

The Effect of Kuchala (Arum Korolkowii Regel, 1873) Tuber Tincture to Increase of the Serum Testosterone in the Adult Male Guinea Pigs (Cavia Porcellus Linnaeus, 1758)

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Research Article

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Abstract

Background: Kuchala (*Arum korolkowii* Regel) is a medicinal plant often used in folk medicine in the Kyrgyz Republic. As a medicinal raw material, the tuber's tincture is used in small doses to increase human sexual potency. However, there is no scientific evidence in support of the medicinal effects of kuchala. For these reasons, we decided to study the pharmacological effect of kuchala tuber tincture on the sexual potency of adult male guinea pigs. We analyzed the effect of kuchala at the age of ± 48 -months, in 12 male guinea pigs. A preparation of 10% tuber tincture of kuchala in 70% ethanol was administered perorally to the male guinea pigs in the form of a once-daily dose of 150 μ l for 30 days. The study data were obtained by ethological, hematological and serum biochemistry, gross anatomical, histological and statistical methods.

Results: The hematological and serum biochemistry parameters were significantly different between the control and the experimental group. The neutrophils' percentage in the experimental group was significantly lower ($^dP < 0.001$) than in the control group. On the other hand, the lymphocyte counts were significantly higher in the experimental group ($^dP < 0.001$). The RBC counts, Hgb, Hct, MCH and MCHC were significantly higher in the experimental group ($^dP < 0.001$; $^dP < 0.001$; $^cP < 0.01$; $^dP < 0.001$; $^dP < 0.001$ respectively) than in the control group. In contrast, the color indicator and the mean platelet volume were higher ($^bP < 0.05$) and significantly higher ($^dP < 0.001$) respectively in the control group than in the experimental group. The ALT and AST levels were lower in the experimental than in the control group (both $^dP < 0,001$). The testosterone concentration in serum was much higher ($^dP < 0,001$) in the experimental group. Microscopically, some structural damages were found in the liver of the experimental animals indicating a metabolic disorder. However, the testes showed an improvement in spermatogenesis in the experimental compared with the control group.

Conclusions: The 10% kuchala tuber tincture in 70% ethanol has a positive effect in terms of improving the sexual potency of adult guinea pigs by increasing the production of testosterone and increasing spermatogenesis.

Background

Since ancient times, people have successfully used folk medicine about medicinal plants as an important therapeutic agent in both human and veterinary medicine [1–3]. Many of the treatment methods have been passed down through families for generations, and some of these have been adapted for use in modern medical practice. Among others, Kyrgyz folk medicine has occupied an important place in the nomadic civilization of the Kyrgyz people.

The Kyrgyz Republic is a mountainous country in central Asia. Due to the extreme environment and climate, there is a diverse range of species of plants, including more than 200 species of medical plants. Many of the medical plants used in Kyrgyz folk medicine have not been studied using modern scientific techniques [4]. *Arum korolkowii* Regel is one of the medicinal plants often used in the folk medicine of Central Asia, which has not lost its relevance, even today. The vernacular name of this medicinal plant is kuchala. *A. korolkowii* R., 1873 belongs to the genus *Arum* L. of the family *Araceae* Juss. and grows in soil pockets on rocky hillsides, beneath low scrub. Its native range is Central Asia, North-Western China, Northern Iran and Afghanistan. *A. korolkowii* R. is a perennial tuberous herb sprouting in early spring from a discoid, vertically-orientated tuber. It has a well-described biological characteristic [5, 6]. However, there is another plant also called kuchala or kuchla or Chinese kuchla

(*Strychnos nux-vomica*). *S. nux-vomica* is an evergreen tree up to 25 m height. Its dried seeds (*Nux vomica*) are used in modern and traditional medicine [7, 8].

A. korolkowii R. is a very poisonous herb. In folk medicine it is used as a medicinal raw material in the form of tincture of tubers which is administered in small doses in order to increase human potency, to treat infertility and stomach ulcers, diseases of the nasopharynx and the respiratory tract, and to eliminate fatigue and give strength. The powdered tuber is used to treat poisonous snake and scorpion bites, fungal skin diseases and hemorrhoids [5]. The medicinal properties of kuchala were mentioned in the works of Avicenna and in such Kyrgyz folk epics as «Manas» [9] and «Semetey» [10]. According to the above-mentioned sources, the milky and sour-milky (kumys) tinctures of the tubers of this plant are often used among the elderly (over 70 years) as a drug that increases the sexual potency of men. However, there are no modern scientific data proving the medicinal properties of kuchala, especially, in terms of its effect on human potency. Besides, the *A. korolkowii* R. chemical composition has not yet been studied. In this regard, we decided to experiment with kuchala tuber tinctures on laboratory animals, specifically with adult male guinea pigs.

Many characteristics make the guinea pig an ideal model for biomedical research [11]. Guinea pigs have been comprehensively studied as laboratory animals using biological, morphological and physiological approaches. Its size and lifestyle make the guinea pig easy to keep and to conduct experimental studies on and, importantly, it has many of the same morphological and physiological characteristics as humans [11, 12], including in terms of reproduction [13–18]. Consequently, guinea pigs are frequently used as a biological model in studies of a number of infectious bacterial and noninfectious diseases [12, 19].

Blood evaluations are a prime means of diagnosis in both human and animal medicine. This is because hematological and serum biochemistry data can show changes in physiological disturbances such as systemic inflammation, renal or hepatocellular disorders [11]. The aim of the present study was to explore the pharmacological effect of the kuchala (*Arum korolkowii* Regel) tuber tincture on the hematological and serum biochemistry parameters, and on the testis and liver structures of the adult male guinea pigs (*Cavia porcellus* Linnaeus, 1758).

Results

Physical characteristics of tubers and tincture

Dried kuchala tubers were discoid (Fig. 1a) in the main, 2–6 cm across, and 2-2.3 cm thick. The color of the peel was light brown and hard. When cleaning of the peel, it flaked off into small, hard scales. Under the hard peel of the tuber was a soft, thin, and easily removed shell of a yellowish-white color. On transverse sections, the tuber was yellow-white color (Fig. 1b), easily cut and was soft consistency similar to that of plasticine. Tubers could be pressed easily and formed a mushy oily mass. The kuchala tubers did not have a pungent smell. The taste was not immediately apparent, but a few moments later, a strong bitter taste of spicy pepper was felt, which lasted for a long time. The prepared 10% tincture of tubers on 70% ethanol was transparent and viscous, yellowish-reddish in color (Fig. 1c). The tincture has a special bitter smell, different from the smell of alcohol.

