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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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ABSTRACT
Kuchala (*Arum korolkowii* Regel) is a medicinal plant commonly used in folk medicine among the Kyrgyz people. The tuber tincture of kuchala is traditionally used in small doses to enhance human sexual potency. However, there is no scientific evidence supporting these medicinal effects. Therefore, we decided to study the effect of kuchala tuber tincture on the sexual potency of adult male guinea pigs. We investigated the effect of kuchala on 12 male guinea pigs, each approximately 48 months old. A 10% tuber tincture of kuchala in 70% ethanol was prepared and administered orally at a daily dose of 150 µl for 30 days. The study employed ethological, hematological, serum biochemistry, gross anatomical, histological, and statistical methods to collect and analyze the data. The hematological and serum biochemistry parameters showed significant differences between the control and experimental groups. In the experimental group, the percentage of neutrophils was significantly lower ($^{d}p < 0.001$) than in the control group, while lymphocyte counts were significantly higher ($^{d}p < 0.001$). Additionally, RBC counts, hemoglobin (Hgb), hematocrit (Hct), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were all significantly higher in the experimental group ($^{d}p < 0.001$; $^{d}p < 0.001$; $^{c}p < 0.01$; $^{d}p < 0.001$; $^{d}p < 0.001$, respectively) compared to the control group. Conversely, the color indicator and mean platelet volume were higher ($^{b}p < 0.05$) and significantly higher ($^{d}p < 0.001$) in the control group than in the experimental group. The levels of alanine transaminase (ALT) and aspartate transaminase (AST) were lower in the experimental group than in the control group (both $^{d}p < 0.001$). Notably, the serum testosterone concentration was much higher ($^{d}p < 0.001$) in the experimental group. Microscopic examination revealed minor structural damage in the liver tissue of the experimental group, indicating a metabolic disorder. However, the testes in the experimental group showed an improvement in spermatogenesis compared to the control group, suggesting a positive effect on reproductive health. The 10% kuchala tuber tincture in 70% ethanol has a positive effect on improving the sexual potency of older guinea pigs by increasing testosterone production and enhancing spermatogenesis.

Background

Since ancient times, people have successfully used folk medicine, which contains an inexhaustible wealth of information about medicinal plants as important therapeutic agents in both human and veterinary medicine [1, 2, 3, 4, 5]. Many treatment methods have been passed down through generations and have been adapted for use in modern medical practice. Kyrgyz folk medicine, in particular, 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 its extreme environment and climate, there is a diverse range of plant species, including more than 200 species of medicinal plants. Many of these medicinal plants used in Kyrgyz folk medicine have not been studied using modern scientific techniques [6]. *Arum korolkowii* Regel is one of the medicinal plants often used in the folk medicine of Central Asia, and it remains relevant today. The vernacular name of this medicinal plant is kuchala. A. korolkowii, described in 1873, belongs to the genus Arum L. of the family Araceae Juss.

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It grows in soil pockets of rocky hillsides, beneath low scrub. Its native range includes Central Asia, North-Western China, Northern Iran, and Afghanistan. *A. korolkowii* is a perennial tuberous herb that sprouts in early spring from a discoid, vertically oriented tuber. It has well-described biological characteristics [7, 8].
However, there is another plant also called kuchla or Chinese kuchla (*Strychnos nux-vomica*). *S. nux-vomica* is an evergreen tree that can reach up to 25 meters in height. Its dried seeds (Nux vomica) are used in both modern and traditional medicine [9, 10].
Arum korolkowii Regel is a very poisonous herb. In folk medicine, a tincture of its tubers is used in small doses as a medicinal raw material to increase human potency and immunity, treat infertility and stomach ulcers, and address diseases of the nasopharynx and respiratory tract. It is also believed to eliminate fatigue and increase strength. The powdered tuber is used to treat poisonous snake and scorpion bites, fungal skin diseases, and hemorrhoids [7]. The medicinal properties of kuchala are mentioned in the works of Avicenna and in the Kyrgyz folk epics "Manas" 11] and "Semetey" [12] .
According to these sources, milky and sour-milky (kumys) tinctures of the tubers are often used among the elderly (over 70 years) to increase male sexual potency. However, there are no modern scientific data proving the medicinal properties of kuchala, particularly its effect on human potency. Additionally, the chemical composition of *A. korolkowii* has not been studied yet. In this regard, we decided to experiment with kuchala tuber tinctures on laboratory animals, with a particular focus on adult male guinea pigs. Thus, the purpose of this study was to investigate the effect of tincture of kuchala tubers (*Arum korolkowii* Regel) on hematological and biochemical parameters of blood serum, as well as on the structure of the testes and liver of adult male guinea pigs (*Cavia porcellus* Linnaeus, 1758).

