Journal of Medicinal and Chemical Sciences

ISC
Islamic World Science Citation Center

Indexed by
Scopus

www.jmchemsci.com

Editorial Board

Editor-in-Chief

Professor Dr. Syed A. A. Rizvi
Department of Pharmaceutical Sciences, College of Pharmacy and Health Sciences, Mercer University, Atlanta, GA. USA.

Professor of Pharmaceutical Sciences

pharmapps.nova.edu/profile.cfm?BioID=srizvi
srizvi@nova.edu
+1 954-262-8311
0000-0002-2385-5672
h-index: 30
+More

Editor-in-Chief

Professor Dr. Rassoul Dinarvand
Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran PO Box 14155-6451, IRAN

Professor of Pharmaceutics, drug delivery, nanomedicine

isid.research.ac.ir/Rassoul_Dinarvand
dinarvand@tums.ac.ir
+98 21 66959095
0000-0003-0694-7556
h-index: 66
+More

Co-Editor-in-Chief

Professor Dr. Ali Nokhodchi
Pharmaceutics Research Laboratory, School of Life Sciences, University of Sussex, Brighton BN1 9QJ, UK

Professor of Pharmaceutics and Drug Delivery

www.sussex.ac.uk/lifesci/nokhodchilab/index
a.nokhodchi@sussex.ac.uk
+44 1273872811
0000-0002-3244-2482
h-index: 65
+More

Editorial Board

Professor Dr. Khosro Khajeh
Dept. of Biochemistry, Faculty of Biological Sciences, Tarbiat Modares University, P.O.Box: 14115, Tehran,Iran

Professor of Bichemistry

ibj.pasteur.ac.ir/files/site1/files/CV-Khajeh.pdf
khajeh@modares.ac.ir
(+98-21) 82884718
0000-0002-5916-0338
h-index: 42
+More

Associate Editor

Dr. Zeinab Arzehgar
Department of Chemistry, Payame Noor University, PO BOX 19395-4697 Tehran, Iran.

Assistant Professor in Organic chemistry

arzehgar@yahoo.com
+98 84 32226101
0000-0003-3774-4348
h-index: 13

Director-in-Charge

Dr. Sami Sajjadifar
Department of Chemistry, Payame Noor University, PO BOX 19395-4697 Tehran, Iran.

Assisitant Professor in Organic Chemistry

chemsajjadifar.blogsky.com
ss.sajjadifar@gmail.com
+98 84 32226101
0000-0001-8661-1264
h-index: 24

Senior Editor

Professor Dr. Mohammad Mansoob Khan
Chemical Sciences, Faculty of Science, Universiti Brunei Darussalam, Jalan Tungku Link, Gadong, BE 1410, Brunei Darussalam, Tanzania.

Professor in Inorganic Chemistry

expert.ubd.edu.bn/mansoob.khan.php
mansoob.khan@ubd.edu.bn
+673 246 0922 / 246 0923
0000-0002-8633-7493
h-index: 55
+ More

Senior Editor

Professor Dr. Ali Delpisheh
Department of Child and Reproductive Health, Liverpool School of Tropical Medicine, Liverpool, UK.

Professor of Clinical Epidemiology

www.feedage.com/feeds/2454625/most-cited-full-text-articles
alidelpisheh@yahoo.com
+98-841-3334060
h-index: 41
+ More

Editorial Board

Dr. Ahmad Reza Moosavi-Zare
Hamedan University of Technology, Hamedan, 65155, Iran.

Associate Professor of Organic Chemistry

che.sjau.ac.ir/%D8%AF%DA%A9%D8%AA%D8%B1-%D8%A7...
moosavizare@yahoo.com
08133117804
0000-0003-0321--9326
h-index: 46
+ More

Editorial Board

Dr. Ali Maleki
Department of Chemistry, Iran University of Science and Technology (IUST), Tehran 16846-13114, IRAN

Associate Professor of Organic Chemistry

www.iust.ac.ir/find.php?item=20.10930.19490.fa
maleki@iust.ac.ir
0098 (21) 77240540
0000-0001-5490-3350
h-index: 69

Editorial Board

Dr. Majid Darroudi
Department of Modern Sciences and Technologies & Nuclear Medicine Research Center, School of Medicine, Mashhad University of Medical Sciences (MUMS), Mashhad, Iran.

Assistant Professor of Nanomedicine

isid.research.ac.ir/Majid_Darroudi
majiddarroudi@gmail.com
05138002286
0000-0002-2624-7242
h-index: 51
+ More

International Editorial Board

Professor Dr. Nenad L. Ignjatovic
Centre for Fine Particles Processing and Nanotechnologies, Institute of Technical Sciences of the Serbian Academy of Sciences and Arts, Knez Mihailova 35/4, 11000 Belgrade, Serbia.

Professor of Biomaterials

www.itn.sanu.ac.rs/nenadignjatovicen.htm
nenad.ignjatovic@itn.sanu.ac.rs
+381 63 8115494
0000-0002-5749-094X
h-index: 35
+ More

Editorial Board

Professor Dr. Behrooz Maleki
Department of Organic Chemistry, University of Mazandaran, Babolsar, Iran

Professor of Organic Chemistry

b.maleki@umz.ac.ir
h-index: 46
+ More

Editorial Board

Professor Dr. Ghodsi Mohammadi Ziarani
Department of Chemistry, Alzahra University, Tehran, Iran.

Professor of Organic Chemistry

gmohammadi@alzahra.ac.ir
0000-0001-5177-7889
h-index: 45
+ More

Editorial Board

Professor Dr. Mehrdad Hamidi
Zanjan University of Medical Sciences (ZUMS)disabled, Zanjan, Iran

Professor of Pharmaceutics

isid.research.ac.ir/Mehrdad_Hamidi
hamidim@zums.ac.ir
0000-0001-7977-5252
h-index: 40
+ More

International Editorial Board

Dr. Yasser Fakri Mustafa Hussein
Department of Pharmaceutical Chemistry, College of Pharmacy, University of Mosul, Mosul, Iraq.

