# *The use of experimental animals in spa research*

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A laboratory rat is a rat of species Rattus norvegicus which is bred and kept for scientific research. Laboratory rats have served as an important animal model for reaserch in psychology medicine , and other fields.

Laboratory rats share origins with their cousins in domestication , the fancy rats. In 18th century Europe , wild Brown rats ran rampant and this infestation fueled the industry of rat-catching .Rat-cathcers would not only make money by trapping the rodents , but also by turning around and selling them for food , or more importantly , for rat-baiting . Rat-baiting was a popular sport which involved filling a pit with rats and timing how long it took for a terrier to kill them all.

The first time one of these ratwas brought into a laboratory for a study was in 1928 , in an experiment on fasting. Over the next 30 years rats were used for several more experiments and eventually the laboratory rat became th first animal domesticated for purely scientific reasons.

Over the years, rats have been used in many experimental studies, which have added to our understanding of genetics , diseases , the effects of drugs , and other topics in healt and medicine. Laboratory rats have also proved valuable in psychological studies of learning and other mental processes. The historical importance of this species the scientific research is reflected by amount of literature on it, roughly 50% more then on mice.

The Wistar rats is currently one of the most popular rat strains used for laboratory research. It is characterized by its wide head, long ears , and having a tail length that is always less than its body length. mentale. Wistar rats are an outbred strain of albino rats belonging to the species Rattus norvegicus. This strain was developed at the Wistar Institute in 1906 for use in biological and medical research , and is notably the first rat strain developed to serve as model organism at a time when laboratories primarily used Mus musculus. Utilizarea de modele animale, permite cercetătorilor să investigheze etiologia

More than half of all laboratory rat strains are descended from the original colony estabilished by physciologist Henry Donaldson scientific administrator Milton J. Greenam , and genetic researcher\embryologist Helen Dean King. Use of animals (Wistar rats), allows researchers to investigate the etiology, disease and disease evolution in a way that would be inaccessible to a human patient, performing procedures that involve a level of damage would not be considered ethical man to be produced

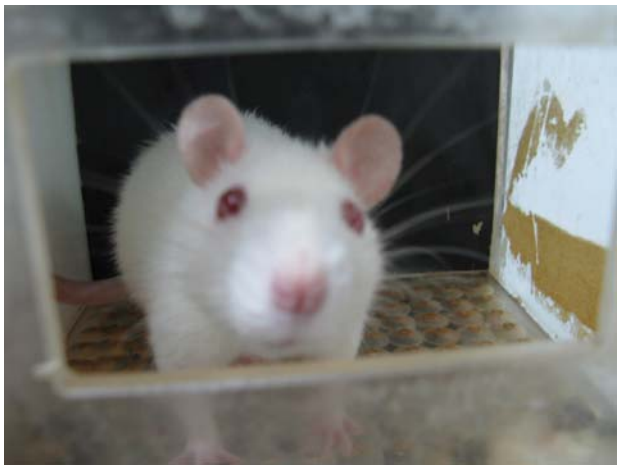
**Animal** models are used to learn more about a disease -diagnosis and treatment, easy to handle, 99% of genes (genetic manipulation), low cost, high rate of reproduction.

Although humans and animals (technically) can show different physiological and anatomical level are similar

We share about 99% of our DNA with mice, and in addition we use mice"knockout" - the effect through activation of common genes and seeing the effect on mice. By recreating the human genetic disease-treatment. The average litter size of the Wistar rat is , the adult body weight is 250-300 g for females , and 450-520 g for males. The typical life span is 2,5 - 3,5 years. Growth, maintenance and use in experiments and other scientific purposes of laboratory animals (Wistar rats) is performe on amaterial basis of fully respecting the legal provisions and regulations in the field. All activities related to growth, maintenance and

use of laboratory animals run on the basis of recognizedprocedures, well documented and in strict accordance with the recommendations FELASA. Base spaces allow adequateseparation of activities, i.e maintenance, quarantine, housing, animals during experiments\ tests, execution of experimental procedures.

Animals (Wistar rats) are housed in cages (number of animals per cage is dependent on their size or weight and size of cage), the rooms where animals are kept and are illuminated a controlled temperature. Animal health is monitored daily by personnel in units.



Animals in cages have access to food and water, feedresearch must be bought from an authorized certifying that each batch of product was manufactured in a way that is stored and shipped properly and that it contains elements adequate nutrition.Weight should be recorded on the sheet of daily activity.

Hygiene is very important because it can cause major problems forresearch groups and can lead to false results.