Materials and Methods

Kuchala tubers and tincture

Dried kuchala tubers of 5 pieces (49.62 g) were procured from local markets in Bishkek, Kyrgyz Republic. Each tuber piece underwent cleaning with warm water followed by 70% ethanol, and subsequently dried in ambient air at room temperature. The tubers, including their peels, were then shredded using a manual grinder. The ground tuber material was weighed using an electronic Precisa scale (Switzerland) and used to prepare a 10% tincture in 70% ethanol. The resulting tincture was poured into a dark glass bottle with a tight seal and stored in a dark place at room temperature. Twice daily, the tincture was agitated, and this process continued for 14 days until the tincture reached readiness. On the 15th day, the tincture was filtered through dense gauze and subsequently filtered through filter paper. The prepared 10% tincture in 70% ethanol was then stored in a refrigerator (+4 °C) and utilized for the experimental study.

Experimental animals and husbandry

We acquired 22 male Abyssinian breed guinea pigs, all approximately 48 months old and with an average weight of 682 g (ranging from 489 to 792 g), from a private guinea pig producer. The animals were clinically healthy at the time of purchase. They were housed in two isolator cages: one measuring 98.7 cm x 347.89 cm x 54.3 cm for 10 control animals and the other measuring 110.3 cm x 398.73 cm x 54.6 cm for 12 experimental animals. The cages were handmade and contained sun-dried clean straw bedding, as well as cardboard huts for enrichment. Bedding was changed every two days or more frequently if necessary. The animals were kept in a room with a temperature ranging from 22 to 26 °C and humidity between 40% and 75%. Guinea pigs had ad libitum access to rodent chow and water. The conditions of animal maintenance and nutrition were in accordance with previously described guidelines [13,14].
The animals were allowed to acclimate in the vivarium for 10 days after delivery before they were used for the study. The research protocol was approved by the Ethics Committee for Animal Experimentation of Kyrgyz-Turkish Manas University, Kyrgyz Republic (№7, 20/12/2019).

Treatments and handling

During the 10-day adaptation period, we closely observed the behavior of the guinea pigs, monitoring their physical activity and body weight. Based on these observations, the experimental animals were divided into two groups. Both the control and experimental groups were formed according to the aforementioned principle, with the control group consisting of 10 male animals and the experimental group consisting of 12 male animals. Both groups were maintained under the same conditions.
Each day, from 8:00 to 9:00 a.m., the experimental animals received 150 µl of kuchala tubers tincture orally, while the control group received 150 µl of normal water orally, for a duration of 30 days. Subsequently, we observed the behavior of the animals through a window in the adjacent room and documented any changes in their behavior.

Blood collection

Blood samples were collected from each guinea pig twice: once five days before the start and once after the completion of administering the tuber tincture, while the animals were under isoflurane anesthesia (3% to 5%). Blood was drawn from the cranial vena cava using a method previously well described in the literature [15]. Throughout the phlebotomy procedure, strict adherence to aseptic and antiseptic protocols was maintained. A 25-gauge, 5/8-inch needle attached to a 3 ml syringe (Zhejiang Huafu Medical Equipment Co. LTD, China) was used to obtain blood from the cranial vena cava. The collected blood was immediately transferred into Gel/Clot Activator (GD060SGC) tubes for serum collection and EDTA.K3 (GD060EK3) tubes for general blood analysis. Additionally, blood smears were prepared for cytological examination.

