and stomach mucous membranes on being histologically studied demonstrated no deviations.

Guinea pigs: water solution of Ramon was perorally injected daily for 10 days in doses 150 and 300mg/kg. The autopsy brought out no pathological changes in gastrointestinal tract. Intestinal and stomach mucous membranes on being histologically studied demonstrated no deviations.

Rabbits: Ramon water solution was injected subcutaneously and intramuscularly daily for 10 days in the dose 40mg/kg. Histological study of intestinal and stomach mucous membranes brought out no deviations. Perorally Ramon was used daily for 30 days in the dose 100mg/kg. The autopsy revealed viscera not to be changed both for experimental and control animals. Histological study confirmed the fact.

Ramon effect to isolated rabbit stomach was studied according to Manguse [4]. Slight concentrations resulted in smooth spasm of musculature therewith 2 to 3 minutes after injection the spasm discontinued and amplitude of penduliform reductions increased. High concentrations (5%) provoked intestinal motor system retarding due to smooth musculature weakening. 20 trials were carried out.

Pathomorphological changes in viscera of mice, rats, rabbits and dogs were studied at the moment and sometime after single and repeated injections of Ramon-lyo [2].

Mouses and rats on being injected singly intraperitoneally with Ramon in intolerable doses died in depressive status in 1 to 5 days. Autopsy demonstrated blood vessels of liver, spleen, heart; peritoneum brimmed over with blood, succulent lungs. Viscera of mice and rats on being histologically studied vessels blood plethora, hemorrhage and edema of tissues. Visible dystrophy of liver cells epithelium, covering epithelium and stomach flooring glands epithelium was observed in kidneys [5]-[6]. Destructive and necrobiotic changes were also noticed in liver so as defradmentation of muscular fibres in heart.

Macroscopic study of rats died from intolerable doses injected intraperitoneally repeatedly brought out plethora of liver and spleen, marked hyperemia of peritoneum blood vessels, tympanism of intestine. Therewith-histological study of viscera brought out dystrophy of hepatic balks, local hepatic hemorrhage. Dystrophy of epithelium and locally epithelium tympanism were marked in twisted renal tubules [5], [6]. Lungs demonstrated break of interalveolaric barriers, perivascular inflammatory infiltration, vessels blood plethora and local hemorrhage. Moderative dystrophy of covering and flooring glands epithelium with inflammatory effect was viewed from the side of gastrointestinal tract.

Macroscopic study of dog viscera animals being repeatedly treated with Ramon perorally in low intolerable dose hasn't brought out visible pathological changes [6]. Microscopic study showed suppression of red and white spleen pulp, marked sclerosis of vessels walls and wide hemorrhage into spleen tissues. In liver: discomplexed balks, hepatocytes with sharply marked dystrophy up to holonuclear cells emergence, moderative plethora of dilated blood vessels. In kidneys: moderative dystrophy of straight and twisted tubules epithelium, uniformly dilated lumens of tubules, moderatively marked capillary glomerulus. There was also observed hyperplasia of intestine epithelium cells with moderatively rebuilt submucous layer.

Morphological study of rats' viscera and bladder animals being singly and repeatedly treated intravesically with Ramon-lyo in tolerable doses hasn't brought out substantial pathological changes. Application of 10% water solution induced slight edema of urine bladder and insignificant hardening of mucous epithelium. The facts were not elicited 5% glucose being used as solvent. Ramon in intolerable doses provoked erosive inflammation of bladder cervix mucous with groups of dilated capillars just under erosive regions of subepithelium layer and slight infiltration of lymphoplasmacytes impured with small amounts of leucocytes and fibroblasts. Pathological changes were not found in back wall of urine bladder. When Ramon was injected intravesically in toxic doses histological study elicited necrotic changes: sclerosis of all cells, local break of blood vessels, sharp edema of urine bladder. In other organs pathology wasn't brought out.

Autopsy of rabbits being singly instillated into bladder with 10% water solution of Ramon in tolerable doses hasn't exhibited any deviations as viewed from bladder as from viscera. Histological study confirmed the fact.

10% solution of Ramon in water and 5% glucose when singly injected to dogs (males and females) in tolerable doses

hasn't induced any pathological changes according to anatomical and histological study data.