- Aprotected environment for scientists and laboratoryanimals
- Strict hygiene
- Management of laboratory animals.

Sanitary conditions should be of great concern for researchers.

- The accumulation of ammonia may affect the experiment.
- Diseases may occur.

The best way to determine if sanitary conditions are poor is to see if any:

- Accumulation of excreta or
- A strong smell urine.

Another important role is the stress laboratory animals, Theanimals are less stressed laboratory will result in more reliableresearch. It is therefore important that animals feel better in theirclosed though, to be disturbed as little as possible. Research will be conducted with care and respect necessary to protect the environment welfare of animals (cruelty is excluded) used for experimental purposes. The goal is always to ensure that animals suffer as little possible from the experimental subject.

* Each research will be clearly stated.
* It will detail the study design and how to perform each procedure.
* This protocol will be submitted for review, comments, guidanceand approval if a special ethics committee, which must be independent investigator, sponsor or any influence.

Male albino rats of the Wistar strain were

used in this experiment . The animals with weights between 250 and 350 g and of same age , were kept in individual plastic cages until time time determined for surgical procedures and euthanasia.

Animals were kept under natural light cycles, at appropriate temperatures , noise and humidity conditions , receiving proper food with free access to food and water throughout the experiment . Animals were numbered, by

simple drawing and weighed before the first surgical procedures .The animals n=46 were distributed in two groups. Group A experiment n=36 and Group B biochemical control n=10. Group A was further divided into 6 experimental subgroups n=6 . Each is described below :

Group A – n=36 Animals in this group were subjected to surgery with right hepatic duct ligature and euthanasia at the end of the waiting time of each sub-group with the later histological assessment of the liver and biochemical assessment of the blood.

Subgroup A1- n=6 In this subgroup , animals were subjected to surgery with the ligature of the right hepatic duct and euthanasia after 7 days for the histological assessment of the liver and biochemical assessment of the blood.

Subgroup A2- n=6 In this subgroup , animals were subjected to surgery with the ligature of the right hepatic duct and euthanasia after 14 days for the histological assessment of the liver and biochemical assessment of the blood.

Subgroup A3- n=6 In this subgroup , animals were subjected to surgery with the ligature of the right hepatic duct and euthanasia after 21 days for the histological assessment of the liver and biochemical assessment of the blood .

Subgroup A4- n=6 In this subgroup , animals were subjected to surgery with the ligature of the right hepatic duct and euthanasia after 28 days for the histological assessment of the liver and biochemical assessment of the blood .

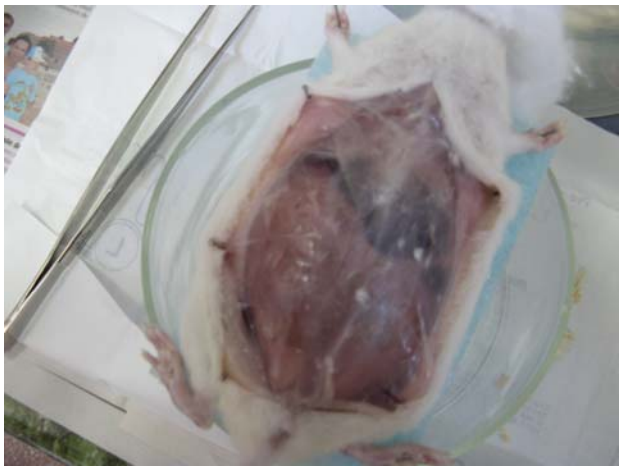
Subgroup A5- n=6 In this subgroup , animals were subjected to surgery with the ligature of the right hepatic duct and euthanasia after 60 days for the histological assessment of the liver and biochemical assessment of the blood .

Subgroup A6- n=6 In this subgroup , animals were subjected to surgery with the ligature of the right hepatic duct and euthanasia after 90 days for the histological assessment of the liver and biochemical assessment of the blood.

Group B – n=10 In this subgroup , animals underwent anesthesia and had 2ml of blood drawn by cardiac puncture for biochemical analysis of level of bilirubins , transaminases TGO and TGP , lactic dehydrogenase LDH , alkaline phosphatase AP , and gama-glutamyl-transpherase GGT .

***Procedures***

Before surgery, animals underwent general anesthesia induced by inhalation of ethylic ether and maintained with a solution of Ketamine and hydrochloride 2-(2.6 xylidine ) -5.6-dyhidro-4H-1.3-thiazine, in the does of 100mg/kg and 10mg/kg respectively , with intramuscular application on the inner side of the left thigh. After reaching plane of anesthesia with no response to pain stimuls applied on the adipose pad of animal s paws and absence of corneal reflex , animals were weighed and positioned , horizontally in dorsal decubitus with all paws held by sticky tape . Next , rats were shaved on the anterior wall of the abdomen and antisepsis was carried out with a solution of alcohol and 2% iodine .