Blood analysis

The collected blood samples were stored for up to 2-3 hours before processing in a refrigerator at 4°C. Subsequently, the blood samples were submitted to a human clinical-diagnostic laboratory for processing and analysis. Hematological analysis was performed using an APUIA 560 Hematology System (Siemens, Germany) for 1–2 minutes, while serum biochemistry analysis was conducted using a Beckman Coulter AU 480 analyzer (USA-Japan) for 15-20 minutes. Immune chemiluminescence tests were analyzed using an ImmuLite 2000 XPi Immunoassay system (Siemens, Germany) for 1 hour and 6 minutes. These machines undergo routine calibration every 6 months by service professionals using commercial calibration standards. An Erythrocyte Sedimentation Rate (ESR) was determined manually. Blood smears were prepared and stained with MGG Quick Stain (04-090805, Bio Optica Milano s.p.a.) using a flooded slide preparation method for microscopic examination.

Necropsy

The anesthetized animals were euthanized by exsanguination and then underwent necropsy following standard procedures [16]. The heart, liver, and testes were extracted, and their gross anatomy data, including color, consistency, and blood filling, were studied. Morphometric parameters of the liver and testes, such as length, width, and thickness, were recorded. The weight of the organs was measured using an electronic weight scale (Precisa, Switzerland).

Histology

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

Statictical analysis

The hematological and serum biochemical data obtained were subjected to statistical processing using software (Microsoft Excel). Mean, standard deviations (SDs), median, Student's t-test, minimum, and maximum values were calculated. A p-value of less than 0.05 was considered statistically significant.

Results

Physical characteristics of tubers and tincture

The dried kuchala tubers are primarily discoid, ranging from 2 to 6 cm across and 2 to 2.3 cm thick. The peel is light brown and hard, flaking off into small, hard scales during cleaning. Beneath the hard peel, there is a soft, thin, easily removable shell of yellowish-white color. Upon transverse sectioning, the tuber exhibits a yellow-white color, is easily cut, and has a soft consistency akin to plasticine. The tubers can be easily pressed, forming a mushy oily mass. They do not emit a pungent smell, but upon tasting, a strong bitter taste reminiscent of spicy pepper develops after a few moments, lingering for an extended duration. The prepared 10% tincture of tubers in 70% ethanol is transparent, viscous, and yellowish-reddish in color. It possesses a distinctive bitter smell, distinguishable from the odor of alcohol.

Animal behavior

The behavior of animals in both the control and experimental groups was observed from an adjacent room through a window, and the observations were recorded. Both groups exhibited an active lifestyle, frequently engaging in running, playing, and occasional fighting among themselves. They also displayed healthy appetites and consumed food well. However, over time, a noticeable increase in appetite and activity was observed in the experimental animals compared to the control group. Experimental animals displayed heightened aggression, often engaging in fights among themselves and occasionally climbing on the sidewalls of the isolator cage.

Hematology

We observed no adverse effects in terms of clinical signs of anemia or other disorders after guinea pigs were phlebotomized under isoflurane anesthesia. Common hematology parameters including 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 revealed several significant differences in blood parameters between the control and experimental groups (Fig. 1).

Neutrophil percentage in experimental animals was significantly lower ($^{d}p<0.001$) than in control animals, while lymphocyte counts were significantly higher in experimental animals ($^{d}p<0.001$). RBC counts, Hgb, Hct, MCH, and MCHC were significantly higher in experimental animals ($^{d}p<0.001$; $^{d}p<0.001$; $^{c}p<0.01$; $^{d}p<0.001$; $^{d}p<0.001$, respectively) compared to control guinea pigs. However, the color indicator and mean platelet volume were significantly higher in control animals ($^{b}p<0.05$; $^{d}p<0.001$, respectively) compared to experimental animals.

Other hematological parameters such as WBC, monocytes, eosinophils, basophils, MCV, erythrocyte sedimentation rate, and platelets between the animal groups were not statistically significant.

Note: The hematological and serum biochemistry parameters of the animals obtained before the experiment showed almost no difference compared to the animals in the control group. Therefore, only the parameters of the control group animals were presented.