Chronic experiment with rabbits (males and females) being injected with 6% water solution of Ramon for 30 days in tolerable doses (30mg/kg) according to data of females autopsy resulted in: rough and irregularly hyperemired mucosa of bladder, upper wall of uterus swollen with moderatively hyperemired mucosa, no visible changes in kidneys, uterine appendages and adrenal glands. Histologically there were elicited plethora of bladder blood vessels, edema of mucosa and submucosa layers, plethora of uterus blood vessels with mucosa preserved, kidneys and adrenal glands non-changed, plethora and rough folding in ureter [5], [6]. Autopsy of males exhibited situation with bladder identical to females' one, absence of visible changes in testicles (this was showed histologically as well), edema of mucosa epithelium cells and plethora of blood vessels in bladder. Other organs demonstrated absence of pathological changes. Morphological picture being studied in distant time stayed eternal.

Treatment of rabbits (males and females) with 30-multiple intolerable dose (50mg/kg, 10% water solution of Ramon intravesically) according to autopsy data resulted in: bladder rough folding, rough surface of mucosa with lucid pinkish secretion, sharp hyperemia of mucosa, local dystrophic changes in kidneys, absence of visible changes in adrenal glands, hyperemia of ureter mucosa with rough folding surface, hyperemia and swelling of uterus upper wall mucosa. Testicles of males were hardened with no other visible changes [7]. Back wall of urine bladder when histologically studied demonstrated hyperplasia of epithelium cells, degeneration of villus, rebuilding of submucosa, edema of blood vessels and plethora, hardening of uterus back side mucosa with rebuilding of submucosa layer, relevant tissue infiltration and plethora, desquamation of ureter mucosa covering epithelium with loose state, edema and plethora of submucosa, dystrophic changes in male testicles, absence of pathological changes in adrenal glands and viscera. All mentioned changes tended to normalize 30 days after injection of Ramon in intolerable dose.

Dogs being treated with Ramon intravesically in tolerable doses (including MTD) during chronic trials were slaughtered by injection of sodium thiopental intrapleurilly. Autopsy of two control and six experimental dogs didn't exhibited any macroscopically visible pathology. Liver, kidneys, myocardium, lungs, spleen, bladder and other organs were histologically studied.

Urine bladder: pathology wasn't elicited in cuts of wall of two control and the first, the third, the forth and the sixth dogs therewith two pieces of bladder wall as from cervix as from the low wall were studied. The second and the fifth experimental dogs demonstrated slight mucosa erosion in bladder preparations. Concentration of dilated capillars just under erosive zones in subepithellium layer and moderative lymphoplasmocyte infiltration with small amount of leucocytes and fibroblasts has been brought out. At the same time any pathology was elicited in pieces of bladder wall taken from its back wall.

All other investigated organs demonstrated no pathology as in experimental as in control groups.

So, 10% solution of Ramon in water and 5% glucose on being repeatedly intravesically injected in tolerable doses hasn't induced any macroscopically changes viewed from viscera of dogs. Some erosion mentioned above seems to result from mechanical irritation (daily catheterization of bladder for 30 days).

Autopsy of slaughtered animals (mouses, rats) treated by Ramonintraperitoneally in intolerable doses brought out polyhemia of viscera (liver, spleen, heart, etc.), edema of the lungs, hyperemia of peritoneum blood vessels and intestine tympanism. Histological study found out that there were destructive and dystrophic changes in liver, spleen, kidneys, tissues that resulted in the toxic effect.

Autopsy of rats, rabbits and dogs being singly and repeatedly treated by 6, 10 and 15% water solutions of Ramon in intolerable doses elicited sharp edema of urine bladder, erosive inflammation of its cervix mucosa. Necrotic changes as epithelium hyperplasia, vasodilatation, local dystrophic changes and hyperemia in kidneys were elicited histologically. Other organs appeared to have no substantial pathology.

10% solution of Ramon in water and 5% glucose being repeatedly intravesically injected to dogs in highly tolerable doses hasn't induced any micro- or macroscopic changes in viscera. Slight erosion of bladder cervix mucosa was probably due to mechanical irritation by daily catheterization for 30 days. Urine bladder and viscera of rats and dogs being intravesically treated by Ramon-lyo in tolerable doses appeared to have no pathological changes according to data of histological study.

It is necessary to provide the control of functional status of kidneys and urinary tract when fulfilling clinical trials of Ramon-lyo.

