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Cardiac puncture as a survival and multiple blood collection method in laboratory rats

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Abstract

Background Blood collection is a vital tool for diagnosis and treatment. Cardiac puncture is considered a suitable terminal technique to attain a single large and good quality blood sample at the end of an experimental period. The present study aimed to modify the cardiac puncture technique to make it a non-terminal multiple blood sampling method. Forty-eight adult Wistar rats of both sexes were randomly divided into four equal groups, each containing six animals for each sex. Cardiac puncture blood sampling, under anesthesia by 3–5% isoflurane, was executed once (group I), twice (group II), three times (group III), and four times (group IV), with a week interval from the first, second, third, and fourth groups, respectively, throughout five weeks. By the end of each group, rats were euthanized under anesthesia with a ketamine/xylazine cocktail to get the heart tissue samples. The hematological parameters, including red blood cell (RBC) and platelet (PLT) counts, hemoglobin (Hb) content, packed cell volume (PCV), and blood indices were investigated. Serum activities of lactate dehydrogenase (LDH), creatine kinase (CK), and aspartate aminotransferase (AST) were estimated. The cardiac levels of malondialdehyde (MDA), glutathione (GSH), and nitric oxide (NO) were reported. Histopathological examination of the cardiac tissue was conducted.

Results We found no significant differences in any of hematological, biochemical, or oxidative stress parameters. Histopathological analysis demonstrated relatively moderate, non-progressive cardiac alterations following repeated sampling, suggesting minimal tissue injury.

Conclusions The 3Rs principle for animal welfare is implemented through the use of the heart puncture technique, which can be modified to serve as a non-terminal approach for collecting multiple blood samples.

Keywords Cardiac puncture, Rats, Survival technique, Oxidative stress, Hematological parameters, Histology

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Introduction

The blood sampling is a useful method for health monitoring and investigating the impact of different experimental factors (Harikrishnan et al., 2018). For each 100 g of rodent body weight, the volume of circulating blood is about 6.3 to 8.0 ml (Everds, 2007). To collect blood samples safely from the rodents, the maximum allowed volume is limited to 10–15% of the total blood volume or less than 1% of the total body weight (Kusmeirczyk et al., 2020). Blood volume can be restored within 24 h. In laboratory rats, the volume of extracted plasma varies from 15.3 to 40.4 ml/kg (Baker et al., 2006).

There are several methods for blood sampling during animal life (non-terminal) or after euthanasia (terminal). It is documented that sampling techniques can affect various parameters in laboratory animals, such as stress hormone level, tissue integrity (Meyer et al., 2020), body weight, daily water consumption, and feed intake (Teilmann et al., 2014).

Blood collection from the retrobulbar plexus/sinus (Harikrishnan et al., 2018), facial vein, and sublingual vein (Gjendal et al., 2020) are common examples for non-terminal blood sampling. Unfortunately, the blood volume obtained by these methods is relatively small and mostly contaminated (Tsai et al., 2015). Collecting blood from the jugular vein by exsanguination is considered a terminal method (Svete et al., 2012). In rodents, the blood volume collected via exsanguination is typically equal to half of the total blood volume. The average blood volume of rodents is dependent on their body weight. Cardiac puncture allows the collection of up to 10 ml of blood from a single rat weighing 150 g (Beeton et al., 2007). For a long time, cardiac puncture was considered a non-survival method since all the previous cardiac puncture techniques targeted the heart through penetrating the diaphragm and subsequently caused internal bleeding as well as serious damage to the internal organs such as the liver, lung, and stomach.