Professor in Medicinal Chemistry

www.researchgate.net/profile/Yasser_Mustafa3
dr.yassermustafa@uomosul.edu.iq
+9647701615864
0000-0002-0926-7428
h-index: 31
+ More

Editorial Board

Dr. Gholamabbas Chehardoli
Department of Medicinal Chemistry, School of Pharmacy, Hamadan University of Medical Science, Hamadan, Iran

Associate Professor of Organic Chemistry

cheh1002@gmail.com
+98 811 8381594
0000-0002-8760-3837
h-index: 21

International Editorial Board

Dr. Ali H. Jawad Al-Taie
Faculty of Applied Sciences, University of Technology MARA (UiTM) Shah Alam, Selangor, Malaysia

Associate Professor in Biochemical and Environment

fsg.uitm.edu.my/v1/research/194-center-of-coals-a-biomass-...
ali288@salam.uitm.edu.my
(+603)55211721
0000-0002-4827-9093
h-index: 53
+ More

Editorial Board

Professor Dr. Asghar Mesbahi
Medical Physics Department, Medical school, Tabriz University of Medical Sciences, Tabriz, Iran

Professor at School of Medicine

isid.research.ac.ir/Asghar_Mesbahi
amesbahi2010@gmail.com
0000-0001-9159-2168
h-index: 26
+ More

Editorial Board

Dr. Masoud Mohammadi
Department of Chemistry, Faculty of Science, Ilam University, P.O. Box, 69315516, Ilam, Iran.

Assistant Professor of Organic Chemistry

chemistrypnu9.blogfa.com/
tbr.masoud@gmail.com
0000-0002-1043-3470
h-index: 23
+ More

International Editorial Board

Dr. Azahar Ali
Affilaiation: Electrical and computer Engineering, Coover Hall, Iowa State University, Ames, IA-50011, USA.

Associate Professor of Nanoscience and Nanotechnology

www.info.iastate.edu/individuals/info/259711/Ali-Azahar
azahar@iastate.edu
+1-515-598-6440
0000-0001-5752-8808
h-index: 37
+ More

Editorial Board

Professor Dr. Ali Reza Modarresi-Alam
Department of Chemistry, Faculty of Science,
University of Sistan and Baluchestan, Zahedan, Iran,
Postcode: 9816745785,

Professor in Organic Chemistry

modaresi@chem.usb.ac.ir
+98-54-33431146
0000-0003-4055-4633
h-index: 20
+ More

International Editorial Board

Professor Dr. Yogesh Chandra Tripathi
Chemistry and Bioprospecting Division, Forest Research Institute, P.O. New Forest, Dehradun-248006, India.
RG=82.77

Professor of Chemistry and Medicinal Chemistry

www.researchgate.net/profile/YOGESH_TRIPATHI/
tripathiyc@icfre.org
+91-135-224207
0000-0003-1367-5122
h-index: 19
+ More

Editorial Board

Dr. Majid Hajifaraji
Dept. of Nutrition and Food Policy & Planning Research, National Nutrition & Food Technology, Research Institute (NNFTRI), Shahid Beheshti University of Medical Sciences (SBUMS), Iran.

Associate Professor in Nutrition

www.linkedin.com/in/majid-hajifaraji-06bb5138/
m.hajifaraji@nnftri.ac.ir
+98(21) 22357486
0000-0002-4353-7866
h-index: 21
+ More

International Editorial Board

Professor Dr. Roberto Acevedo
University of Virginia, Santiago Province, Chile.

Professor Dr. in Chemistry

www.roberto-acevedo.cl/
roberto.acevedo.llanos@gmail.com
+569 4209-5982
h-index: 15

International Editorial Board

Professor Dr. Ali Sabea Hammood
Head of Biomedical Materials Engineering Track-Faculty of Engineering-University of Kufa-Iraq.

Professor in Biomedical Materials Engineering

www.researchgate.net/profile/Ali_Hammood
alis.altameemi@uokufa.edu.iq
00964(0)7801035379
0000-0002-0047-2900
h-index: 10
+ More

International Editorial Board

Professor Dr. Ehab AlShamaileh
Department of Chemistry, School of Science,
The University of Jordan, Amman 11942, Jordan

Professor of Physical Chemistry

ehab@ju.edu.jo
+9626 5355000 ext 22133
h-index: 15

Language Editor

Dr. Behroz Jamalvandi
Ilam Farhangyan University, Ilam, Iran.

University lecturer in applied linguistics

behrouzjamlvaandi@gmail.com
0000-0003-4162-2052
h-index: 5

Language Editor

Dr. Fatemeh Ramezani
Ph.D. Student, EFL Teaching, Tehran university, Tehran, Iran.

ramezani.tvu@yahoo.com

Language Editor

Dr. Nadereh Shirvani
Ph.D. Student at Ilam University, Ilam, Iran.

n.shirvani865@gmail.com

Journal of Medicinal and Chemical Sciences 6 (2023) 2348-2356

Journal of Medicinal and Chemical Sciences

Journal homepage: http://www.jmchemsci.com/

Original Article

Effect of Forest Bee Honey (*Apis dorsata*) Supplementation on Expression of HIF-1α, SOD, and TNF-α in Rats (*Rattus norvegicus*) Liver Exposed to Physical Stress

Hani Plumeriastuti[1]*, Widjiati Widjiati[2], Mey Vanda Pusparina Sajida[2], Annise Proboningrat[1]

[1]*Veterinary Pathology Division, Department of Veterinary Medicine, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia*
[2]*Veterinary Anatomy Division, Department of Veterinary Medicine, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia*

ARTICLE INFO

Article history

Receive: 2023-03-05
Received in revised: 2023-04-10
Accepted: 2023-05-14
Manuscript ID: JMCS-2304-2018
Checked for Plagiarism: **Yes**
Language Editor:
Dr. Fatima Ramezani
Editor who approved publication:
Dr. Mehrdad Hamidi