Animal behavior

The behavior of animals in both groups was observed through a window from the next room, and the results of the observation were recorded. Both the control and the experimental group led an active lifestyle. They often ran and played, fought among themselves, and ate well. However, gradually, day after day, an increase in the appetite and activity of the experimental animals was observed when compared to the control group. The experimental animals often fought among themselves and were more aggressive. Sometimes they climbed on the sidewalls of the isolator cage.

Hematology

We observed no adverse effects in terms of the clinical signs of anemia and other disorders after the guinea pigs were phlebotomized under isoflurane anesthesia.

Common hematology parameters such as WBC, neutrophils, lymphocytes, monocytes, eosinophils, basophils, RBC, Hgb, Hct, MCV, MCH, MCHC, color indicator, erythrocyte sedimentation rate, platelets and mean platelet volume were evaluated (Table 1). The results of the study showed that several parameters of the blood were significantly different between the control and the experimental groups (Fig. 2). Neutrophils's percentage in the experimental animals was significantly lower ($^dP < 0.001$) than in the control animals. Lymphocyte counts on the other hand were significantly higher in the experimental animals ($^dP < 0.001$). RBC counts, Hgb, Hct, MCH and MCHC were significantly higher in the experimental animals ($^dP < 0.001$; $^dP < 0.001$; $^cP < 0.01$; $^dP < 0.001$; $^dP < 0.001$ respectively) than in the control guinea pigs. However, the color indicator and the mean platelet volume were higher ($^bP < 0.05$) and significantly higher ($^dP < 0.001$) respectively in control animals than in experimental animals. Other hematological parameters such as WBC, monocytes, eosinophils, basophils, MCV, erythrocyte sedimentation rate, platelets between animal groups were not statistically significant. Note: the hematological and serum biochemistry parameters of the animals obtained before the experiment and the animals in the control group showed almost no difference. Therefore, only the parameters of the control group animals were shown.

Table 1
Hematological parameters for control and experimental guinea pigs

Blood parameters	Control group (n = 10)					Experimental group (n = 12)				
	Mean	SD	Median	Min	Max	Mean	SD	Median	Min	Max
WBC (x10 ⁹ /L)	7.24 ± 0.343	1.084	7.1	5.5	9.1	7.94 ± 1.00	3.48	7	4	15.2
Neutrophils (%) ^d	53.6 ± 1.258 _d	3.978	53	48	61	27.08 ± 2.62	9.07	26.5	14	46
Lymphocytes (%) ^d	39.4 ± 0.872	2.757	40	35	43	56.08 ± 3,08 ^d	10.66	56.5	40	71
Monocytes (%)	4.8 ± 0.291	0.919	5	4	7	4.58 ± 0,89	3.09	3.5	2	11
Eosinophils (%)	2.4 ± 0.499	1.578	3	0	4	5.42 ± 1,65	5.71	4	0	20
Basophils (%)	0.4 ± 0.163	0.516	0	0	1	0.08 ± 0,08	0.29	0	0	1
RBC (x10 ¹² /L) ^d	4.53 ± 0.110	0.347	4.6	3.9	4.9	5.38 ± 0,03 ^d	0.10	5.35	5.3	5.6
Hgb (g/dL) ^d	144.5 ± 0.934	2.953	145.5	139	148	154.58 ± 1,14 ^d	3.96	154.5	147	160 ^d
Hct (%) ^c	43.6 ± 0.340	1.075	44	42	45	45.33 ± 0.37 ^c	1.27	45.1	44	48 ^c
MCV (fL)	76.6 ± 1.222	3.864	77	70	81	74.61 ± 0.95	3.30	73.8	70.4	84.2
MCH (pg) ^d	24.27 ± 0.495	1.566	24.2	21.9	26.3	53.89 ± 5.94 ^d	20.57	51.5	25.7	95.5
MCHC (g/dL) ^d	32.36 ± 0.264	0.834	32.45	30.9	33.3	752.83 ± 132.66 ^d	459.54	685	352	1910
Color indicator ^b	0.88 ± 0.007 ^b	0.022	0.88	0.83	0.9	0.85 ± 0,00	0.01	0.855	0.83	0.87
Erythrocyte sedimentation rate (mm/hour)	2.6 ± 0.163	0.516	3	2	3	2.25 ± 0.13	0.45	2	2	3
Platelets (x10 ⁹ /L)	301.9 ± 2.364	7.475	303	289	310	343.50 ± 22.05	76.38	335.5	235	494

Note: ^bP < 0.05; ^cP < 0.01; ^dP < 0.001.

Blood parameters	Control group (n = 10)					Experimental group (n = 12)				
	Mean	SD	Median	Min	Max	Mean	SD	Median	Min	Max
Mean platelet volume (fL) ^d	7.43 ± 0.037 ^d	0.116	7.4	7.3	7.7	4.58 ± 0.30	1.04	4.8	3.1	6
Note: ^b P < 0.05; ^c P < 0.01; ^d P < 0.001.										

Serum biochemistry

In this study, some serum biochemical parameters such as ALT (alanine aminotransferase), AST (alanine aminotransferase), glucose and testosterone were evaluated (Table 2). As a result, three of them (ALT, AST and testosterone), were significantly different in terms of the control and the experimental groups of animals (Fig. 3). ALT and the AST percentages in the experimental animals were significantly lower than those in the control animals (both ^dP < 0.001). The testosterone concentration was considerably higher (^dP < 0.001) in the experimental guinea pigs and the glucose percentage in the serum was not statistically significant between the animal groups studied.

Table 2
Serum biochemistry parameters for control and experimental guinea pigs

Serum parameters	Control group (n = 10)					Experimental group (n = 12)				
	Mean	SD	Median	Min	Max	Mean	SD	Median	Min	Max
ALT (U/L) ^d	63.65 ± 0.521 ^d	1.647	64.25	60.7	65.4	55.82 ± 1.33	4.59	55.6	48.6	63.93
AST (U/L) ^d	78.29 ± 3.096 ^d	9.790	80.05	58.2	90.9	60.78 ± 2.00	6.94	62.85	47.67	69.9
Glucose (mmol/L)	9.877 ± 0.621	1.963	9.92	6.91	13.5	9.20 ± 0.39	1.35	9.5	6.99	11.08
Testosterone (nmol/L) ^d	9.533 ± 0.184	0.583	9.615	8.63	10.4	21.73 ± 2.11 ^d	7.32	20.95	11.4	31.5
Note: ^d P < 0.001										

Gross anatomy and histology

In order to make a comparison, all internal organs were visually studied, especially the heart, liver, kidneys and testes in both the control and the experimental groups. Results of a visual examination of the color, consistency and degree of blood filling of the above-mentioned organs concerning the control and experimental groups of necropsied guinea pigs did not reveal any significant differences. Comparative morphometric studies (organ weight, width, length and thickness) of the liver and testes in animals of both groups were also not statistically significant. Therefore, we did not provide comparative morphometric data of the studied organs.