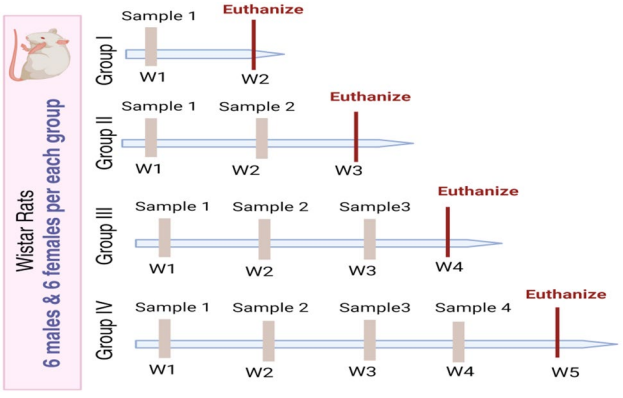

Fig. 1 Schema for experimental design animal groups

Williams and his colleagues (2020) suggested that death is not a requirement for cardiac puncture use. In terms of animal welfare, they propose that heart puncture is the most beneficial method for blood samples. Unlike other blood sample procedures, this method does not increase small rodents' susceptibility to infection or have any adverse effects on their vision or lives. Accordingly, the goal of this study is to improve the heart puncture blood collection technique to ensure its continued existence as a survival method, thereby adhering to the 3Rs principle.

Materials and methods

Material

Ethyl alcohol (C_2H_5OH, 70% volume/volume) and chlorhexidine ($C_{22}H_{30}C_{l2}N_{10}$, 99.9% purity) were purchased from Perfect chemical company for medicinal and cosmetic products, Sadat city, Egypt. Gauze and 24-gauge syringes were purchased from the international company for medical necessities, Industrial zone, Abu Tig, Assiut, Egypt. Isoflurane ($C_3H_2ClF_5O$, > 99%) was obtained from the Arab company for gelatin and pharmaceutical product, 6th October, Egypt.

Animals

Males and females Wistar rats (*Rattus norvegicus*) weighing 160 ± 10 g, were utilized in the present study. Rats were obtained from the Research Institute of Medical Entomology, Agouza, Giza, Egypt. Rats were transported in specially designed cages in air-conditioned vehicle to the UResearch Animal Facility (URAF) for the onset of the experiments. Rats were received and kept for one week in the quarantine room for monitoring of their health status and acclimatization. Rats were housed in polyacrylic cages with six animals per cage in a well-ventilated animal holding room, on a 12 h/12 h light-dark cycle, at room temperature (22–25 °C).

Experimental design

Fourty-eight healthy adult Wistar rats of both sexes (males and females) were randomly divided into four main groups with 12 rats per group (6 males and 6 females). The four groups were treated as shown in Fig. 1. Group I subjected the rats to a single heart puncture blood sample, while Group II, Group III, and Group IV underwent blood samples two times, three times, and four times consecutively, each with a one-week interval between each successive sample. All rat groups were euthanized.

Cardiac puncture technique

1. All animals were anesthetized with isoflurane, at 3–5%, and then the left side of the chest was shaved.

2. The shaved area was cleaned and decontaminated with chlorhexidine 5% then alcohol 70%, successively.
3. The animals were restrained in dorsal recumbency.
4. By a 24G syringe, the needle was inserted into the intercostal muscles of the left side chest between rib numbers 7 and 9 near the sternum (Fig. 2).
5. The heartbeats were felt with the hand to confirm the position.
6. The needle's direction was slightly perpendicular to the heart.
7. If the needle correctly penetrates the left ventricle of the heart, a blood dot is found at the base of the needle.
8. At this point, blood was withdrawn slowly with a maximum volume of 1 ml.

Animal handling and collection of the samples

Blood was drawn from all animal groups and placed in EDTA-containing tubes for hematological analysis and serum tubes for biochemical analysis. For oxidative stress analysis, the heart tissues were sliced and stored at -80 °C. Another sample of cardiac tissue was suspended in 10% formal saline for preparative fixation for histological fixation.

Hematological parameters

The hemoglobin (Hb) content, red blood cell count (RBC), packed cell volume (PCV), platelet count (PLT), and blood indices including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were computed using an animal blood counter (Micros analyzer model ABC vet #907-AB-6012, France).