DOI:10.26655/JMCHEMSCI.2023.10.10

KEYWORDS

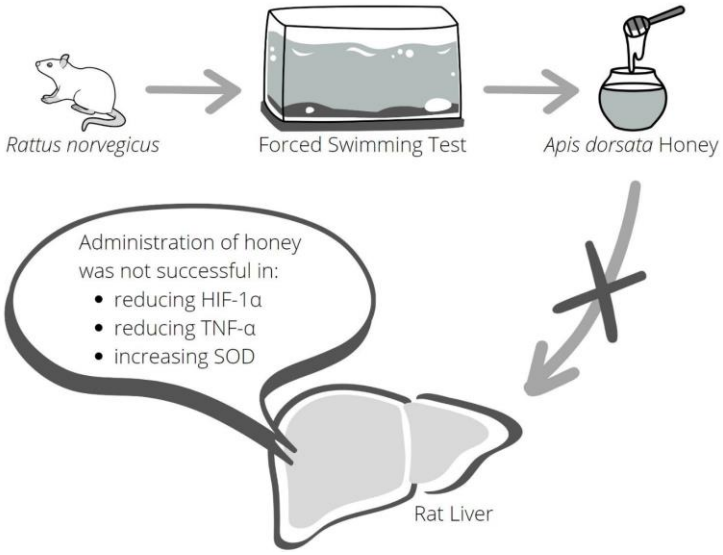
Forest bee honey
Hypoxia-inducible factor
Physical stress
Superoxide dismutase
Tumour necrosis factor-α

ABSTRACT

Physical activity in general improves metabolic processes by stimulating muscles to contract actively, improving blood circulation, and supplying oxygen. On the other hand, it will create stressful conditions, which will increase cell damage and inflammatory responses in the liver. This study aimed to determine the effect of forest bee honey supplementation on the expression of HIF-1α, SOD, and TNF-α in rats that underwent the forced swimming test (FST) as a physical stress model. The physical activity conducted is at risk of disrupting to several organs due to the stress it causes in addition to the obtained health benefits. A total of 24 adult female rats were divided into four groups: Control (C) with FST only, (T1) FST and honey 2 g/day, (T2) FST and honey 4 g/day, (T3) FST and honey 6 g/day. A forced swimming test was conducted for five minutes per day for 14 days. The collected liver organs were histopathologically prepared by immunohistochemical staining for HIF-1α, SOD, and TNF-α proteins. The results showed that hepatic HIF-1α and TNF-α expression decreased in the honey-supplemented group, while hepatic SOD expression increased, although all three showed insignificant differences from each other. This study concludes that honey supplementation is incapable of increasing the expression of SOD as well as reducing the expression of HIF-1α and TNF-α in the liver of rats modelled by physical stress.

* Corresponding author: Hani Plumeriastuti
✉ E-mail: hani-p@fkh.unair.ac.id

GRAPHICAL ABSTRACT



Introduction

Physical activity in general has a positive effect on metabolic processes, stimulating muscles to actively contract, improving blood circulation, and supplying oxygen [1]. On the other hand, physical activity will also induce stressful conditions, which will subsequently increase cell damage and inflammatory responses in the liver [2, 3]. The production of the cortisol hormone marks the occurrence of stressful conditions in the body [4]. The production of cortisol in the body suppresses the production of brain-derived neurotrophic factor (BDNF), which protects against malondialdehyde (MDA) toxicity as a marker of oxidative stress caused by reactive oxygen species (ROS) [5]. Physical activity could trigger an increase in malondialdehyde (MDA) levels and decrease Superoxide Dismutase (SOD) levels in the body [6, 7]. The body naturally has an antioxidant system that aims to prevent damage caused by ROS, including the enzymes Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), and Catalase [8]. ROS in sufficient levels is beneficial in the cellular immune response, but when produced in excessive amounts beyond the capacity of antioxidant enzymes, it will cause oxidative stress conditions that are detrimental to the cell membrane [9].

Oxidative stress will trigger the activation of Hypoxia Inducible Factor (HIF-1α) through the MAPK pathway [10]. In physiological conditions, activation of HIF-1α plays an important role in wound healing because it triggers angiogenesis. On the other hand, HIF-1α production is often associated with the pathogenesis of various hepatic disorders, such as fibrosis, hepatitis, and even cancer [11, 12]. Hepatic damage can be induced by an acute inflammatory response due to physical stress characterized by an increase in Tumor Necrosis Factor-α (TNF-α) [13]. TNF-α production in the liver is carried out by Kupffer cells, which are involved in inflammation and hepatocyte apoptosis through caspase activation [14]. Just like HIF-1α, TNF-α production in the liver is related to the pathogenesis of chronic hepatic inflammation that leads to fibrosis.

Stress due to physical activity is usually not realized by the individual and is often inevitable because it is a consequence of the work undertaken, such as in the case of sports athletes [14, 15].

Physical activity done with a sufficient portion is beneficial in the metabolic process of the individual while if done with high intensity will cause an increase in cortisol production and oxidative stress [16, 17]. To compensate for the decrease in endogenous antioxidants due to physical stress, exogenous antioxidants derived from food or beverages are needed.

Antioxidants are substances that can protect biological components from harm caused by chemical processes involving free radicals. They work by breaking down chains or stabilizing molecules [18]. Thus, the best approach to

alleviating oxidative stress is to reduce free radicals or optimize the body's defences by multiplying antioxidants. Furthermore, antioxidants protect the tissue from oxidative damage [19].

Indonesia is rich in biodiversity and has many natural resources that can be beneficial to treat various illnesses [20].

Honey is one of the natural ingredients that can be easily obtained and contains ascorbic acid, carotenoids, phenolic acids, flavonoids, and simple sugars [21]. Antioxidants contained in honey act as free radical scavengers under oxidative stress conditions and were proven to increase the production of GPx, SOD, and CAT as endogenous antioxidants in rats and improve the histological structure of the liver [22, 23]. Many animal models of stress and depression have been made using the forced swimming test along with observations on the liver, such as oxidative damage [17], MDA levels [24], and glycogen levels [25] of the liver [17]. This study attempted to determine whether the administration of wild bee honey supplements increased the expression of endogenous antioxidants, especially SOD, and decreased the expression of HIF-1α and TNF-α proteins in the livers of rats modelled by physical stress.