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
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: $^{b}P < 0.05$; $^{c}P < 0.01$; $^{d}P < 0.001$.

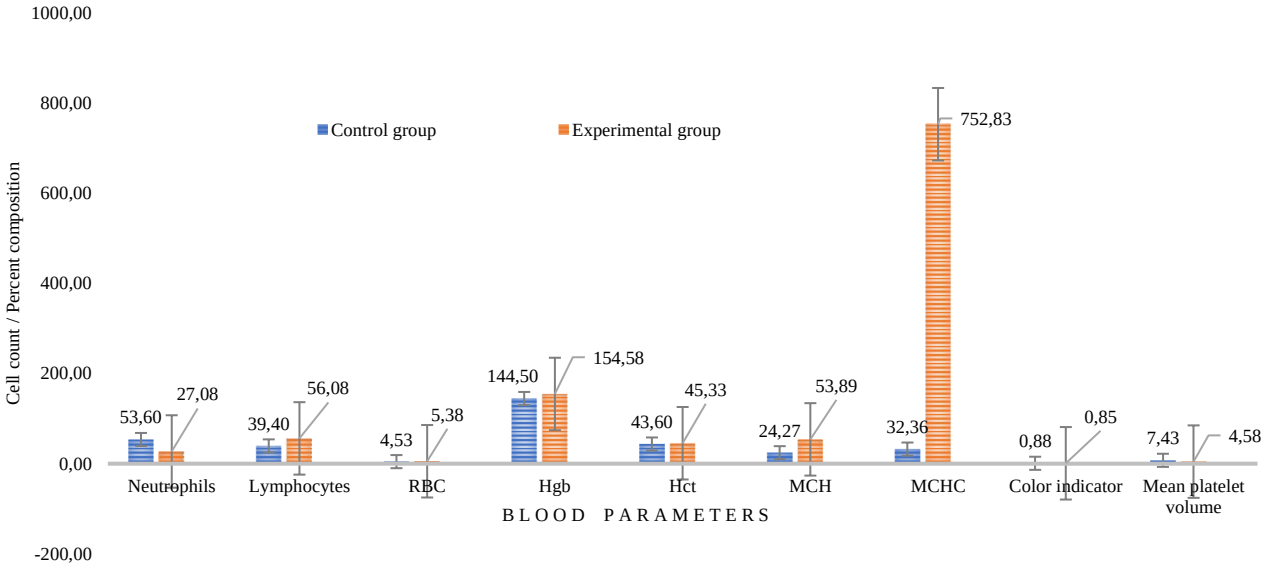

Fig 1 Hematological parameters (mean±s.e.m.) that were significantly different between the control and experimental groups of guinea pigs. Notable differences were observed in the percentage of neutrophils ($^{d}p<0.001$), lymphocytes ($^{d}p<0.001$), RBC ($^{d}p<0.001$), Hgb ($^{d}p<0.001$), Hct ($^{c}p<0.01$), MCH ($^{d}p<0.001$), MCHC ($^{d}p<0.001$), color indicator ($^{b}p<0.05$), and mean platelet volume ($^{d}p<0.001$).

Serum biochemistry

In this study, several serum biochemical parameters including ALT (alanine aminotransferase), AST (aspartate aminotransferase), glucose, and testosterone were evaluated (Table 2). As a result, three of these parameters (ALT, AST, and testosterone) showed significant differences between the control and experimental groups of animals (Fig. 2). ALT and AST percentages in experimental animals were significantly lower than those in control animals (both $^{d}p<0.001$). Additionally, the testosterone concentration was considerably higher ($^{d}p<0.001$) in experimental guinea pigs. However, the glucose percentage in serum was not statistically significant between the studied animal groups.

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: $^{d}P < 0.001$.