Serum biochemical parameters

The activity of aspartate aminotransferase (AST) was estimated using the colorimetric technique described by Young (1990). AST stimulates the transfer of amino group from L-aspartate to 2-oxoglutate, resulting in oxaloacetate. In the presence of 2,4-dinitrophenylhydrazine, oxaloacetate hydrazone is formed, and its level is directly proportional to the activity of AST.

The activity of creatine kinase (CK) was estimated kinetically, according to Friedman and Young (2001). In

Fig. 2 Cardiac puncture by 24G syringe

presence of creatine phosphate, CK catalyzes the phosphorylation of ADP into ATP. CK activity can be indirectly determined by measuring nicotinamide adenine dinucleotide phosphate (NADPH) levels resulting from the coupled reactions by hexokinase and glucose-6-phosphate dehydrogenase, at λ = 340 nm. Lactate dehydrogenase (LDH) was estimated as described by Van der Heiden et al. (1994). LDH catalyzes the conversion of pyruvate into L-Lactate, with accompanied conversion of $NADH + H^+$ into NAD^+. Initially, the activity of LDH is directly proportional to the concentration of the resultant NAD^+. Thus, LDH activity is estimated by measuring the decrease in absorbance of $NADH + H^+$, at λ = 340 nm. The kits of AST, CK and LDH were purchased from Spectrum-Diagnostic (Egyptian Company for Biotechnology, Cairo, Egypt).

The levels of nitric oxide (NO) were determined based on the method suggested by Montogomery and Dymock (1961).

In biological samples, NO can be determined via nitrite. NADPH reduces nitrate (NO_3^-) in the sample into nitrite (NO_2^-). The resultant nitrite reacts with sulfanilamide and N-(1-napthyl) ethylenediamine dihydrochloride to form red-violet diazo dye. The absorbance of the produced dye is read at λ = 540 nm. The kit for measuring NO was obtained from Biodiagnostic Company, Giza, Egypt.

Determination of oxidative stress parameters

For the determination of malondialdehyde (MDA) content, a small part of heart tissue was homogenized in 50 mM cold potassium phosphate buffer (PBS, pH 7.5), and then was centrifuged at 4000 rpm for 15 min. The level of MDA was measured in the supernatant as described by Okhawa et al. (1979). In an acidic pH, MDA reacts with thiobarbituric acid (TBA) to form a TBA-reactive product with a pink color that can be measured at λ = 534 nm. The remaining part of the heart was homogenized in 50 mM cold PBS (pH 7.5) containing 1mM EDTA, then centrifuged at 9000 rpm for 15 min at 4 °C. The produced supernatant was used for measuring the glutathione (GSH) content according to the methods described by Beutler et al. (1963). GSH reduces 5,5`dithiobis – 2 - nitrobenzoic acid into a yellow chromogen. The concentration of the produced chromogen is directly proportional to GSH level, and its absorbance is read at λ = 405 nm. The levels of MDA, and GSH were assayed using biodiagnostics kits (Dokki, Giza, Egypt).

Histological examination

Heart specimens were collected and fixed in 10% neutral buffered formalin. The processing of tissue was then performed using an ascending concentration of ethanol and xylene. Tissues were embedded in paraffin and sectioned into 4 µm thick sections using a rotary microtome (Leica 2135). Tissue sections were stained with hematoxylin and eosin stain and examined using a light microscope with ab installed digital camera.

Statistical analysis

Data were analyzed using the International Business Machines – Statistical Package for the Social Sciences (IBM-SPSS) version 23 software. The Kolmogorov–Smirnov test was executed to illustrate the population distribution. The normally distributed data were analyzed using an independent t-test. The data is displayed as a mean ± standard error (SE). A P-value < 0.05 is considered a significant difference.

Results

Effect on the studied hematological parameters

The results of Hb content, PCV, RBC count, and PLT count in all groups are displayed in Table 1. There were non-significant differences between the investigated parameters. Changes in blood indices including (MCV, mean MCH and mean MCHC) of all groups are demonstrated in Table 2. The results of all the studied hematological parameters revealed insignificant changes among the different experimental groups.