A microscopic examination of the blood smears also revealed no noticeable differences or changes between animals in the control and experimental groups. Only the WBC of the guinea pigs in the experimental group were

reflected (Fig. 4) without a description of the structural features of each of them.

Microscopic examination of the liver of the control guinea pigs visualized normal hexagonal hepatic lobules in various sizes having hepatocytes, a central vein with blood cells, sinusoids along with empty spaces, and not clearly visible macrophages (Kupffer cells), lining different places of the sinusoids (Fig. 5a). The liver sections of the experimental animals were stained poorly when compared to the control group. Liver cords, sinusoids, intensive stained Kupffer cells, several apoptotic figures and apoptosomes (Fig. 5a*) were clearly visible.

A histological examination of a section of the control guinea pigs' testes showed tubular glands and intertubular connective tissue by specific intestinal or Leydig cells. The round-oval seminiferous tubules were of various sizes, and they surrounded by loose vascular connective tissue forming the lobules of the testis. The coiled seminiferous tubules were lined with multilayered spermatogenic cells in different developmental stages, and sustentacular or Sertoli cells (Fig. 5b). There was a decrease in the number of spermatogenic cells in seminiferous tubules. The experimental testis section showed a restoration of spermatogenesis in the seminiferous tubules (Fig. 5b*). The amount of spermatogonias, primary spermatocytes, and spermatids had increased. The lumen of the seminiferous tubule was filled with developing spermatozoa.

Discussion

This paper is devoted to the scientific exploration of the pharmacological effect of the kuchala (*Arum korolkowii Regel*) tuber which has long been used in Asia folk medicine, on male potency. The Kyrgyz people have used the tubers of this medicinal plant for a long time, adding to the process the preparation of the national drink, kumys (fermented milk product of mare's milk), as a means of increasing the strength and endurance of male warriors [9, 10]. Folk healers, referring to the works of Avicenna, indicate that the kuchala tubers in conjunction with wine, stimulates sexual desire and detoxifies the kidneys. However, the recipe of the kuchala tuber tincture in the kumys and in the wine has not been written in detail, and modern folk healers also keep it in secret. Based on the above, we prepared a 10% tincture of kuchala tubers in 70% ethyl alcohol.

The next important stage of research planning was the choice of the type of laboratory animal for experimental study. To determine this, we analyzed the literature and found that among the different laboratory animals, the guinea pig has many morphofunctional similarities with humans, in terms of the lung physiology [20], and hormonal, immunological and corticosteroid responses [11, 12]. Importantly, the guinea pig has many common features with humans in terms of reproduction related to the accessory glands [14, 15], the characteristics of the placenta [21], and morphological and functional analysis of spermatogenesis [22]. In addition, information exists that the testosterone and androstenedione content in serum and testes were different in guinea pigs in the prenatal [18, 23], and postnatal [24] periods. The concentration of testosterone in plasma reaches its maximum level at 60 days of age in guinea pigs, and then decreases with the increase in age. In the 24–35 month old guinea pigs, there is a 65% decreased in the concentration of testosterone in blood plasma [24]. For centuries, Kyrgyz people have used the kumys tinctures of the kuchala tuber to treat disorders in terms of sexual libido of men over 70 years of age and to strengthen the bone system. In this regard in particular, the adult male guinea pigs were selected at \pm 48-months of age, when their testosterone level was low. Additionally, in guinea pigs the structure of the testicles [25], epididymis [26] and spermatogenesis [27] have been studied in detail. The above-mentioned data served as a justification for the use of guinea pigs in this experimental study.

For the comparative analysis, we studied the hematological and serum biochemical parameters of guinea pigs. Based on the hematological and serum biochemical data of an inbred strain, 13/N guinea pigs were divided into the following age groups - juveniles (0-150 days), adults (151–900 days) and geriatric adults (older than 900 days) [28]. Our selection for the experimental study was 48-month-old guinea pigs. Although from a different breed, they fully met our goal in terms of the choice of adult animals.

In terms of the comparative aspect of our study, we studied sixteen hematological (Table 1) and four serum biochemistry parameters (Table 2) of the blood. Based on our data, we can say with confidence that the goal of this experimental work has been achieved. This is so because the concentration of testosterone in the blood plasma in the experimental animals (21.73 ± 2.11 d) was more than twice that of the control animals (9.533 ± 0.184), which is confirmed statistically ($^dP < 0.001$). In addition, it is confirmed that microstructural changes in the testes showed improvement in terms of spermatogenesis, i.e. increase of spermatogenesis cells in the seminiferous tubules.

However, some hematological parameters such as the lymphocytes, RBC, Hgb, Hct MCH, MCHC, color indicator and mean platelet volume significantly increased in the experimental guinea pigs ($^dP < 0.001$; $^dP < 0.001$; $^cP < 0.01$; $^dP < 0.001$; $^dP < 0.001$; $^dP < 0.001$, $^bP < 0.05$, $^dP < 0.001$ respectively), compared with the control animals. On the other hand, the neutrophils percentage in the experimental animals was significantly lower ($^dP < 0.001$) than in the control animals. Such suspicious data indicates the toxic effect of the tincture on guinea pigs. This is probably due to the dose (0.15 μ l) or the high concentration of the tuber tincture (10%), possibly with the higher diluted solution of ethanol (70%), or the long time (30 consecutive days) over which the tincture was given to the experimental guinea pigs. Hepatocellular injuries can be evaluated using serum biochemistry parameters of alkaline phosphatase (ALP) and alkaline aminotransferase (ALT) [11]. The toxic effect of this tincture on the guinea pigs was also confirmed with regards to a decrease in the concentration of ALT and AST in the plasma biochemistry of the experimental guinea pigs, which was statistically significant ($^dP < 0.001$). Such a process is observed in cirrhosis or necrosis of the liver. The toxic effects of any drug or toxic substance in the organism are expressed by damage to the structure of the liver [29, 30]. The gross anatomy parameters of the liver of both animal groups were similar, which is consistent with the data of other researchers of the liver of guinea pigs [31]. However, microscopic examination revealed some differences in the liver in the experimental animals. The liver sections of the experimental animals were pale-stained, and all the microstructural components of the liver - cords, sinusoids, Kupffer cells [32] - were clearly visible. There were several apoptotic figures and apoptosomes in the liver parenchyma. As is known, pale staining of cell structures indicates low functional activity on the part of the organ.