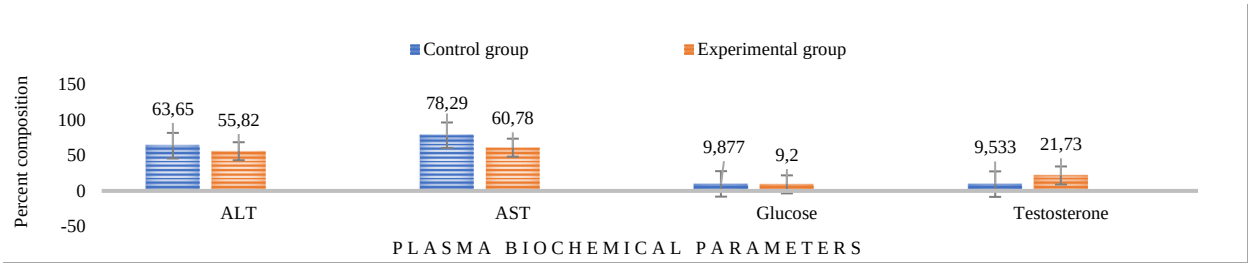

Fig 4 Serum biochemistry parameters (mean ± s.e.m.) of guinea pigs in control and experimental groups are presented. Apart from the percentage of glucose in serum, notable differences were observed in the percentages of ALT ($^{d}p<0.001$), AST ($^{d}p<0.001$), and testosterone ($^{d}p<0.001$).

Gross anatomy and histology

A comparative analysis was conducted on all internal organs, with special attention given to the heart, liver, kidneys, and testes in both control and experimental groups. Results from the visual examination, including color, consistency, and degree of blood filling of the aforementioned organs in necropsied guinea pigs from both groups, did not reveal any significant differences. Similarly, comparative morphometric studies, encompassing organ weight, width, length, and thickness, for the liver and testes showed no statistically significant disparities between the groups. As a result, comparative morphometric data for the studied organs were not provided.

Microscopic examination of blood smears also demonstrated no noticeable differences or changes between the control and experimental groups of animals. The only distinction observed was in the white blood cell (WBC) count of guinea pigs in the experimental group, as depicted in Figure 3, without a detailed description of the structural features of each cell.