Effect on the studied oxidative stress parameters

In Table 3, the levels of MDA, GSH and, nitric oxide (NO) in the cardiac tissue of all the studied groups are presented.The frequency of sampling did not exhibit any significant effect on the lipid peroxidation biomarker, NO level, or GSH content in the cardiac tissue of rats.

Effect on the studied biochemical parameters

The serum activities of LDH, CK, and GOT are reported in Table 4. No significant changes in the activities of LDH, CK, and GOT were reported after multiple samplings of the blood using a cardiac puncture.

Effect on histopathological structure of cardiac tissue

The microscopical investigation of heart tissue in the rats of all groups revealed mild alternations in the heart tissue. Rats in group IV revealed edema with moderate leukocytic edema in the cardiac muscle of males (Fig. 3a), in addition fibrosis was observed between the muscle fibers of females (Fig. 3b). Moreover, rats in group III showed pericarditis and mild leukocytic infiltration in male rats (Fig. 3c), while hemorrhage between the muscle bundles were noticed (Fig. 3d in females). In group II, males showed edema and leukocytic infiltration in the cardiac muscle (Fig. 3e). However, females of group II exhibited mild leukocytic infiltration and fibrosis at the periphery of the cardiac muscle (Fig. 3f). Rats of group I revealed mild lesions close to the heart valve in males (Fig. 3g).

Table 1 The hemoglobin (Hb) content, red blood cell (RBC) count, packed cell volume (PCV) and platelet (PLT) count in the blood of male and female rats after collecting blood from the heart via cardiac puncture technique (once, twice, three- and four-times)

Parameter	Sex	Blood sampling frequency			
		Single	Double	Triple	Quadruple
Hb (g/dL)	Male	14.28 ± 0.40	14.20 ± 0.13	14.68 ± 0.24	14.30 ± 0.44
		–	$p = 0.874$	$p = 0.399$	$p = 0.958$
	Female	13.86 ± 0.22	13.12 ± 0.39	13.78 ± 0.28	13.92 ± 0.27
		–	$p = 0.096$	$p = 0.851$	$p = 0.888$
RBC (10^6/mL)	Male	5.46 ± 0.24	5.26 ± 0.13	5.61 ± 0.09	5.68 ± 0.38
		–	$p = 0.581$	$p = 0.67$	$p = 0.54$
	Female	5.19 ± 0.09	4.88 ± 0.06	5.14 ± 0.09	5.17 ± 0.27
		–	$p = 0.175$	$p = 0.85$	$p = 0.95$
PCV (%)	Male	44.25 ± 1.25	44.02 ± 0.41	45.52 ± 0.74	44.36 ± 1.35
		---	$p = 0.876$	$p = 0.393$	$p = 0.94$
	Female	42.98 ± 0.09	40.68 ± 0.06	42.72 ± 0.09	43.14 ± 0.27
		–	$p = 0.097$	$p = 0.844$	$p = 0.904$
PLT (10^3/mL)	Male	698.50 ± 36.26	784.00 ± 38.17	756.20 ± 40.81	781.60 ± 53.29
		–	$p = 0.199$	$p = 0.379$	$p = 0.211$
	Female	851.80 ± 50.98	933.80 ± 33.92	923.40 ± 27.41	924.00 ± 44.63
		–	$p = 0.169$	$p = 0.227$	$p = 0.223$

Data is presented as a mean ± standard error of the mean. $P > 0.05$: insignificant difference, as compared to the corresponding single blood sampling group in the same row

Table 2 The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) in the blood of male and female rats after collecting blood from the heart via cardiac puncture technique (once, twice, three- and four-times)