Local irritative effect of Ramon was studied with 130 white mongrel rats (subcutaneous, intraperitoneal, intramuscular, per os and intravesical injections), 22 rabbits-Chinchilla (intravesical injection and instillation into eye conjunctival pouch), 16 mongrel dogs (intravesical and per os injections) [2]. Pieces of tissues were taken in points of injection and urine bladder to be morphologically studied 2 to 4 days after single injection and 1 to 3 days after the course of injections and then repeatedly 10-15-30 days after for clarification of process development. Subcutaneous and intramuscular recurrent injections of 5% and 10% water solutions of Ramon in the dose 45mg/kg to rats resulted in necrosis of subcutaneous cellular tissue and muscles. Being singly injected Ramon induced slight edema, which let up 8 to 10 days after.

Peroral application of 5, 10 and 15% water solutions of Ramon in chronic experiments with rats in doses 150 and 200 mg/kg hasn't result in irritation of gastrointestinal tract mucosa. Recurrent intraperitoneal injections of 5 and 10%water solutions in tolerable (40 and 45mg/kg) and intolerable (50mg/kg) doses induced intensive irritation of peritoneum mucosa with secondary effects (see other chapters). 5 to 20ml of ascite was accumulated in peritoneum

of 20 to 70% of rats (depending on the dose) to the end of trial. Peritoneum organs appeared to be covered with grayish-white coating, conglomerate has been formed. 2 and 1% solutions were less irritative to peritoneum mucosa of rats.

5 and 10% water solutions of Ramon were being instillated into rabbit's eye conjunctive pouch daily for 10 days. Irritative effect was observed neither 4 hours after the first procedure nor after the last one and during the following lookout.

Single injection of 10% water solution of Ramon into urine bladder of animals (as males as females) in doses: 250mg/kg (to rats), 1000 and 2000mg/kg (to rabbits and dogs). Morphological study of bladder hasn't elicited any pathological changes. Injection of 10% solution of Ramon to rats in intolerable doses (500 and 1000mg/kg) resulted in hardening (elicited by palpation) in bladder zone. Autopsy brought out strong vasodilatation in bladder walls. 15% water solution induced edema of bladder walls let up in 7-10 days. Morphological study hasn't elicited any pathological changes in walls of rats' bladder.

Recurrent injections of 10% water solution of Ramon into urine bladder of animals. 3 to 4 intravesical injections of Ramon to rats in intolerable doses (200mg/kg induced 50% loss, 300mg/kg–60% loss) resulted as in edema and hardening of external sexual organ of males and urethra of females as in dysuria. It embarrassed intravesical manipulations during the injection. Erosive changes and hardening in cervix mucosa and walls of bladder, injection of its walls blood vessels were elicited for lost and alive rats 10 and 30 days after injection completion. 10% water solution of Ramon in tolerable dose (50mg/kg) was intravesically infused to rats daily for 10 days with following lookout 30 and 45 days after the coarse completion. Morphological study of bladder elicited no pathological changes.

10% water solution of Ramon was intravesically infused to dogs in tolerable dogs (200mg/kg) for 30 days and 6 and 10% water solutions – to rabbits in tolerable dose 20mg/kg for 30 days, the coarse being controlled for 30 days after its completion. Materials for histological study were taken for the third and the thirtieth days after the coarse completion. Two dogs (male and female) from the control group and six (3 males and 3 females) from the experimental one, 3 rabbits (1 female and 2 males) from the control group and 6 (3 males and 3 females) from the experimental one were slaughtered. Two dogs from the control group and 4 from the experimental one demonstrated no pathological changes in shears from the bladder wall, therewith two its pieces were studied (taken from cervix zone and from the nether wall). Two other dogs exhibited slight mucosa erosion in the preparations of bladder cervix. Sometimes it was shallow, sometimes larger-scaled but in every case in visible ranges when magnification was 1x80. Groups of dilated capillars, moderative lymphoplasmocytic infiltration with small amount of leucocytes and fibroblasts could be observed just under erosive zones in subepithellium layer. Pathology was not found in the preparations of bladder back wall of the same dogs. Such changes were absent 45 days after the coarse completion. Ramon being chronically applied in 15% water solution induced more erosion of bladder mucosa. Histological picture of rabbit's bladder after treatment by Ramon was described above.