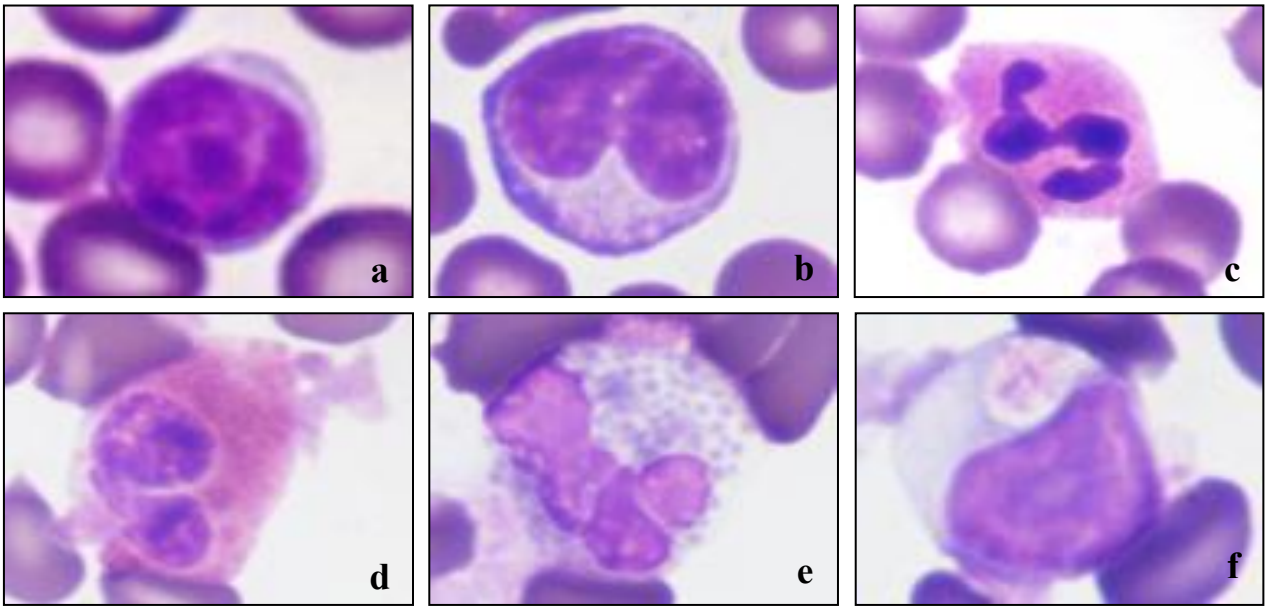

Fig 3 White blood cells (WBCs) commonly observed in guinea pig blood smears. (a) Lymphocyte with the largest nucleus and a small cytoplasmic rim, (b) bean-shaped nuclear monocyte, (c) segmented neutrophil, (d) eosinophil with purple-colored granules in the cytoplasm, (e) basophil with characteristic blue-purple granules, and (f) Foa-Kurloff cell with a pink intracytoplasmic inclusion body. Staining: MGG Quick Stain, magnification x100 (oil immersion).

Microscopic examination of the control guinea pig liver revealed normal hexagonal hepatic lobules of various sizes, containing hepatocytes, a central vein with blood cells, sinusoids with some empty spaces, and Kupffer cells lining the sinusoids in different locations (Fig. 4a). In comparison, liver sections from experimental animals appeared paler than those from the control group. The liver cords, sinusoids, intensely stained Kupffer cells, several apoptotic figures, and apoptosomes were clearly visible (Fig. 4a*).

Histological examination of the control guinea pig testes section showed tubular glands and intertubular connective tissue, characterized by Leydig cells. The round-oval seminiferous tubules varied in size and were surrounded by loose vascular connective tissue, forming testicular lobules. The coiled seminiferous tubules were lined with multilayered spermatogenic cells at different developmental stages and sustentacular (Sertoli)

cells (Fig. 4b). There was a decrease in the number of spermatogenic cells in the seminiferous tubules. In contrast, experimental testis sections showed restoration of spermatogenesis in the seminiferous tubules (Fig.