With a scalpel, an approximately 4cm median incision was performed. Using a Metzembaum scissors, laparotomu was completed with the opening of linea alba and peritoneum .Next an Adson * utostatic retractor was placed to expose peritoneal cavity an inventory of the cavity was carried out by observing all abdominal viscera .Fig 2

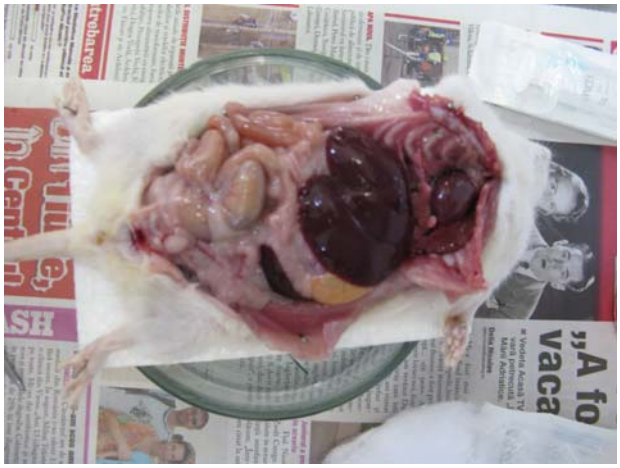
A dissection of the right hepatic duct was performed with the help of a stereoscopic microscope with wide angle lens and 12.5X magnification .Next the small intestine and the colon were eviscerated and wrapped in gauze dampened in a slaine solution .

Exposition of the biliary tract was achieved with the help of small flexible cotton buds dampened in sodium chloride 0.9%(saline).

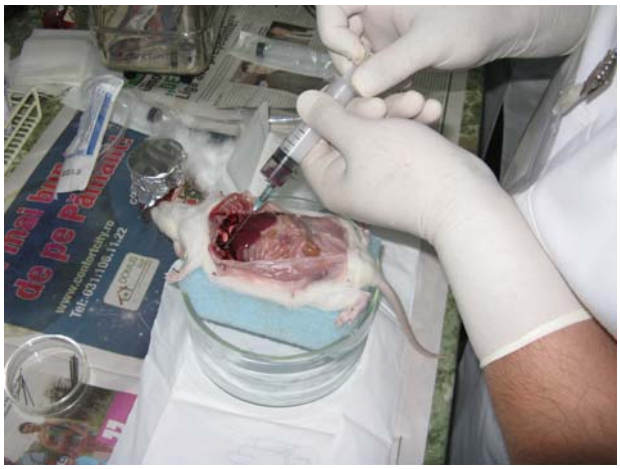
Biliary tract was identified between pancreatic tissue and the hepatic hilum ; right

hepatic duct was identified and double ligated with polypropylene suture, at 1 cm of its exposition , outside the hepatic parenchyma and sectioned between ligatures. The animal viscera were returned to the cavity . The closure of recto-abdominal sheath was performed with 4-0 absorbing thread and continuous anchored suture.

The skin and the subcutaneous mesh were sutured with nylon monofilament thread , with one single plane and continuous anchored suture . After each groups respective waiting time, animals underwent anesthesia and re-opening of the cavity through thoraco-phreno-laparotomy fot cardiac puncture and liver removal .Fig 4.



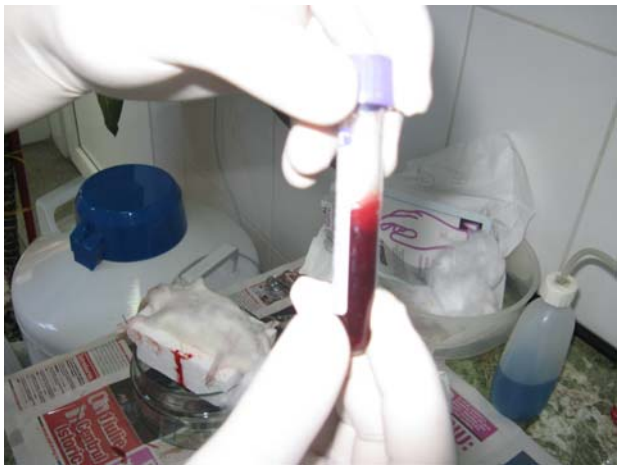
Initially an itracardiac puncture was performed for the collection of 2 ml of blood .