Results and Discussion

The mean protein expression of HIF-1α, TNFα, and SOD in the whole population showed the results, as indicated in Table 1 and Figure 1.

Table 1: The mean expression of HIF-1α, TNFα, and hepatic SOD in all treatment groups. Based on data analysis, it is mentioned that the results do not have significant differences (p>0,05)

Dose Group (grams/rat)	Mean Rank±SD		
	HIF-1α	SOD	TNF- α
0 (C)	17.06±1.97	13.50±1.45	21.44±0.37
2 (T1)	16.56±0.91	14.63±2.17	17.81±0.89
4 (T2)	16.69±1.85	15.63±1.05	14.69±0.29
6 (t3)	15.69±1.78	22.25±0.26	12.06±0,63
Asymp. Sig.	0.993	0.173	0.205

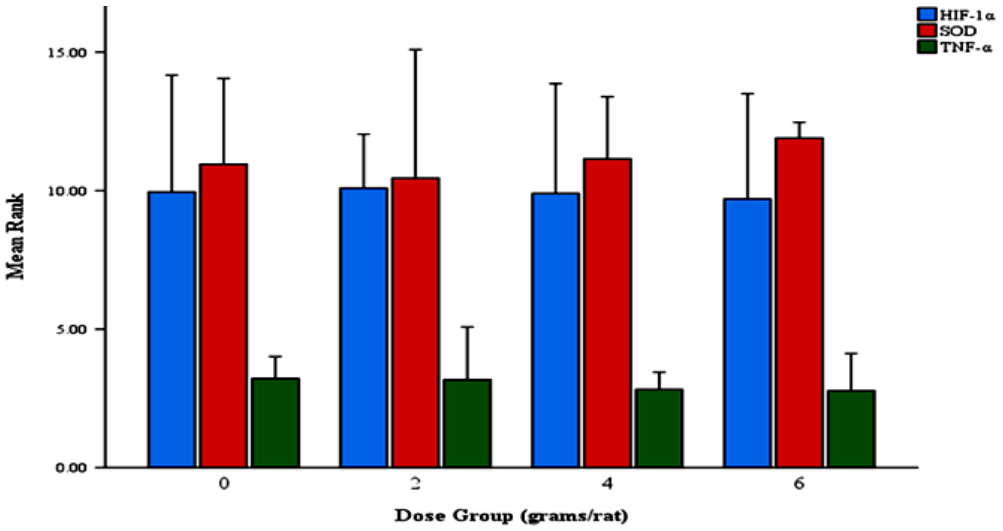


Figure 1: Mean results of hepatic HIF-1α, TNF-α, and SOD protein expression in all treatment groups with honey doses of 0 g (C), 2 g (T1), 4 g (T2), and 6 g (T3). The blue column represents HIF-1α; the red column represents SOD; and the green column represents TNF-α. All study parameters did not appear to differ significantly between treatment groups

The forced swimming test as a physical stress model accompanied by honey supplementation was proven to reduce the expression of HIF-1α and TNF-α in the liver but based on statistical analysis, it was considered insignificant (p>0.05) as can be observed in Table 1 and Figure 1. The

highest expression of HIF-1α and TNF-α was obtained in group C, which only underwent swimming activities without honey supplementation. Based on Table 1 and Figure 1, it can be further observed that between groups of forced swimming test treatment with honey supplementation, hepatic SOD expression gradually increased along with the increase in honey dose, with the least amount in group C, which did not get any honey. Although the values varied, the differences were not significant ($p>0.05$) (Table 1). The expression of HIF-1α, TNFα, and SOD presented in immunohistochemical staining can be further observed in Figures 2, 3, and 4.

Based on the immunohistochemical overview of the liver depicted in Figure 2, it can be observed that there is an expression of HIF-1α in the hepatic parenchyma of rats modelled by physical stress, even though there is no significant difference in expression between groups. ROS that arises due to stress will stimulate MAP/ERK Kinase (MEK) to phosphorylate p300 so that there is an increase in the transcription of the HIF-1α molecule in the liver even under normoxia [11]. While in Figure 3, it is appeared that TNF-α expression is very weak to almost zero in the liver. TNF-α production in the liver is carried out by Kupffer cells, which play a role in inflammation and hepatocyte apoptosis through caspase activation [13]. Apoptosis begins with a decrease in BCl-2 activity on the mitochondrial membrane so that membrane permeability changes. These changes cause the release of cytochrome C into the cytosol, which will activate Apaf-1. This activation is followed by a caspase cascade on pro-caspase 9 to process caspase 3, which will damage DNA [26]. From Figure 4, it can be observed that the expression of SOD in the group with 6 g/day honey supplementation appears to be stronger in colour change when compared to other groups, although in statistical analysis the difference was considered insignificant.