Conclusion

The present study shows the 10% kuchala (*Arum korolkowii* Regel) tuber tincture in 70% ethanol has a positive effect in terms of improving the sexual potency of old male guinea pigs by increasing the production of testosterone and increasing the spermatogenesis. The toxic effects of this tincture on the animal organism can be resolved by reducing the dose. The concentration of the drug tincture in ethanol will be the main aim of our next study.

Materials And Methods

Kuchala tubers and making the tincture

Five pieces (total 49.62 g) dried kuchala tubers were purchased at local markets in Bishkek (Kyrgyz Republic). Each of the tuber pieces was cleaned with warm water and then with 70% of ethanol, and air dried at room temperature. The tubers with peel attached were shredded using a manual grinder. The ground tuber was weighed on a Precisa (Switzerland) electronic weight and prepared as a 10% tincture in 70% ethanol. The tincture was poured into a dark glass bottle which was tightly closed. It was then placed in a dark place at room temperature. The tincture was mixed twice per day, and this process was continued for 14 days until the tincture was ready. On the 15th day, the tincture was filtered through dense gauze and then through filter paper. The 10% tincture in 70% ethanol so prepared was then stored in a refrigerator (+ 4 °C) for use in the experimental study. The main phases of the experimental study, in sequential order, are shown on the following schematic image (Fig. 6).

Experimental animals and husbandry

We purchased clinically healthy 22 male guinea pigs of Abyssinian breeds, all \pm 48-month-old and weighing on the average 682 g (489–792 g) from a private guinea pig breeder. Animals were housed in two handmade isolator cages measuring 98.7 cm x 347.89 cm x 54.3 cm for 10 control animals and 110.3 cm x 398.73 cm x 54.6 cm for 12 experimental animal for the duration of the experiment, with under sun-dried clean straw bedding and cardboard huts. The bedding was changed every two days or more frequently if needed. Animals were housed at a room temperature of 20–26 °C with 40–70% humidity. Guinea pigs had access *ad libitum* to rodent chow and water. Animals were allowed to acclimate in the vivarium for 10 days after delivery before they were used for this study. The research was conducted in accordance with the internationally accepted principles for laboratory animal use and care [33], and ARRIVE (Animal Research: Reporting of *In Vivo* Experiments) guidelines 2.0 [34].

Treatments and handling

We observed the behavior of the guinea pigs during the adaptation period (10 days). Based on their physical activity and body weight, the experimental animals were divided in two groups. Both the control and experimental groups of animals were formed according to the above-mentioned principle. The control group consisted of 10 and the experimental group of 12 male animals, and both groups were kept in the same conditions. Every day from 8:00 to 9:00 a.m. 150 μ l of kuchala tubers tincture were administered to the experimental animals and 150 μ l normal water was administered perorally to the control group over 30 days. After that, we observed the behavior of the animals through a window in the next room and took note of the changes in the behavior of the animals.

Blood collection

Blood samples were collected from each guinea pig on two occasions, five days before the start and after the finish of giving tincture of the tuber, under isoflurane (3–5%) anesthesia. Blood was collected from the cranial vena cava according to a previously well-described method [35]. During the phlebotomy, the rules of asepsis and antiseptic were strictly observed [11]. Blood from the cranial vena cava was obtained using a 25-gauge, 5/8-in needle attached to a 3 ml syringe (Zhejiang Huafu Medical Equip. Co. Ltd China). Collected blood was immediately transferred into Gel/Clot Activator (GD060SGC) tubes for serum collection and EDTA.K3 (GD060EK3) tubes for general blood analysis. In addition, blood smears were taken for cytology analysis.

Blood analysis

Blood was stored for up to 2–3 hours in a refrigerator at 4 °C before processing. The blood samples were then submitted to the human clinical-diagnostic laboratory for processing and analysis. The hematological analysis was carried out using an APUIA 560 Hematology System (Siemens, Germany) for 1–2 minutes, serum biochemistry was analyzed using a Beckman Coulter AU 480 (USA-Japan) for 15–20 minutes, and immune chemiluminescence tests were analyzed using a ImmuLite 2000 XPi (Immunoassay system) (Siemens, Germany) for 1 hour 6 minutes. These machines were calibrated routinely every 6 months by service professionals using a commercial calibrator. An Erythrocyte Sedimentation Rate (ESR) was done manually. Blood smears were stained with MGG Quick Stain (04-090805, Bio Optica Milano s.p.a.) in flooded slide preparation.

Necropsy

The anesthetized animals were euthanized by exsanguination and were necropsied according to standard procedure [36]. The heart, liver and testes were extracted and their gross anatomy data (color, consistency, blood filling) were studied. Liver and testes morphometric parameters were recorded (length, width, thickness) and the weight of organs using a Precisa (electronic weight scale, Switzerland) was measured.

Histology

Tissue samples for the microscopic study from the testes and liver were fixed in neutral buffered 4% formaldehyde (pH 7.4) overnight at room temperature. After standardized histological processing to paraffin, sections (4 µm thick) were cut with an automated Leica RM2255 rotary microtome, followed by staining with hematoxylin and eosin. A Nikon ECLIPSE 50i microscope equipped with a Nikon Digital Sight DS-Fi1 camera was used for observation and photography.

Statistical analysis

The hematological and serum biochemical data obtained were subjected to statistical processing. Mean, SDs, Median, Student's t-test, Min and Max values were calculated with software (Microsoft Excel). A value of $P < 0.05$ was considered as being statistically significant.

Declarations

Ethics approval and consent to participate

Animal Research Local Ethics Committee at the Kyrgyz-Turkish Manas University (Kyrgyz Republic) approved the research (case number EC-KTMU-07, 20-12-2019).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

AN and JJ conceived, designed and realized the study. JJ, ON, KB and SK participated in the experimental work. AN, TA and SR took blood and necropsied the animals. ChK, KG and TA provided academic instruction. AN, TA, KG, BB and ShG conducted data collection and analysis. ChK undertook the statistical processing of the data. AN interpreted and wrote the draft manuscript. All authors read and approved the final manuscript.

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References

1. Suleman S. *et al.* Treatment of malaria and related symptoms using traditional herbal medicine in Ethiopia. *Ethnopharmacol.* 2018. <https://doi.org/10.1016/j.jep.2017.10.034>.
2. Söukand R. and Pieroni A. The importance of a border: Medical, veterinary, and wild food ethnobotany of the Hutsuls living on the Romanian and Ukrainian sides of Bukovina. *Ethnopharmacol.* 2016. <https://doi.org/10.1016/j.jep.2016.03.009>.
3. Tulobaev A.Z. Range of Medicinal Plants Used in Folk Veterinary Medicine in Kyrgyzstan. *Manas J. Agric. Vet. Life Sci.* 2019; 9(2):91–98.
4. Wang Guo-Qiang, Huang Lu-Qi, Xie Dong-Mei. [Introduction of traditional medicinal plants in Kyrgyzstan]. *Zhongguo Zhong Yao Za Zhi* 2014; 39(3):391–396.