Parameter	Sex	Blood sampling frequency			
		Single	Double	Triple	Quadruple
MCV (*f*L)	Male	81.33 ± 1.71	81.90 ± 2.01	81.22 ± 1.10	76.98 ± 1.71
		---	$p = 0.817$	$p = 0.966$	$p = 0.096$
	Female	82.94 ± 1.25	83.40 ± 1.91	83.14 ± 2.17	78.96 ± 2.22
		---	$p = 0.868$	$p = 0.942$	$p = 0.164$
MCH (g/dL)	Male	26.25 ± 0.54	27.06 ± 0.49	26.20 ± 0.36	24.88 ± 1.11
		---	$p = 0.444$	$p = 0.962$	$p = 0.203$
	Female	26.72 ± 0.41	26.88 ± 0.62	26.82 ± 0.69	25.98 ± 0.44
		---	$p = 0.84$	$p = 0.90$	$p = 0.358$
MCHC (%)	Male	32.20 ± 0.07	32.22 ± 0.02	32.24 ± 0.02	32.32 ± 0.12
		---	$p = 0.852$	$p = 0.709$	$p = 0.271$
	Female	32.24 ± 0.02	32.26 ± 0.02	32.26 ± 0.02	32.28 ± 0.02
		---	$p = 0.555$	$p = 0.555$	$p = 0.245$

Data is presented as a mean ± standard error of the mean. $P > 0.05$: insignificant difference, as compared to the corresponding single blood sampling group in the same row

Females of group I showed a normal histological structure of the heart with no evidence of inflammation or fibrosis (Fig. 3h).

Table 3 The levels of malondialdehyde (MDA), glutathione (GSH) and nitric oxide (NO) in the heart tissue of male and female rats after collecting blood from the heart via cardiac puncture technique (once, twice, three- and four-times)

Parameter	Sex	Blood sampling frequency			
		Single	Double	Triple	Quadruple
MDA (nmol/g tissue)	Male	0.64 ± 0.03	0.58 ± 0.11	0.45 ± 0.03	0.46 ± 0.08
		---	$p = 0.60$	$p = 0.078$	$p = 0.10$
	Female	0.56 ± 0.04	0.66 ± 0.05	0.65 ± 0.05	0.63 ± 0.03
		---	$p = 0.117$	$p = 0.192$	$p = 0.262$
GSH (mmol/g tissue)	Male	0.23 ± 0.03	0.18 ± 0.01	0.18 ± 0.04	0.18 ± 0.02
		---	$p = 0.169$	$p = 0.201$	$p = 0.169$
	Female	0.21 ± 0.05	0.13 ± 0.06	0.10 ± 0.03	0.13 ± 0.07
		---	$p = 0.282$	$p = 0.146$	$p = 0.260$
NO (mmol/g tissue)	Male	4.46 ± 0.22	4.45 ± 0.76	5.28 ± 0.38	4.70 ± 0.40
		---	$p = 0.99$	$p = 0.247$	$p = 0.727$
	Female	6.04 ± 0.43	6.97 ± 0.61	5.59 ± 0.42	6.71 ± 0.23
		---	$p = 0.156$	$p = 0.487$	$p = 0.298$

Data is presented as a mean ± standard error of the mean. $P > 0.05$: insignificant difference, as compared to the corresponding single blood sampling group in the same row

Discussion

Blood collection is a regular necessity for preclinical experiments on rodents. The method of blood withdrawal in rats is determined by the volume of blood required, the frequency of sampling, the health status of the animal to be bled, and the technician's skill level. Moreover,

Table 4 The serum activities of lactate dehydrogenase (LDH), creatine kinase (CK) and aspartate aminotransferase (AST) in male and female rats after collecting blood from the heart via cardiac puncture technique (once, twice, three- and four-times)