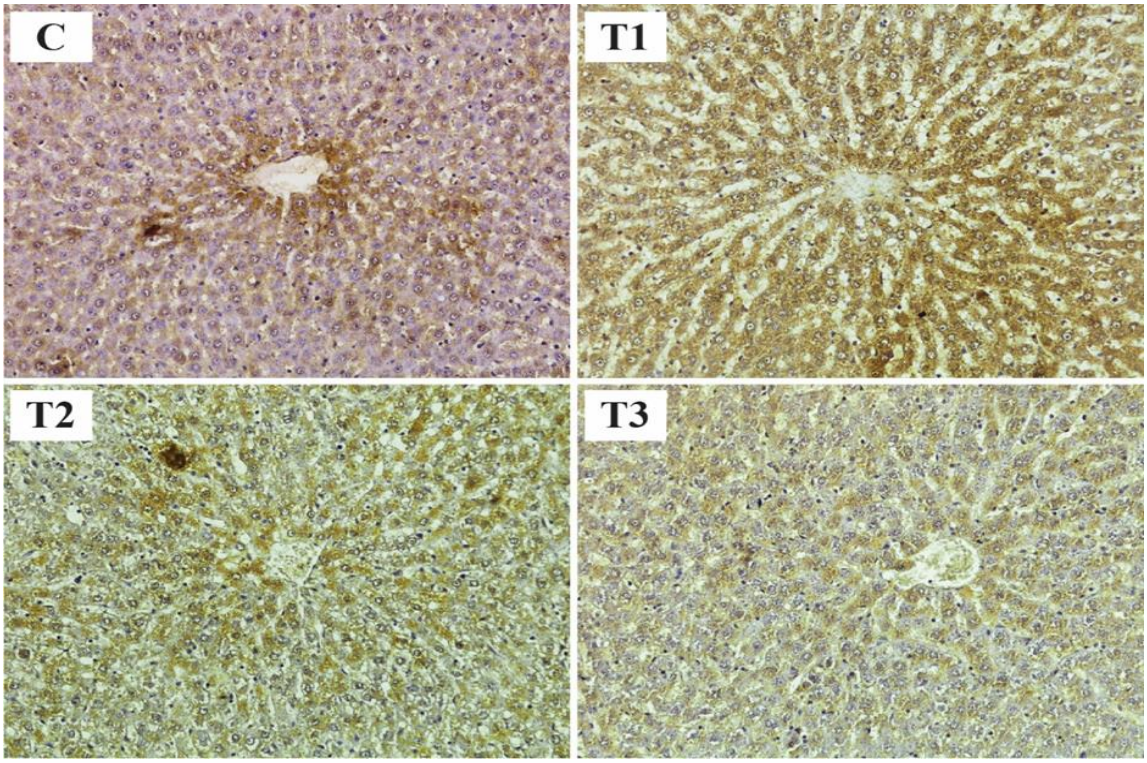


Figure 2: HIF-1α expression in hepatocytes of physical stress model rats by immunohistochemical staining (200x). FST without honey (C); FST with 2 g/day honey (T1); FST with 4 g/day honey (T2); and FST with 6 g/day honey (T3). Brownish-yellow coloration with weak to moderate intensity could be observed in all treatment groups

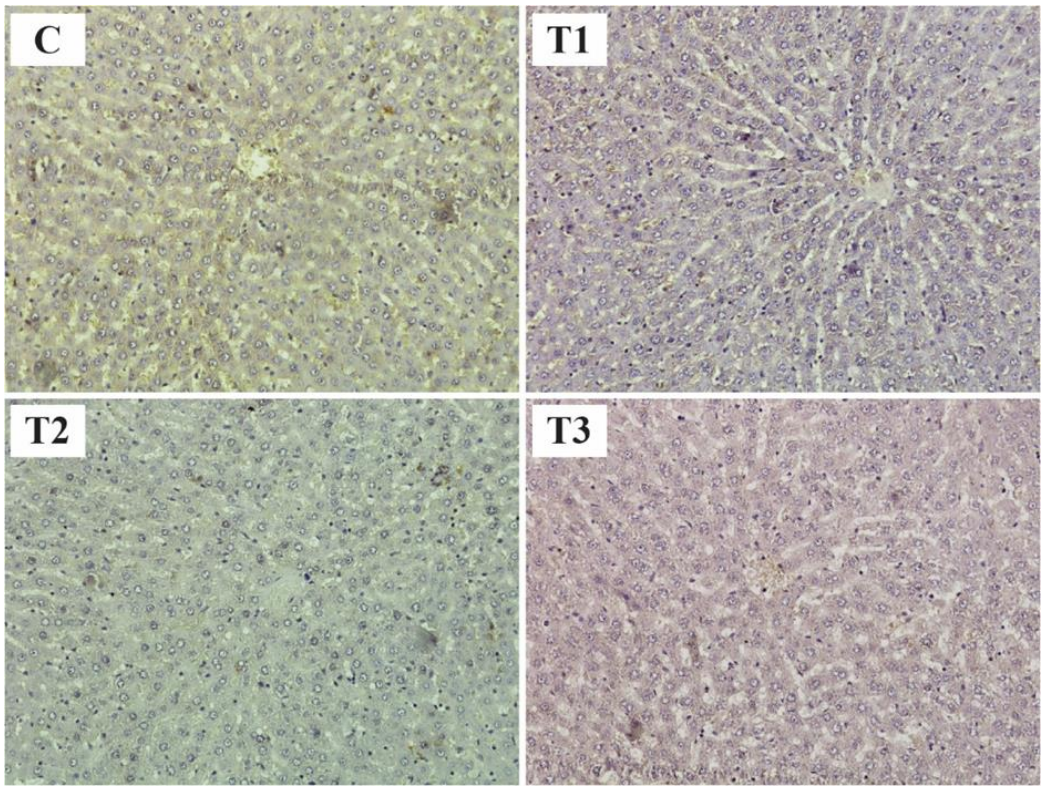


Figure 3: TNF-α expression in hepatic Kupffer cells of physical stress model rats by immunohistochemical staining (200x). FST without honey (C); FST with 2 g/day honey (T1); FST with 4 g/day honey (T2); and FST with 6 g/day honey (T3). A brownish-yellow colour with weak intensity can be observed in group C, while in the honey supplementation group, the intensity is getting smaller to zero