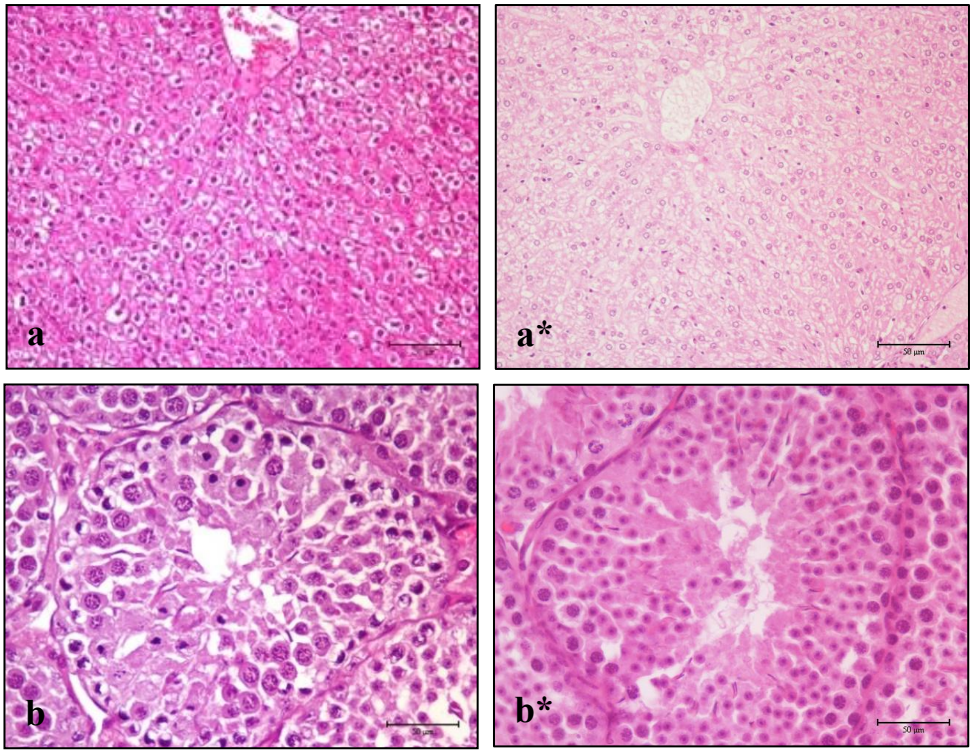

Fig. 4. Photomicrographs of paraffin sections of control (a) and experimental (a*) liver, and control (b) and experimental (b*) testis of guinea pigs. (a) Intensely stained normal hepatic lobule with hepatocytes, sinusoids, and some macrophages. (a*) Pale-stained experimental hepatic lobule with clearly visible sinusoids, some intensely stained Kupffer cells, and apoptotic figures. (b) Control testis section showing round-oval seminiferous tubules with multilayered spermatogenic cells and sustentacular cells. (b*) Experimental testis section showing a similar picture (b*), with an increase in spermatogenic cells. Staining: Hematoxylin and eosin, x20 (a, a*) and x40 (b, b*). 4b*). The quantity of spermatogonia, primary seminiferous tubule was filled with developing spermatozoa.spermatocytes, and spermatids increased, and the lumen of the seminiferous tubule was filled with developing spermatozoa.

Discussion

Our study delves into the medicinal efficacy of kuchala (*Arum korolkowii* Regel) tuber, a traditional remedy with roots in Asian folk medicine, particularly among the Kyrgyz people. By exploring its effects on male potency and reproductive health, we bridge the gap between traditional knowledge and modern scientific inquiry.

The traditional use of kuchala tubers in preparing kumys, a revered fermented mare's milk drink, underscores its significance in Kyrgyz culture [11,12]. References to ancient texts, such as those of Avicenna, further highlight its purported benefits in stimulating sexual desire and cleansing the kidneys. Despite its longstanding use, detailed recipes for kuchala-based remedies remain somewhat elusive, adding an aura of mystique to its therapeutic potential.

Utilizing guinea pigs as our experimental model offers several advantages [17-18]. Their morphofunctional similarities to humans, particularly in reproductive physiology [19-25], make them invaluable for studying male potency and related disorders. Furthermore, hematological and serum biochemical analyses provide valuable insights into physiological changes induced by experimental interventions [15,26].

Our study's focus on adult male guinea pigs, mirroring age-related declines in testosterone levels akin to those observed in aging men, strengthens the relevance of our findings to human health. The observed increases in testosterone levels and improvements in spermatogenesis among experimental animals treated with kuchala tuber tincture underscore its potential as a natural remedy for enhancing male reproductive function.

However, the presence of aberrant hematological parameters raises concerns regarding potential toxic effects of the tincture. This highlights the importance of dosage optimization and careful consideration of administration protocols to minimize adverse outcomes. The observed alterations in liver microstructure and

serum biochemistry parameters further emphasize the need for cautious experimentation and thorough assessment of safety profiles.

Our study contributes to the scientific validation of traditional remedies while also advocating for further research to refine dosage regimens and elucidate mechanisms underlying therapeutic effects. By bridging traditional knowledge with modern scientific inquiry, we pave the way for the development of safer and more effective treatments for male reproductive disorders.

In conclusion, our findings underscore the potential of kuchala tuber tincture as a promising avenue for enhancing male potency and reproductive health. Through rigorous experimentation and collaborative efforts between traditional healers and modern researchers, we can unlock the full therapeutic potential of natural remedies like kuchala, enriching our understanding of human health and well-being.

Conclusion

Our study demonstrates that the administration of a 10% kuchala (Arum korolkowii Regel) tuber tincture in 70% ethanol has a beneficial impact on enhancing the sexual potency of aged male guinea pigs. This effect is attributed to the increased production of testosterone and enhanced spermatogenesis observed in the experimental group.

Furthermore, our findings suggest that any potential toxic effects of this tincture on the animal organism can be mitigated by reducing the dosage. Future research endeavors will focus on determining the optimal concentration of the drug, specifically the tincture in ethanol, to maximize its therapeutic benefits while minimizing adverse effects.

These results provide valuable insights into the potential use of kuchala tuber tincture as a natural remedy to address age-related sexual potency issues in male guinea pigs. Further exploration in this direction holds promise for the development of novel therapeutic interventions for similar conditions in humans.

Competing interest

The authors declare that they have no conflict of interests.

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