Parameter	Sex	Blood sampling frequency			
		Single	Double	Triple	Quadruple
LDH (U/mL)	Male	1.93 ± 0.22	1.38 ± 0.19	1.38 ± 0.17	1.53 ± 0.11
		---	$p = 0.05$	$p = 0.058$	$p = 0.142$
	Female	1.07 ± 0.16	1.32 ± 0.25	1.36 ± 0.05	1.23 ± 0.24
		---	$p = 0.374$	$p = 0.309$	$p = 0.599$
CK (U/mL)	Male	0.66 ± 0.04	0.63 ± 0.13	0.60 ± 0.08	0.67 ± 0.04
		---	$p = 0.805$	$p = 0.692$	$p = 0.934$
	Female	0.45 ± 0.02	0.48 ± 0.09	0.48 ± 0.06	0.62 ± 0.11
		---	$p = 0.791$	$p = 0.83$	$p = 0.172$
AST (U/L)	Male	37.33 ± 1.33	38.75 ± 2.50	35.00 ± 1.95	35.80 ± 7.93
		---	$p = 0.862$	$p = 0.765$	$p = 0.844$
	Female	34.90 ± 0.10	34.80 ± 5.19	33.25 ± 1.03	30.80 ± 4.20
		---	$p = 0.987$	$p = 0.80$	$p = 0.513$

Data is presented as a mean ± standard error of the mean. $P > 0.05$: insignificant difference, as compared to the corresponding single blood sampling group in the same row

Institutional Animal Care and Use Committee (IACUC) approval requires that animals be subjected to as little suffering, stress, and pain as possible when obtaining blood samples (Parasuraman et al., 2010). A substantial variety of in vitro and in vivo experiments require the collection of large blood volumes from rodents. The most common blood collection approach in rats, which allows for greater blood volumes (e.g., 0.2-1 mL) (Diehl et al., 2001), is retro-orbital bleed, a survival way, and heart puncture, a terminal method. Despite the development of new and improved approaches for obtaining blood samples from small laboratory animals, obtaining enough blood from small animals is not always straight forward. Thereby, the present study aims to prove that cardiac puncture may be a suitable technique to obtain repeated, large, good quality blood samples from rodents as a non-terminal and multiple method.

The mortality rate would likely raise ethical concerns for continued performance amongst many ethics committees (Clough, 1982). Because it can be altered by sickness, trauma, or environmental concerns, the mortality rate is a regularly used statistic that provides a retrospective assessment of welfare. In consonance with the report of Williams and his colleagues (2020), the present study showed that death is not a requirement for blood collection using serial cardiac puncture methods. Several preclinical studies have identified much potential mortality for serial blood sample collection. These include the study of Forbes et al. (2010), who confirmed that a death rate of 4/214 mice (2%) was recorded in retrospective research with serial facial vein sampling. In addition, when blood samples were serially collected from