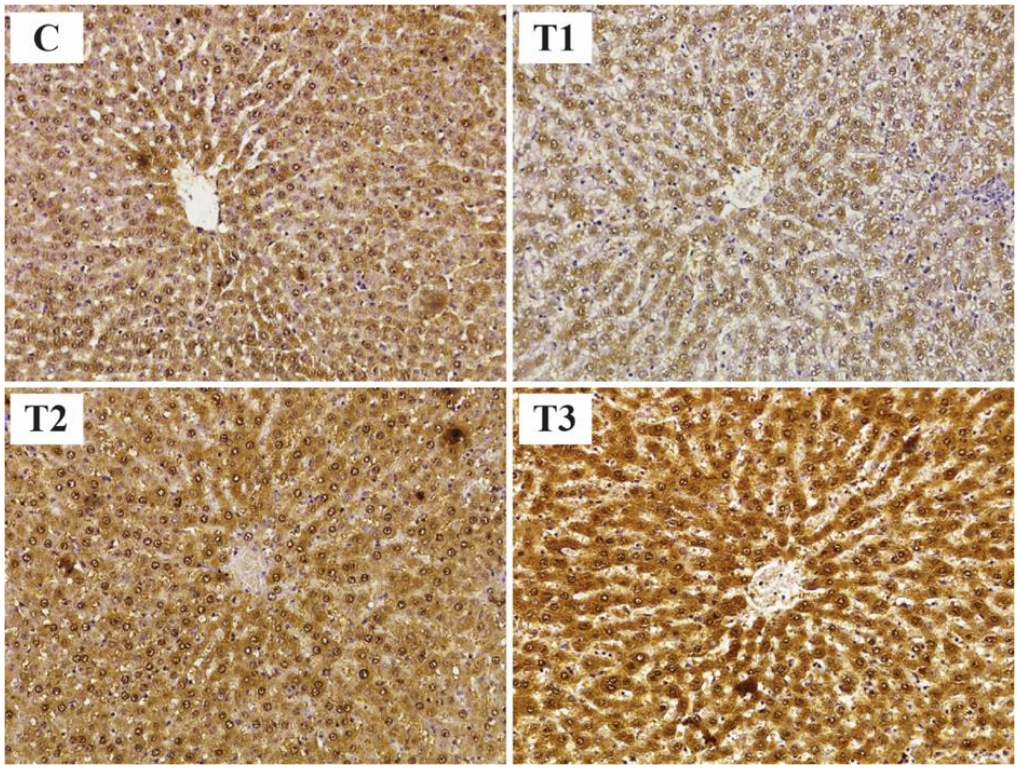


Figure 4: SOD expression in hepatocytes of rats modeled by physical stress by immunohistochemical staining (200x). FST without honey (C); FST with 2 g/day honey (T1); FST with 4 g/day honey (T2); and FST with 6 g/day honey (T3). A brownish-yellow color with moderate to strong intensity can be observed in all groups, with the strongest intensity seen in group T3

The FST procedure used in this study was shown to be associated with HIF-1α production in the liver. This result is in line with other studies that revealed that physical stress of swimming can increase the ROS production, which will induce the activation of HIF-1α in the liver [27]. In addition, the FST procedure proved to be positively correlated with hepatic TNF-α production, following other studies that prove that physical stress can increase TNF-α expression in the liver [28]. The antioxidant content of honey is known to suppress cell damage due to physical stress and ROS [29, 30]. Furthermore, honey has also been shown to increase the production of endogenous hepatic antioxidants SOD as well as GPx, GSH, and catalase [22, 23].

However, honey efficacy was not significantly proven in this study. This may be due to the concentration of ROS being too high, so the dose level used in this study was insufficient to compensate for the cell damage that occurred even though there was an increase in SOD expression. Other studies have shown that excessive consumption of honey can distort the hepatic sinusoid circuit and necrosis of hepatocytes [31]. Similar results also revealed that honey consumption can cause haematological changes as well as damage to hepatic and renal cells [32]. This damage is thought to be due to the accumulation of heavy metals such as Pb and Cd in honey from the environment where the honey is taken. The accumulation of these metals then initiates higher ROS [32]. Therefore, the length of time honey is given and the amount of dose given should be of utmost concern so that honey consumed provides benefits to the body and not the other way around. The dose level of honey and the duration of the study in this study are not sufficient to prove that honey supplementation can prevent general hepatic damage in rats modelled under physical stress. However, the observed parameters in this study do not fully represent the hepatic quality of an individual; other supporting data such as AST, ALT, and GGT profiles are needed for higher validity.

Materials and Methods

Ethical approval

This study had been approved by the Ethics Commission of the Faculty of Veterinary Medicine, Universitas Airlangga (1.KEH.041.04.2022).

Experimental animals

The experimental animals used were three-month-old female Wistar rats (*Rattus norvegicus*) with an average body weight of 200 grams. The total population of 24 rats was divided into four treatment groups using a complete randomization system so that each group contained six rats. Rats were acclimatized with adequate food and water for seven days. During the study, the rats were placed in cages measuring 53 × 30 × 17 cm with a base of wood chips and placed in a room with a temperature of 34 °C and 50% humidity. The forced swimming test is a method used to model animal stress and depression, according to Porsolt [34]. Rats were exercised in a 50 cm diameter and 60 cm high barrel filled with water to approximately 2/3 of the height of the barrel for five minutes every day for 14 days.

Experimental procedure

There were four treatment groups: C with the forced swimming test (FST) alone; T1 with the FST and 2 g/day honey; T2 with the FST and 4 g/day honey; and T3 with the FST and 6 g/day honey. Honey was given by oral gavage. FST was performed for 14 days, and the rats were sacrificed on day 15. Rats were sacrificed with a combination of ketamine and xylazine injections. A laparotomy procedure was performed to collect the liver.

Tissue processing

The organs were prepared for histological examination as follows [33]: each animal's liver was fixed in 10% neutral buffered formalin. The fixed tissues were cleared with xylol after being dehydrated in a graded series of alcohols. The tissues were then infiltrated with molten paraffin at 56–60 °C. From a solid block of tissue, serial

sections of 3 µm thickness were cut, washed, and placed in object glass.

Immunohistochemistry staining

The tissues were deparaffinized and rehydrated. Then the tissue slides were incubated with hydrogen peroxide for 10-15 minutes, and blocking was performed for 5 minutes. Antibody incubations were conducted using primary antibodies (HIF-1α (1:200), TNFα (1:50), and SOD (1:200)) for 60 minutes and secondary antibodies for 30 minutes. Subsequently, the slides were incubated with streptavidin peroxidase for 10 minutes. Furthermore, the addition of DAB chromogen and substrate was carried out for 15 minutes. Likewise,counterstaining was carried out using Mayer's hemalum solution.

Statistical analysis

Hepatic immunohistochemical protein expressions were documented using a Nikon Eclipse Ci microscope at 200x magnification and interpreted using the IRS scoring system. Expression was considered positive if there was a brownish-yellow colour change due to antigen-antibody binding in hepatocytes. Observations were made in five fields of view, and the results were averaged. The data obtained were then statistically analysed by ANOVA using SPSS for Windows.