5. Eisenman S.W., Zaurov D.E. and Struwe L. *Medicinal plants of Central Asia: Uzbekistan and Kyrgyzstan*. Springer; 2013. <https://doi.org/10.1007/978-1-4614-3912-7>.
6. Haigh A. et al. *Arum korolkowii* Regel. *Plants of the World online*. 2011. <http://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:86049-1>.
7. Guo R. et al. Botany, Phytochemistry, Pharmacology and Toxicity of *Strychnos nux-vomica* L.: A Review. *J. Chin. Med.* 2018. <https://doi.org/10.1142/S0192415X18500015>.
8. Patel K., Laloo D., Singh G.K., Gadewar M., Patel D.K. A review on medicinal uses, analytical techniques and pharmacological activities of *Strychnos nux-vomica* Linn.: A concise report. *J. Integr. Med.* 2017. <https://doi.org/10.1007/s11655-016-2514-1>.
9. Orozbekov S. *Manas II*. Bishkek: Kyrgyzstan; 1995. <https://new.bizdin.kg/kniga/manas-eposu-4-kitep>.
10. Mamai J. Zhusup Mamais variant of Semetey. 2017. https://new.bizdin.kg/media/books/Эпос_Семетей_Вариант_Жусупа_Мамая.pdf.
11. Williams W.R., Jonston M.S., Higgins S., Izzo A.A., Kendall L.V. Blood profiles in unanesthetized and anesthetized Guinea pigs (*Cavia porcellus*)," *Lab Anim. (NY)*. <https://doi.org/10.1038/labam.911>.
12. Padilla-Carlin D.J., McMurray D.N. and Hickey A.J. The guinea pig as a model of infectious diseases. *Med.* 2008; 58(4):324–340.
13. Suzuki O., Koura M., Noguchi Y., Takano K., Yamamoto Y., Matsuda J. Optimization of superovulation induction by human menopausal gonadotropin in guinea pigs based on follicular waves and FSH-receptor homologies. *Reprod. Dev.* 2003. <https://doi.org/10.1002/mrd.10242>.
14. Gradela A. et al. Morphological and morphometric study of the prostate of guinea pigs (*Cavia porcellus*, Linnaeus, 1758) during postnatal development. *Biotemas* <https://doi.org/10.5007/2175-7925.2013v26n4p221>.
15. Gradela A. et al. Morphologic and morphometric description of the guinea pigs vesicular gland during postnatal development. *Vet. Bras.* 2013. <https://doi.org/10.1590/S0100-736X2013000700017>.
16. Rodríguez-Casuriaga R., Geisinger A., Santiñaque F.F., López-Carro B., Folle G.A. High-purity flow sorting of early meicytes based on DNA analysis of guinea pig spermatogenic cells. *Part A* 2011. <https://doi.org/10.1002/cyto.a.21067>.
17. Rodríguez R.E., Wettstein R.M. Quantitative Study on Guinea Pig Spermatogenesis Shows a Relative High Percentage of Early Meiotic Prophase Stages. *Rec. - Part A Discov. Mol. Cell. Evol. Biol.* 2004. <https://doi.org/10.1002/ar.a.20037>.
18. Nunes A.K.R. et al. Morphological development of the testicles and spermatogenesis in Guinea pigs (*Cavia porcellus* Linnaeus, 1758). *Morphol. Sci.* 2017. <https://doi.org/10.4322/jms.107816>.
19. Acosta S., Dizeyi N., Feinstein R., Pierzynowski S., Abrahamsson P-A. Long-term testosterone stimulation induces hyperplasia in the guinea-pig prostate. *Prostate Cancer Prostatic Dis.* <https://doi.org/10.1038/sj.pcan.4500744>.
20. Noonan D.E. The Guinea Pig (*Cavia porcellus*). *Genet.* 1975; no. September:275–307.
21. Card S.E., Brien J.F. No effect of chronic ethanol administration of the activity of alcohol dehydrogenase and aldehyde dehydrogenases in the near-term pregnant guinea pig. *J. Physiol. Pharmacol.* 1989. <https://doi.org/10.1139/y89-096>.

22. Nunes AKR et al. Morphological and functional analysis of spermatogenesis in guinea pigs (*Cavia porcellus*) from pre-puberty to post-puberty. *Vet. Bras.* 2013; 33:1–7.
23. Pelardy G. and Delost P. Secretion of the androgens in the male guinea-pig during the perinatal period," *Acta Endocrinol. (Copenh)*. [https://doi.org/ 10.1530/acta.0.0890770](https://doi.org/10.1530/acta.0.0890770).
24. Rigaudiere N., Pelardy G., Robert A., Delost P. Changes in the concentrations of testosterone and androstenedione in the plasma and testis of the guinea pig from birth to death. *Reprod. Fertil.* 1976. <https://doi.org/10.1530/jrf.0.0480291>.
25. Simões L.S. *et al.* The quantification of testicular cells during the postnatal development in two Caviomorph rodents: The guinea pig (*Cavia porcellus*) and the cutia (*Dasyprocta agouti*). 2017. <https://doi.org/10.1590/0001-3765201720170038>.
26. Uppal V., Bansal N., Pathak D., Kumar A. Histomorphochemical studies on the epididymis of guinea pig. *Indian J. Anim. Sci.* 2009; 79(8):809–812.
27. Simões L.S. *et al.* Ultrastructural analysis of the spermatogenesis in the guinea pig (*Cavia porcellus*). *Vet. Bras.* 2016. <https://doi.org/10.1590/S0100-736X2016001300013>.
28. Genzer S.C., Huynh T., Coleman-McCray J.A.D., Harmon J.R., Welch S.R., Spengler J.R. Hematology and clinical chemistry reference intervals for inbred strain 13/N Guinea pigs (*Cavia porcellus*). *Am. Assoc. Lab. Anim. Sci.* 2019. <https://doi.org/10.30802/AALAS-JAALAS-18-000118>.
29. Fan Y., Liu S., Chen X., Feng M., Song F., Gao X. Toxicological effects of Nux Vomica in rats urine and serum by means of clinical chemistry, histopathology and 1H NMR-based metabonomics approach. *Ethnopharmacol.* 2018. [https://doi.org/ 10.1016/j.jep.2017.06.027](https://doi.org/10.1016/j.jep.2017.06.027).
30. Uche F., Obianime A., Gogo-Abite M. Effects of Vanadium Pentoxide on the Histological and Sperm Parameters of Male Guinea Pigs. *Appl. Sci. Environ. Manag.* 2010. <https://doi.org/10.4314/jasem.v12i3.55512>.
31. STAN F.G. Comparative Study of the Liver Anatomy in the Rat, Rabbit, Guinea Pig and Chinchilla. *Univ. Agric. Sci. Vet. Med. Cluj-Napoca. Vet. Med.* 2018. <https://doi.org/10.15835/buasvmcn-vm:002717>.
32. Rosas C.C., Vásquez B.P., del Sol M. Histological and Histochemical description of the liver of the guinea pig (*Cavia porcellus*). *J. Morphol.* 2010. <https://doi.org/10.4067/S0717-95022010000100021>.
33. Garber J.C. et al. *GUIDE FOR THE CARE AND USE OF LABORATORY ANIMALS*. 8th ed. 21(3). Washington; 2011.
34. Percie du Sert N. *et al.* The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *PLoS Biol.* <https://doi.org/10.1371/journal.pbio.3000410>.
35. Williams W.R. and Kendall L.V. Blood collection in the guinea pig (*Cavia porcellus*). *Lab Anim. (NY)*. <https://doi.org/10.1038/labam.787>.
36. Kopteva K.E. et al. The technique of autopsy and removal of organs in laboratory animals. *Anim. Sci. Res.* 2019. <https://doi.org/10.29296/2618723X-2019-02-05>.