Blood was stored in a specific vial for blood collection and sent immediately for biochemical analyses . Fig 5

Next the whole liver was removed by section with scissors-cutting of ligaments , at splenic and hepaticangles .

All other remaining adhesions, and /or bands were removed in blocs, along with the hepatic ducts and diaphrahm muscle. Fig 6 Later, with the animal under anesthesia, euthamasia ny exsanguinations was performed . The carcass was place inside a specific plastic bag and properly disposed of in the experimental surgery hospital garbage . Liver ,after being removed , had its lobes recognized in its right , left , casual and median divisions .The left and right lobes were separated and sectioned transversally in its biggest diameter with the help of a 23 steel blade , and remaind in 10% formaldehyde solution for 24 hours .



After fixation the specimens were rinsed with water and immersed in a 70% alcohol solution. Specimenss were then code-numbered and sent to the histology lab to undergo routine histological procedure and obtain 7 to 10mm serial paraffin-embedded , hematoxylin and eosin stained cuts . Ten histologic cuts were obtained for each of the hepatic lobes .

The following criteria were taken into consideration in the analysis :

- Presence or absence of morphologic alterations in the hepatic lobes.
- Mean size of 2 hepatic lobes in each cut of the sloce , measured with an optic ruler previously calibrated according to the method proposed by Mandarim Lacerda for linear measurements.
- Presence or absence of granulation tissue and /or increase in the number of polymorphonuclears,and/or mononucleras in the examined cuts .

Histological alteration of the connective tissue present in the portal-space and in the centrilobular area of each analyzed lobe. For the biochemical study , the following analyses were carried out: Alkaline phospatase AF, gamma-glutamyl –transferase GGt, total bilirubin TB, direct bilirubin DB, indirect bilirubin IB , transaminases, aspartate aminotransferase AST . alanine amino transferase ALT, lactic dehydrogenase LDH.

In the histologic study right and left lobes of same liver were abalyzed –left serving as control for the right one –through 10 semiserial cute apiece, 7 to 10 in thickness and 200mm clearance between cuts. The analysis of slides was carried out on a conevtional optic microscope using 10X and 40X magnification. The following parameters were assessed in this analysis .

With 1 10X magnification lens :

a. Presence or absence of fibrosis on the hepatic lobe , lymphotyc infiltration in any area of the cut lobe and histologic alterations around biliary ducts and portal-spaces.
b. Size, in micrometer of at least two hepatic lobes transversally cut within each hitologic cut using an optic ruler previously calibrated according to the method proposed by Mandarim Lacerda for linear measurements .

With a 40X magnification lens the following procedures were carried out.

Focusing on one hepatic lobe , transversally sectioned within the histologic cut a wide-angle lens was placed on the border of the connective tissue existing in one of the portal-spaces of the said lobe , delimiting a counting area towards the central vein of the chosen lobe. Next a counting was carried out for the number of polymorphonuclears, mononuclears, or macrophagic cells existing within the area delimited by wide-angle After the histologic analysis the codes for each slide were revealed and tables were put togheter following the protocol for histological analysis. All biochemical and histological results were tabulated on Ms Excel software and statistically analyzed using the Spss 8.0 program. Since biochemical measurements did not present a normal distribution non-parametric tests were applied. Was pplied in the comparasion between of each of groups in the experiment and the control group and the differences were tested in a 95% significance level ($p<0.05$).

***Results***

The table indicates the mean values of all sub-goups used in the present study No significant difference ($p<0,05$) was found, except fot the DB values in the A6 sub-group . Values of the control group were adopted as normal . The table below demonstrates that no parenchyma necrosis and no color alteration on the hepatic surface of any liver in the experiment was found. A single nodule in animal of sub-group A 4 was found. The table below indicates the presence of hilum adhesions in all operated animals and hilum fibrosis in 9 cases, three for which in sub-group A5.

The table below indicates that it was not possible to observe any significant histological alteration in the size og the hepatic. lobes analyzed through H.E. stained cuts. The sequence of microphotography below indicates that it was not possible to find any histological alterations worth of closer observation among the several groups of analyzed animals . The first sequence shows 10X magnified H.E. stained slides of 4 group.Fig 8. The second sequence shows 40X magnified H.E. stained slides of groups .Fig 9

***Conclusion***

The present study did not identify histological or biochemical alterations that expressed significant differences between animals that underwent ligature of right hepatic duct and yhose in the control group in regards to hepatic parenchyma lesion .