Conclusion

The conclusion that can be drawn from this study is that supplementation of *Apis dorsata* honey at the dose level given is unable to reduce the expression of HIF-1α and TNFα and increase the SOD expression in physical stress model rats. In future studies, it is suggested to use a more customized dose of honey and to determine the overall hepatic profile, other research parameters such as AST, ALT, and GGT are needed.

Acknowledgments

The authors express their gratitude to Agung Budianto Achmad, DVM, M.Sc., who supported the data analysis in this study.

Disclosure Statement

No potential conflict of interest was reported by the authors.

Funding

This study was funded by Universitas Airlangga with Grant No. 251/UN3/2022.

Authors' Contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

ORCID

Hani Plumeriastuti
https://orcid.org/0000-0002-4540-811X
Widjiati Widjiati
https://orcid.org/0000-0002-8376-1176
Mey Vanda Pusparina Sajida
https://orcid.org/0000-0002-4079-9967
Annise Proboningrat
https://orcid.org/0000-0001-7939-9402

References

[1]. Malm C., Jakobsson J., Isaksson A., Physical Activity and Sports—Real Health Benefits: A Review with Insight into the Public Health of Sweden, *Sports*, 2019, **7**:127 [Crossref], [Google Scholar], [Publisher]
[2]. Czarkowska-Paczek B., Piekarczyk-Persa J., Wyczatkowska-Tomasik A., Zendzian-Piotrowska M., Paczek L., Increased TNF-α and TGF-β concentrations in rat liver after intense exercise, *Polish Annals of Medicine*, 2018, **25**:98 [Crossref], [Google Scholar], [Publisher]
[3]. Dab H., Hamed S.B., Hodroj W., Zourgui L., Combined diabetes and chronic stress exacerbates cytokine production and oxidative stress in rat liver and kidney, *Biotechnology & Biotechnological Equipment*, 2023, **37**:250 [Crossref], [Google Scholar], [Publisher]
[4]. Mahabadi N., Doucet A., Wong A.L., Mahabadi V., Glucocorticoid Induced Hypothalamic-Pituitary Axis Alterations Associated with Hypogonadotropic Hypogonadism, *Osteology and Rheumatology Open Journal*, 2019, **1**:30 [Crossref], [Google Scholar], [Publisher]

[5]. Markham A., Bains R, Franklin P., Spedding M., Changes in mitochondrial function are pivotal in neurodegenerative and psychiatric disorders: how important is BDNF?, *British Journal of Pharmacology*, 2014, **171**:2206 [Crossref], [Google Scholar], [Publisher]
[6]. Trofin F.P., Ciobica A., Cojocaru D., Chirazi M, Honceriu C., Trofin L., Serban D., Timofte D., Cojocaru S.I., Anton E., Increased oxidative stress status in rat serum after five minutes treadmill exercise, *Central European Journal of Medicine*, 2014, **9**:722 [Crossref], [Google Scholar], [Publisher]
[7]. Sinaga F.A., Purba P.H., Sinaga R.N., Silaban R., Effects of Red Fruit Oil on Exercise Endurance and Oxidative Stress in Rats, *Proceedings of 5th International Conference on Physical Education, Sport and Health (ACPES)*, 2019, **362** [Crossref], [Google Scholar], [Publisher]
[8]. Pham-Huy, L.A., He H., Pham-Huy C., Free radicals, antioxidants in disease and health, *International Journal of Biomedical Science*, 2008, 4:2 [Crossref], [Google Scholar], [Publisher]
[9]. Husen S.A., Winarni D., Salamun, Ansori A.N.M., Susilo R.J.K., Hayaza S., Hepatoprotective Effect of Gamma-mangostin for Amelioration of Impaired Liver Structure and Function in Streptozotocin induced Diabetic Mice, *IOP Conference Series: Earth and Environmental Science*, 2019, **217**:012031 [Crossref], [Google Scholar], [Publisher]
[10]. Wilson G.K., Tennant D.A., McKeating J.A., Hypoxia inducible factors in liver disease and hepatocellular carcinoma: Current understanding and future directions, *Journal of Hepatology*, 2014, **61**:1397 [Crossref], [Google Scholar], [Publisher]
[11]. Lee J., Bae S., Jeong J., Kim S., Kim K., Hypoxia-inducible factor (HIF-1)α: its protein stability and biological functions, *Experimental and Molecular Medicine*, 2004, **36**:1 [Crossref], [Google Scholar], [Publisher]
[12]. Iommarini L., Porcelli A.M., Gasparre G., Kurelac I., Non-Canonical Mechanisms Regulating Hypoxia-Inducible Factor 1 Alpha in Cancer, *Frontiers in Oncology*, 2017, **7**:286 [Crossref], [Google Scholar], [Publisher]
[13]. Yang Y.M., Seki E., TNFα in liver fibrosis, *Current Pathobiology Reports*, 2015, **3**:253 [Crossref], [Google Scholar], [Publisher]
[14]. Khadim I., Tabassum Y., Butt M.Z.I., Prevalence of Reproductive Disorders in Sportswomen, *Global Educational Studies Review*, 2020, **5**:308 [Crossref], [Google Scholar], [Publisher]
[15]. Castelo-Branco C., Reina F., Montivero A.D., Colodron M., Vanrell J.A., Influence of high-intensity training and of dietic and anthropometric factors on menstrual cycle disorders in ballet dancers, *Gynecological Endocrinology*, 2006, **22**:31 [Crossref], [Google Scholar], [Publisher]
[16]. Raastad T., Bjoro T., Hallen J., Hormonal responses to high and moderate-intensity strength exercise, *European Journal of Applied Physiology*, 2000, **82**:121 [Crossref], [Google Scholar], [Publisher]
[17]. Souza L.M.V., Aidar F.J., de Matos D.G., Marcal A.C., de Souza R.F., dos Santos J.L., Wartha E.R.S.A, da Silva A.N., Estevam C.S., de Araujo S.S., Analysis of oxidative stress in Wistar rats submitted to high-intensity interval training, *Motricidade*, 2020, **16**:2764 [Crossref], [Google Scholar], [Publisher]
[18]. Husen S.A., Setyawan M.F., Syadzha M.F., Susilo R.J.K., Hayaza S., Ansori A.N.M., Alamsjah M.A., Ilmi Z.N., Wulandari P.A.C., Pudhiastuti P., Awang K., Winarni D., A Novel Therapeutic effects of *Sargassum ilicifolium* Alginate and Okra (*Abelmoschus esculentus*) Pods extracts on Open wound healing process in Diabetic Mice, *Research Journal of Pharmacy and Technology*, 2020, **13**:2764[Crossref], [Google Scholar], [Publisher]
[19]. Ansori A.N.M., Susilo R.J.K., Hayaza S., Winarni D., Husen S.A., Renoprotection by *Garcinia mangostana* L. pericarp extract in streptozotocin-induced diabetic mice, *Iraqi Journal of Veterinary Sciences*, 2019, **33**:13 [Crossref], [Google Scholar], [Publisher]
[20]. Tacharina M.R., Ansori A.N.M., Plumeriastuti H., Kusnoto, Kurnijasanti R., Hestianah E.P., Bene cial Effect of Grinting Grass (*Cynodon dactylon*) on the Streptozotocin Induced Diabetes Mellitus in the Mice, *Indian Veterinary Journal*, 2020, **97**:35 [Google Scholar], [Publisher]