Figures



Figure 1

View of the outside (a) and on transvers sections (b) dried kuchala (*Arum korolkowii* Regel) tubers and prepared 10% tincture (c).

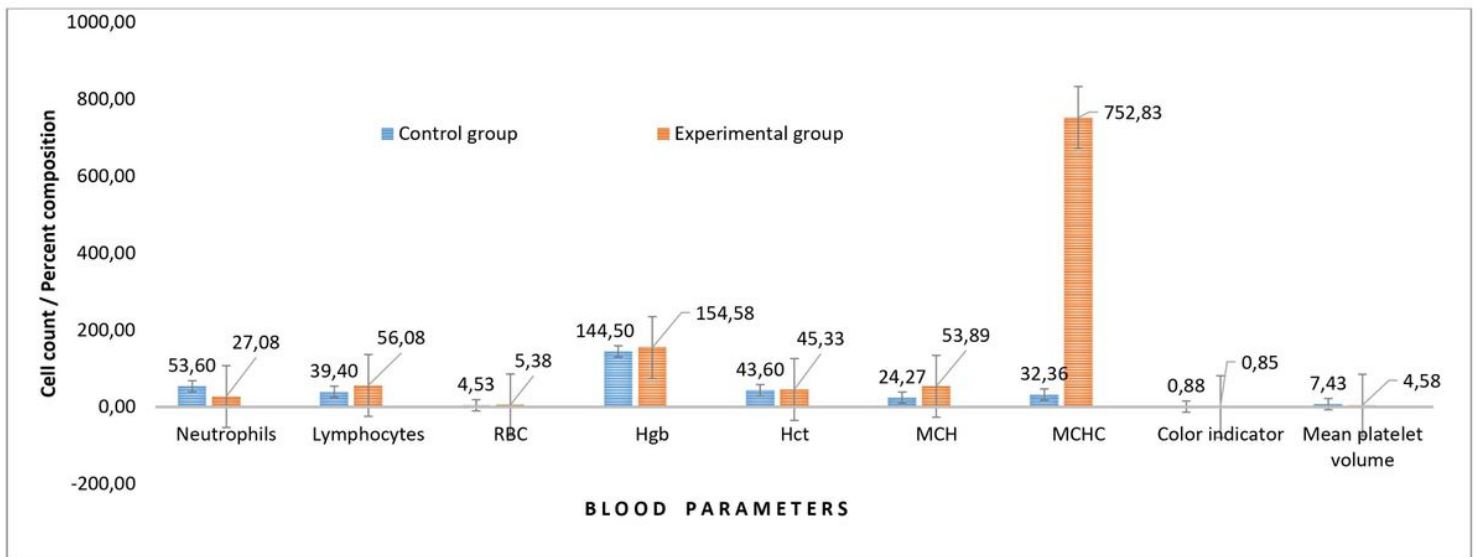


Figure 2

Hematological parameters (mean \pm s.e.m.) which were significantly different between the control and the experimental groups of guinea pigs. Notable differences were observed in percentage of neutrophils (dP < 0.001), lymphocytes (dP < 0.001), RBC (dP < 0.001), Hgb (dP < 0.001), Hct (cP < 0.01), MCH (dP < 0.001), MCHC (dP < 0.001), Hct (bP < 0.05) and mean platelet volume (dP < 0.001).

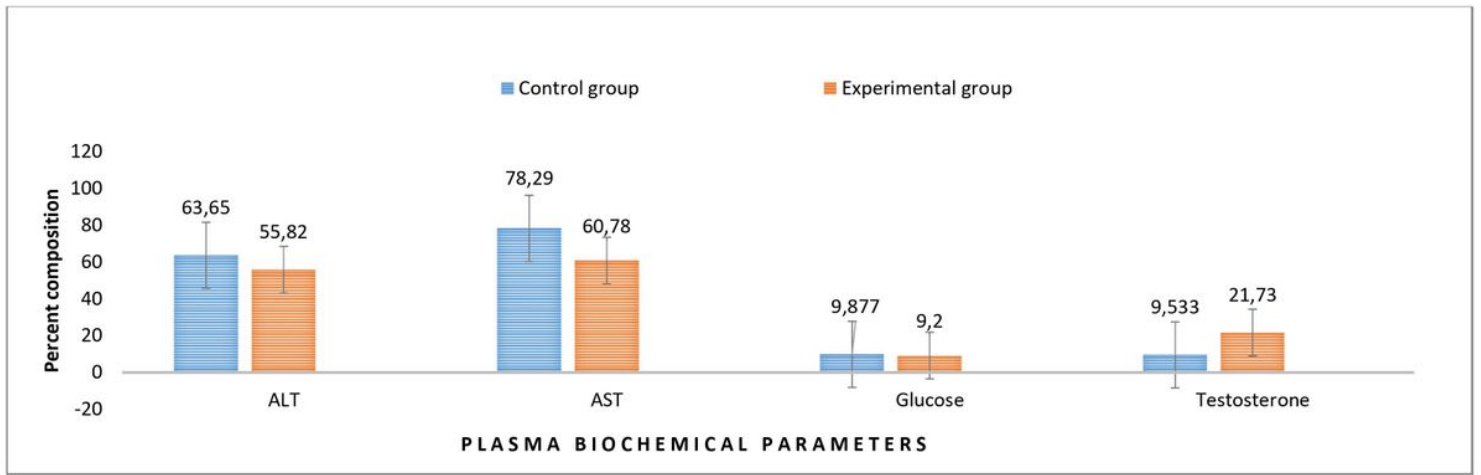


Figure 3

Serum biochemistry parameters (mean \pm s.e.m.) of guinea pigs in control and experimental groups. Besides percentage of glucose in serum, notable differences were observed in percentage of ALT (dP < 0.001), AST (dP < 0.001) and testosterone (dP < 0.001).