So, 10% water solution of Ramon on being intravesically infused to dogs in the dose 200mg/kg and to rabbits in the dose 20mg/kg hasn't induced micro- and macroscopically pathological changes viewed from bladder of animals. Slight erosions elicited in bladder cervix mucosa were probably due to mechanical irritation (daily catheterization of bladder for 30 days). Rabbits appeared to be more sensitive to Ramon infused into bladder.

Thus, 10% water solution of Ramon for instillations into bladder may be considered to be acceptable for clinical trials.

Allergic effect of Ramon was studied with 45 guinea pigs [6]. Cattle serum was used for sensitization and resolution. Sensitization was carried out by subcutaneous injection of 25 ml serum before and after injection of Ramon. Resolutary dose of serum was subcutaneously injected 14 days after sensitization in volume 0.5ml. 10% water solution of Ramon was infused to animals intraperitoneally singly or daily for 10 days.

Index of animals' loss and anaphylaxis (according to 4-ball system) was regarded as criterion of guinea pigs reaction to recurrent injections of the alien protein.

Two trials were carried out. The dose of serum in the first one was 0.5ml. Animals of the first group were infused with sodium chloride isotonic solution for 10 days, animals of the second one were sensitized with 0.5ml of serum subcutaneously, of the third one – with single dose of Ramon (45mg/kg) daily for 10 days before sensitization with serum and of the forth one – with the same coarse of Ramon injections but after sensitization.

0.25ml of serum was sensitizing dose and 0.5 n8 – dose for resolution in the second trial. Animals of the first group were sensitized by serum (this was concerned as positive control). Animals of the second group were treated daily for 10 days with single dose 25mg/kg before sensitization, the third group – with the same course of Ramon but after sensitization, the forth group – with single dose of 250mg/kg Ramon before sensitization, the fifth group – with the same dose but after sensitization[see Table II].

TABLE II
SCHEME OF TRIALS SETTING AND RESULTS OF RAMON ALLERGIC EFFECT STUDY

Groups number	Reactions type and sequence x number of injections	Response	Exodus, lost/ altogether
	The first trial		
1	Isotonic sodium chloride solution x 10	0	0/6
2	Cattle serum (CS)	+++	2/6
3	Ramon, 45 mg/kg x 10 + CS	0	0/6
4	CS + Ramon, 45 mg/kg x 10	+++	3/6
	The second trial		
1	CS	++	1/6
2	Ramon, 25 mg/kg x 10 + CS	+	0/6
3	CS + Ramon, 25 mg/kg x 10	++	0/6
4	Ramon, 250 mg/kg x 1 + CS	+	0/6
5	CS + Ramon, 250 mg/kg x 1	++	0/6

Ramon appears not to induce allergic effect i.e. not to enhance anaphylaxis in case when animals are treated as singly as recurrently after sensitization with serum.

Pyrogenic properties of Ramon were studied in experiments with 8 rabbits (4 males and 4 females) in accordance with Instructions State Pharmacopoeia of Republic of Kazakhstan. Medicinal form of Ramon has been shown not to possess pyrogenic properties.

Reaction of Ramon to immunogenesis was studied by modeling of the immune answer of intact rats and rats of line "August" with transplantated Plisslymphosarcoma to erythrocytes of sheep [2]. The maximal tolerable dose of the preparation (50mg/kg) on being injected simultaneously with antigen (sheep erythrocytes) hasn't produce certain effect to synthesis of specific antibodies bur has substantially diminished amount of peripheral blood lymphocytes which identified functional-metabolic activity of neutrophylic leucocytes (according to NST-test) in the reaction of spontaneous E-rosette formation with native erythrocytes of guinea pigs. Middle therapeutic doses (40mg/kg) have produced less immunodepressive effect.

Middle therapeutic doses of Ramon didn't influence intensity of immunologic reconstruction of organism in the case of experimental animals with transplantated Plisslymphosarcoma in 5, 10 and 15 days. Experimental data were 23.2±0.5; 24.0±0.3; 25.2±0.5 correspondingly in comparison with control 21.0±0.3; 20.0±0.9; 22.2±0.8 excluding slight diminishing of macrophage function on the fifteenth day of treatment (10.5±1.15 in experimental group and 13.2±0.38 in control one according to NST-test).

