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OPEN PEER REVIEW

Effects of Hirudotherapy in Alloxan-induced Diabetic Male Rats: Histopathological and Biochemical Changes

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1 Original submission

2 comments · 27 Dec 2021 – 15 Jan 2022

Referee 1 Reviewer

REJECT 27 Dec 2021

1. The histopathological images are of very low quality, and the claimed findings are practically invisible in the submitted images.
2. The levels of liver enzymes in animals in the negative control group (healthy animals) are much higher than normal, so the results of these observations cannot be applied to the other groups.
3. The rationale for selecting the parameters evaluated in the present study is not clear. For example, if the aim of the study was to investigate the effect of salvage therapy on alloxan-induced diabetes, the selection of specific and related parameters in this field is recommended.

Editor-in-Chief

MAJOR REVISION 15 Jan 2022

Dear Authors,

Your manuscript has been scrutinized by three different reviewers; major revision is required. We will reconsider your manuscript if you decide to revise the manuscript according to the reviewers' comments. Please upload the revised version along with a point-by-point response to the reviewers.

Kind regards

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Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Dear Editor-in-Chief (JJNPP),

Thank you for reviewing the article **Effects of Hirudotherapy in Alloxan-Induced Diabetic Male Rats: Histopathological and Biochemical Changes**, and your suggestions as it helps us improve the quality of our work. Please see below for the response to the reviewer's comments.

Respectfully,

A.N

2/26/2022

The first referee's answer

The quality of histopathological images is very inadequate and low and the claims are .1 practically invisible in the submitted images

The images have been replaced with better quality images and an explanation was added. In addition, attempts were made to photograph the specific lesions again in order to make it as .clear as possible

The level of liver enzymes in animals of the negative control group (healthy animals) is .2 much higher than normal, so the results of these observations cannot be invoked in other groups

The results obtained from the activity of serum enzymes under the laboratory condition and .using Pars kits made in Iran and BT automatic analyzer on Wistar rats serum

Given that previous studies on leech therapy had not been performed on diabetic rats, the results were based on the results obtained from the autoanalyzer and the analyses. However, it should be noted that geographical regions and the climatic conditions of the region may .also have some effects on the serum enzymatic activity, which requires comparative studies

The parameters evaluated in the present study are not clear on what rationale they were .3 selected. For example, if the aim of the study was to investigate the effect of salvage therapy on alloxan-induced diabetes, the selection of specific and related parameters in this field is .recommended

The title of the article was changed from “Benefits and Hazards of Hirudotherapy in Alloxan-Induced Diabetic Male Rats: Histopathological and Biochemical Changes”, to “Effects of Hirudotherapy in Alloxan-Induced Diabetic Male Rats: Histopathological and Biochemical Changes”, to determine the main purpose of the study, and considering that in studies previously done on traditional treatments for diabetes examined changes in the biochemical and histopathological parameters of liver, kidney, and pancreatic tissues. In diabetic people in some parts of the world, more is done to heal the wounds of the extremities and to avoid amputation. However, in the present study, the possible effects of leech therapy on enzymatic and tissue activities were investigated.

The second referee's answer

It is a good handwriting

.The authors of the article are very grateful for your attention to this manuscript

It is recommended to set more tables
for example
Table ( ) –The results obtained from ….. analysis of ……. Parameters are summarized in -1
…..
….The animals were randomly separated into …. Groups of ….. as follows-2

Due to the journal's guidelines and the limited number of tables and images, the measured biochemical parameters, which were somewhat related, were consolidated so the readers could understand them better. In addition, the design of the grouping of animals was .modified, which is marked in red in the text of the article

The first referee's answer

What was the logic of the study? As far as I know, there's no evidence/ history of diabetes .1 ?treatment by Leech in Iranian traditional medicine

The title of the article was changed from The Benefits and Hazards of Hirudotherapy in Alloxan-Induced Diabetic Male Rats: Histopathological and Biochemical Changes. To the Effects of Hirudotherapy in Alloxan-Induced Diabetic Male Rats: Histopathological and Biochemical Changes, in order to specify the main purpose of this study. Lines 42 to 52 refer to the applications of leech therapy, especially improving the healing process of foot ulcers in diabetics and preventing blood clotting due to compounds in leech saliva. Insulin-dependent diabetics inject insulin to prevent platelet aggregation and thrombosis formation and increase the function of fibrinolytic mechanisms, which in the case of leech therapy in the text of lines 54-55, 57-58 and 244-246 as much as possible. The subject is mentioned. However, after leech treatment, the substances in leech saliva may enter the systemic bloodstream, disrupting the activity of biochemical parameters. Finally, towards the end of the introduction, the .purpose of the study on diabetic and non-diabetic rats is mentioned

Impairment of blood clotting is common in diabetic patients. Leech also impairs blood .2 clots. Thus, there's no reason for using leech to control/treat diabetes

As mentioned in the response to the question of the *Reviewer* referee, people with insulin-dependent diabetes mellitus, which was induced with alloxan, based on the reference 25, increased platelet aggregation and thrombosis formation in insulin-dependent diabetics reference 4. In addition, one of the complications for people with diabetes is ulceration and gangrene of the extremities, especially in the feet. That said, leech therapy is used to improve the repair process and prevent platelet aggregation and thrombosis, but studies on the effect of leech therapy were short of examining the biochemical activities of serum, blood sugar, and fat profiles, which in the present study were evaluated in both healthy and diabetic rats. .However, further studies on the coagulation factors and Fibrinolytic systems are required

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.Title of the manuscript does not reflect the results and conclusion .3

The title of the article was modified as follows

.Text of the results is too long .4

An attempt was made to summarize the text of the results as much as possible

As a fact, renal toxicity does not induce within one month or less .5

Because kidney damage occurs in uncontrolled diabetics, kidney damage appears to be exacerbated following leech therapy. Microscopic lesions have been reported for less than a

.month (references 44 and 45)

Animal grouping isn't enough. To compare the effect of leech a positive group (to receive .6 antihyperglycemic) was needed

The injection of alloxan in rats causes insulin-dependent diabetes mellitus, so the use of anti-diabetic drugs such as metformin and glibenclamide is relatively ineffective and the purpose wasn't of comparative evaluation of leech therapy and the effectiveness of anti-diabetic .drugs

Keywords aren't enough. Add study subject to the list .7

.The keywords were changed after the article title was changed and marked in red font

Oddly enough using two doses of alloxan on two days in a row to induce diabetes. A dose .8 .of alloxan is enough

According to reference number 25, injection of a dose of alloxan 170 to 200 mg / kg causes terrible fatality in rats, so in order to observe ethical considerations and prevent fatality and reduce the cost of research study subcutaneous injection with two doses of 100 mg / kg was performed on two consecutive days

Data presented as mean+-SEM, not STD. It is necessary to re revise it .9

Statistical information, as mentioned in the statistical analysis section of the text, was in the form of mean ± SEM, which was incorrectly inserted under the mean ± SD tables, which was .corrected

2 Revision 1

1 comment · 24 Dec 2022

Associate Editor

ACCEPT 24 Dec 2022

The revised version of the manuscript is accepted.