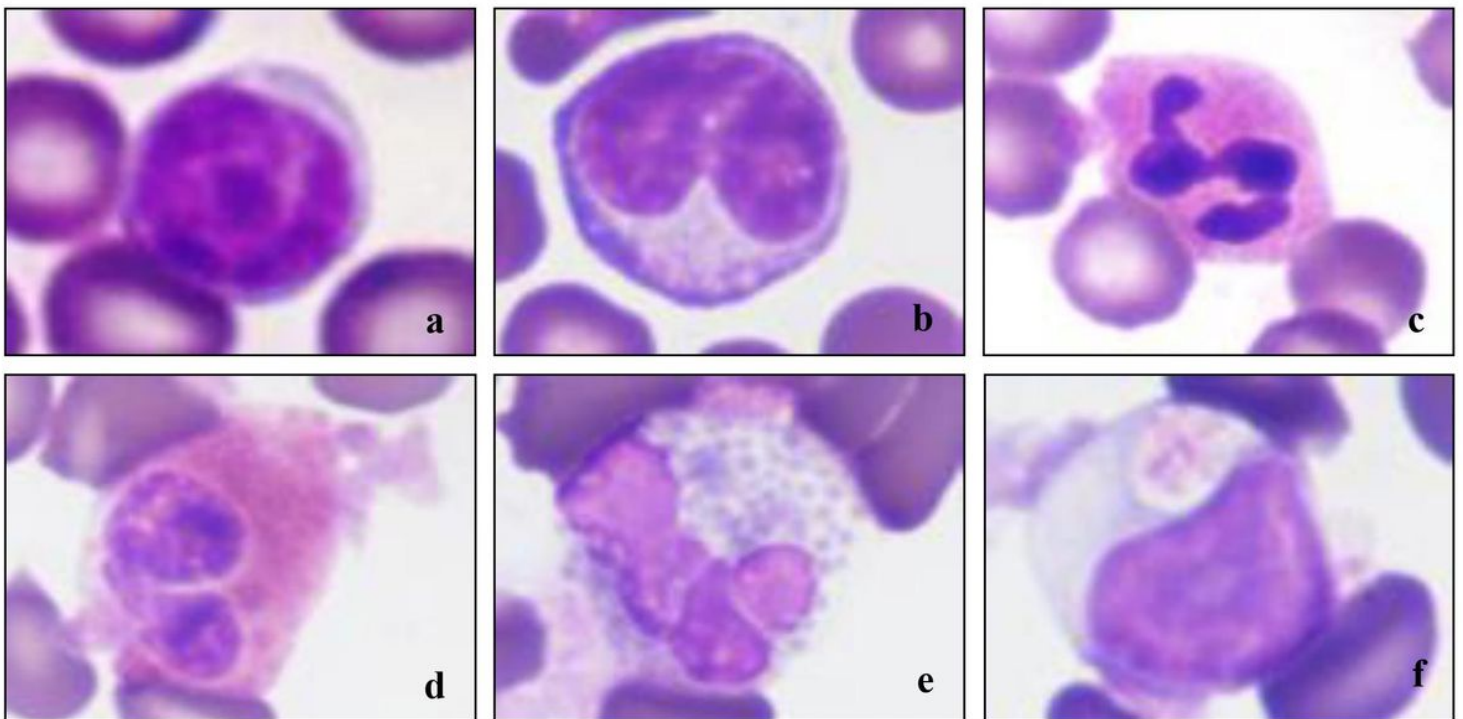


Figure 4

WBC are commonly found in guinea pig blood smears. Lymphocyte with biggest nucleus and small cytoplasmic rim (a), bean-shaped nuclear monocyte (b), segmented neutrophil (c), and eosinophil with purple-colored granules in the cytoplasm (d), basophil with characteristic blue-purple granules (e) and Foa-Kurloff cell with pink intracytoplasmic inclusion body (f). MGG Quick Stain staining, x100 (oil immersion).