At the same time rats with Plisslymphosarcoma when treated by maximal tolerable doses of Ramon demonstrated substantial growth of ability to induce immunologic reconstruction. This appeared higher titers of antibodies in comparison with control group of animals (not treated by Ramon): log 2.97±0.07 and 2.3±0.1 correspondingly. Moreover indexes of circulating T-lymphocytes and functional activity of phagocytes in all time of the experiment were higher (24.8±7.5; 25.2±4.1; 24.3±1.3 correspondingly on the fifth, tenth and fifteenth day in comparison with control data 20.1±2.4; 17.2±1.4; 18.3±2.1).

So, Ramon has variously affected immunogenesis and factors of non-specific resistance intact and Plisslymphosarcoma transplantated rats' organisms. But in the last case it doesn't produce noticeable immunodepressive effect and even stimulates immunogenesis to T-dependent antigen. This fact demonstrates perspective of Ramon application in combined chemotherapy of tumors.

III. Experimental

Chronic toxicity of Ramon-substance was studied in the experiments with 6 mongrel dogs (3 males and 3 females) with carotid moved out to cutaneous patch. The preparation was perorally injected with food to 3 dogs for 60 days in the dose 67mg/kg. Coarse dose amounted 4020 mg/kg. General status, behavior and survival were observed [4].

Behavior of dogs hasn't changed: as during treatment as 2 months after they demonstrated aggression and good general status. Weight growth ran to 11%.

Arterial tension and breathing. Arterial tension was measured by tonometer in the left carotid (moved to cutaneous patch) before injection and 15, 30, 45, 60 and 90 days after it. There were no substantial changes in the arterial tension in comparison with starting data. Electrocardiogram was also put down during the trials. There were no sure changes as in its characteristics for dogs being treated by Ramon in comparison with starting data as viewed from dogs breathing [5].

Peripheral blood. There were no substantial deviations in morphological blood formulae. As viewed from white blood there was observed slight lowering of leucocytes amount within the limits of its physiological changes. Neutrophyle number prevailed in the leukocyte blood formulae at the expense of lymphocytes decrease [6]-[7].

Biochemical analysis of serum blood showed general protein level not to change in comparison with starting data and analogous indicator for control dogs. As for protein fractions some growth of albumins and lowering of globulins were observed. Protein fractions of treated animals increased to the end of experiment at 14.5%the analogous index of control dogs being stable. Concentration of glucose in blood was within the limits of physiological changes for both groups of animals [2].

Daily diuresis for both groups of animals decreased at the end of the experiment within the limits of confidence bounds.

Study of serum blood and urine ion composition displayed indexes of potassium and sodium ions not to differ from the control ones.

Ramon-lyo in doses 200mg/kg x 30 and 200mg/kg x 60 was intravesically injected to 12 dogs (6 males, 6 females) while 4 dogs (2 males and 2 females) served as control ones. Fresh 10% solution of Ramon-lyo in water and 5% glucose was daily injected into the dogs' bladder through catheter for 30 days (4 males and 2 females) and 60 days (4 males and 2 females). Overall doses came to 6000 and 12000mg/kg respectively. Behavior and general condition of treated dogs appeared not to change as during treatment itself as 60 days after. Dogs didn't reveal aggression. Weight growth in comparison with starting data was 15 to 20%. Any disuric effect was observed.

References

[1] R. A. Muzychkina, *Natural Anthraquinones. Biological Properties and Physicochemical Characteristics*, Moscow: Phasis, 1998.
[2] A. A. Adjei, J. K. Buolamwini, *Novel Anticancer Drug Protocols*, New York: Academic Press, 2011
[3] S. K. Gupta, *Drug Screening Methods*, Kent: Anshan Publishers, 2009.
[4] M. Hidalgo, E. Garrett-Mayer, N. J. Clendeninn, S. G. Ekhardt, *Principles of Anticancer Drug Development*, New York: Springer, 2011.
[5] A. A. Al Fattah Amara, "Methods other than Experimental Animals for Screening Antitumor Compounds," *Am. J. Cancer Sci.*, vol. 2, no. 1, pp. 1-27, 2013.
[6] H. G. Vogel, J. Maas, A. Gebauer, *Drug Discovery and Evaluation: Methods in Clinical Pharmacology*, Berlin: Springer, 2011.
[7] J. Kolman, P. Meng, G. Scott, *Good clinical practice: standard operating procedures for clinical researchers*, New-York: J. Wiley & Sons, 1998.