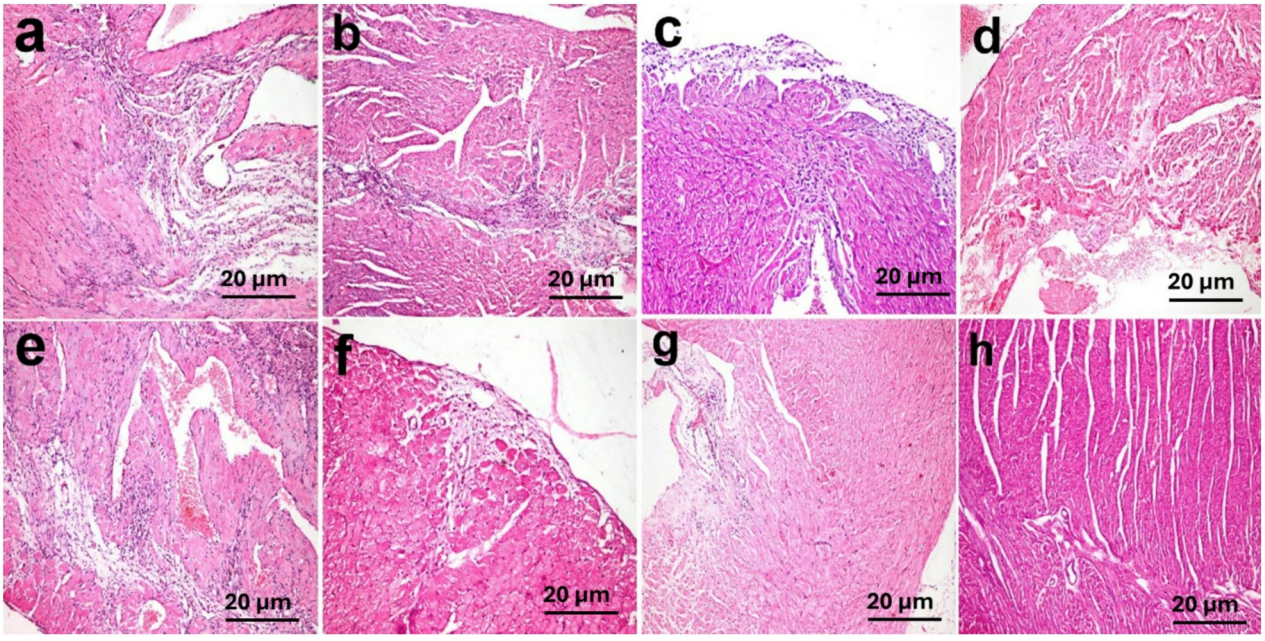

Fig. 3 Histopathology of rat heart after exposure to heart puncture. In group IV, **a** moderate leukocytic edema in the cardiac muscle in males, **b** fibrosis in between the muscle fibers in females, In group III, **c** pericarditis and mild leukocytic infiltration in males, **d** hemorrhage in between the muscle bundles in females, In group II, **e** edema and leukocytic infiltration in the cardiac muscle in males, **f** mild leukocytic infiltration and fibrosis at the periphery of the cardiac muscle in females, In group I, **g** mild leukocytic infiltration and fibrosis close to the heart valve in males, **h** histological structure with no evidence of inflammation or fibrosis in females. Hematoxylin and eosin stain X 100

mice using the facial vein method, Frohlich et al. (2018) recorded a higher mortality rate of (4/12, 33%). In addition, Williams et al. (2020) stated that direct handling mortality rate (3.9%) using cardiac puncture and isoflurane on 6611 white-footed mouse, *Peromyscus leucopus* captures. The present study demonstrated apparent animal survival following serial blood collection via heart puncture in laboratory rats, which may be a consequence of the skills and adequacy of the technique. Moreover, in the experiments that require the collection of large blood volumes from rodents, one of the most important consequences is that more animals would have to be used. Therefore, it is possible to avoid increasing the number of animals required for collecting blood from the cardiac puncture without causing unacceptable distress to the animals.

The evaluation of body weight and food and water intake allowed for an unbiased estimation of the impacts produced to the animals due to painful and stressful blood collection method. Everds et al. (2013) reported that decreased total body weights or body weight gain, altered food consumption and activity are the most important and reliable outcomes of repeated blood collection from rodents. In the present study, following collection of four serial blood samples, the total body weights of the animals either male or female had been significantly increased. This result may confirm the safety of the blood collection using the applied technique.

Hematological and biochemical profiles of blood, particularly hemoglobin and erythrocytes, can provide important information about the internal environment of the organism (Velisek et al., 2005), due to their responsibilities for transportation of nutrition, oxygen, and metabolic wastes (Min & Kang, 2008). There has been concern around performing repeated blood collection from rodents, mainly due to the potential impact on erythroid mass and the potential that the additional stresses may cause a confounding interpretation of safety end points (Wickremsinhe et al., 2016). The present study showed a non-significant change in the primary markers of erythroid mass (erythrocyte count, hemoglobin concentration, and hematocrit) in rats subjected to serial blood sampling via heart puncture, which may be explained by absence of hemorrhage after sample collection and the rapid erythroid regeneration process. Moreover, the direct collection of the blood from heart using our applied technique would have the advantage over other methods of blood sampling as the collected blood could be replaced.

These results were confirmed by our vets and technicians at the animal facility, who confirmed that no abnormal clinical signs were registered or reported. In addition, the present study demonstrated the ability to successfully collect about 1 ml of blood from rats via the heart puncture once a week for four continuous weeks without affecting the health status of the animals.