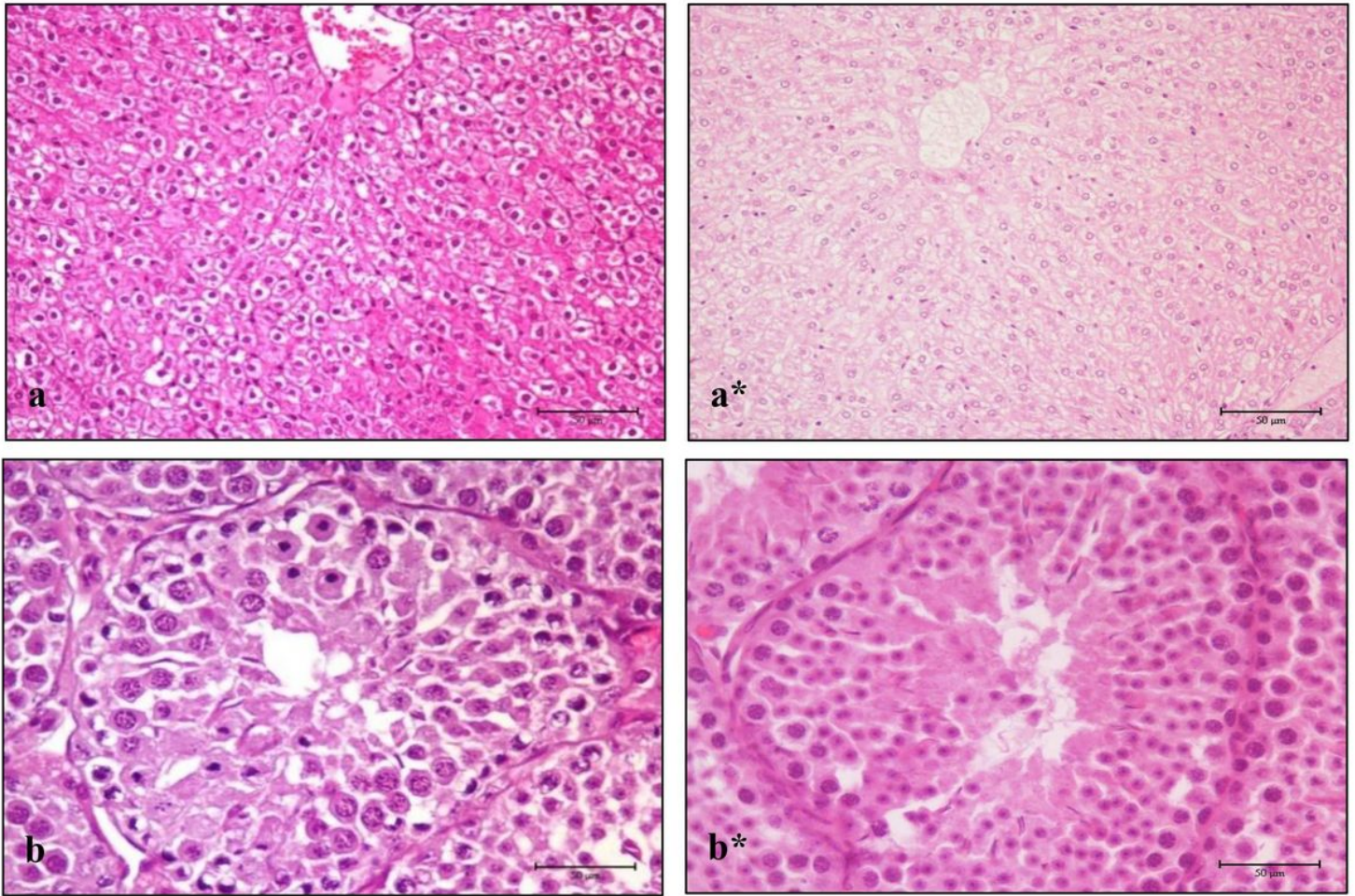


Figure 5

Photomicrographs of paraffin sections of control (a) and experimental (a*) liver, and control (b) and experimental (b*) testes of guinea pigs. Intensive stained normal hepatic lobule with hepatocytes, sinusoids, some macrophages (a) and pale stained experimental hepatic lobules with clearly visible sinusoids, some intensively stained Kupffer cells, apoptotic figures (a*). The control testes section showed the round-oval seminiferous tubule with multilayered spermatogenic cells and sustentacular cells (b). The experimental testes section showed the same picture (b*), but with an increase in spermatogenic cells, hematoxylin and eosin staining, x20 (a, a*) and x40 (b, b*).

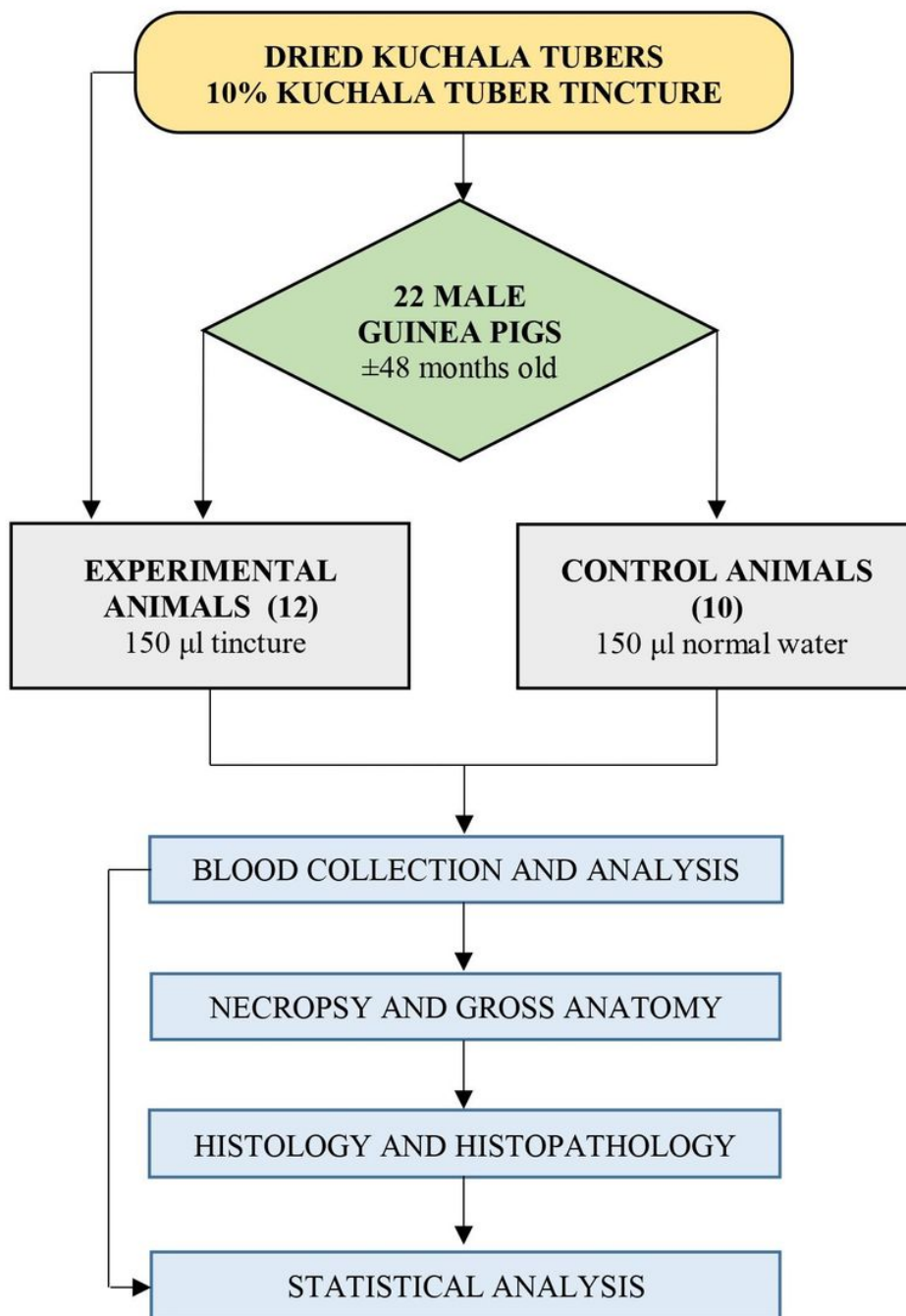


Figure 6

The main phases of the experimental study.

Supplementary Files

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- [Table1and2data.xlsx](#)