The process for collecting blood for analysis is critical and accounts for a considerable number of pre-analytic mistakes (Erdem et al., 2021). It was reported that different methods of blood collection in rats may be accompanied by stress (Wickremsinhe et al., 2016). Monitoring oxidative stress (OXS) markers in rats is constrained seriously by the adverse effects of blood sampling. Therefore, measuring OXS markers is highly demanded in the laboratory. Because it has been related to several adverse effects, OXS is currently the focus of much interest and investigation (Vassalle et al., 2020). Reactive species (RS) cause changes in biomolecules such as DNA, lipids, and proteins during oxidative stress (Knasmüller et al., 2008). Moreover, it has already been proven that most blood sampling methods needs some handling procedures for animal such as picking up animals by the tail that may actually simulate the act of being captured and provoke stress responses (Hurst & Wes, 2010; Resch et al., 2019). The present study confirmed the findings of Gouveia and Hurst (2013) and Roughan and Sevenoaks (2019), who explained that handling of animals is considered a source of stress. In the present study, the handling need for our applied blood collection technique is not considered, which may explain non- significant change in the measutings of OXS markers (MDA, GSH, and NO).

O'Brien et al. (1997), reported cardiac injury arising from various causes such as ischemia/reperfusion, doxorubicin administration, trauma, and cardiac puncture. Creatine kinase (CK) is a dimeric enzyme that catalyzes the ATP-dependent reversible phosphorylation of creatine (Moghadam-Kia et al., 2016). Elevations of serum CK have been previously reported in cardiac injury (Hashimoto et al., 1987). LDH is an important marker of cardiac injury. The present study demonstrates that the determination of serum LDH and CK potentially provides valuable diagnostic information during cardiac injury. Repeated collection of blood via heart puncture may cause some degree of heart tissue trauma. The present study showed that serial blood sampling via heart puncture in rats does not cause any heart injury. Accordingly, heart puncture, as a repeated and non-terminal procedure, may be a viable technique for acquiring repeated, large, high-quality blood samples from rats.

Conclusions

The current study sheds light on the availability of using heart puncture as a safe and survival blood collection technique in laboratory rodents. The present investigation also confirmed the potential of lowering the number of animals utilized for heart puncture blood collection without causing intolerable suffering to the animals. The Hematological analysis (RBCS, Hb, and blood indices)

and biochemical profiles of blood, particularly OXS markers (MDA, GSH and NO), LDH, and CK affirmed the suitability of the studied technique for collection of large volumes of blood and for repeated use without affecting the health status of the animals.

Abbreviations

ADP	Adenosine diphosphate
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
CK	Creatine kinase
GSH	Glutathione
Hb	Hemoglobin
H&E	Hematoxylin and eosin
LDH	Lactate dehydrogenase
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MDA	Malondialdehyde
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
NO	Nitric oxide
PCV	Packed cell volume
PLT	Platelet count
RBC	Red blood cell
RS	Reactive species
TBA	Thiobarbituric acid

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Author contributions
All authors contributed to the conception, design, and execution of the study, as well as data analysis and interpretation. Muhammad Abdel-Fattah Metwally, Atef Ali Ali, and Ayman Mohamed Saber drafted the manuscript. Reham S. Desoky, Manal M. Zaki, Sohair Ramadan Fahmy, Abeer Mahmoud Badr, and Khadiga Gaafar assisted with experiments, data collection, and manuscript revision. All authors read and approved the final version.

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Data availability
The datasets used or analysed during the current study available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Ethical aproval for conducting the experimental procedures was obtained from UResearch's Institutional Animal Care and Use Committee (URAF-IACUC) in Egypt under the approval number of URAF/13/23. All experimental techniques were carried out in accordance with ARRIVE guidelines and in compliance with Guide for Care and Use of Laboratory animals, 8th edition.

Consent for publication
All the authors assure that this research is their own original work, which has not been published before and is not being considered anywhere else. They also confirm that they have gone through the manuscript and agree to have it published in The Journal of Basic and Applied Zoology.

Competing interests
The authors declare no competing interests.

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