[21]. Moniruzzaman M., Khalil M.I, Sulaiman S.A., Guan S.H., Physicochemical and antioxidant properties of Malaysian honeys produced by *Apis cerana*, *Apis dorsata* and *Apis mellifera*, *BMC Complementary and Alternative Medicine*, 2013, 13:43 [Crossref], [Google Scholar]
[22] Waykar B.B., Alqadhi Y.A., Protective Role of Hon.ey and Royal Jelly on Cisplatin Induced Oxidative Stress in Liver of Rat, *International Journal of Pharmaceutical Sciences and Research*, 2019, **10**:3898 [Crossref], [Google Scholar], [Publisher]
[23]. Mohamed N.Z., Aly H.F., El-Mezayen H.A.M., El-Salamony H.E., Effect of co-administration of Bee honey and some chemotherapeutic drugs of dissemination of hepatocellular carcinoma in rats, *Toxicology Reports*, 2019, **6**:875 [Crossref], [Google Scholar], [Publisher]
[24]. Emiliano S., Cecilia L.A., Karen B., Guillermo H., Nancy R., Effect of prenatal stress and forced swimming acute stress on adult rat's skeletal muscle an liver MDA levels, *MOJ Anatomy & Physiology*, 2019, **6**:226 [Crossref], [Google Scholar], [Publisher]
[25]. Morakinyo, A.O., Iranlonye B.O., Ogunsola O.A., Glucometabolic effects of single and repeated exposure to forced-swimming stressor in Sprague-Dawley rats, *Endocrine Regulations*, 2018, **52**:85 [Crossref], [Google Scholar], [Publisher]
[26]. Luqman E.M., Sudiana I.K., Darmanto W, Achmad A.B., Widjiati, Mouse (*Mus musculus*) embryonic cerebral cortex cell death caused by carbofuran insecticide exposure, *Journal of Veterinary Research*, 2019, **63**:413 [Crossref], [Google Scholar], [Publisher]
[27]. Fathi I., Modulatory Effects of Swimming Training on Hypoxia-Induced Factors in Heart Tissue of Rats Exposed to Chronic Stress, *Gene Cell Tissue*, 2022, **9**:e116825 [Crossref], [Google Scholar], [Publisher]
[28]. Gao C., Liu Y., Jiang C., Liu L., Li J., Li D., Guo X., Wang Z., Yang Y., Liu L., Yao P., Tang Y., Intensive Running Enhances NF-κβ Activity in the Mice Liver and the Intervention Effects of Quercetin, *Nutrients*, 2020, **12**:2770 [Crossref], [Google Scholar], [Publisher]
[29]. Mosavat M., Mohamed M., Ooi F.K., Mirsanjari M., Zin A.A.M, Romli A.C., Histological changes of female reproductive organs subjected to different jumping exercise intensities and honey supplementation in rats, *PeerJ*, 2019, **7**:e7646 [Crossref], [Google Scholar], [Publisher]
[30]. Yaman T., Yener Z., Celik I., Histopathological and biochemical investigations of protective role of honey in rats with experimental aflatoxicosis, *BMC Complementary and Alternative Medicine*, 2016, **16**:1 [Crossref], [Google Scholar], [Publisher]
[31]. Wilson J.I., George B.O., Umukoro G.E., Effects of honey on the histology of liver in adult Wistar rats, *Biology and Medicine*, 2011, **3**:1 [Crossref], [Google Scholar], [Publisher]
[32]. Agbatutu A., Asiwe J.N., Adebayo O.G., Liver and Renal Cell Damage Following Excess Bee Honey Consumption in Male Wistar Rat, *Biology, Medicine and Natural Product Chemistry*, 2022, **11**:35 [Crossref], [Google Scholar], [Publisher]
[33]. Proboningrat A., Plumeriastuti H., Utama S., Sudjarwo S.A., Legowo D., Luqman E.M., Widjiati, Effect of *Moringa oleifera* leaf extract on the Histopathological Features of Testicular Seminiferous Tubules of Mice (*Mus musculus*) Exposed to Methylmercury, *Ecology, Environment and Conservation Paper*, 2022, **28**:1 [Crossref], [Google Scholar], [Publisher]
[34]. Porsolt R.D., Le Pichon M., Jalfre M., Depression: a new animal model sensitive to antidepressant treatments, *Nature*, 1977, **266**:730 [Crossref], [Google Scholar], [Publisher]

HOW TO CITE THIS ARTICLE
Hani Plumeriastuti, Widjiati Widjiati, Mey Vanda Pusparina Sajida, Annise Proboningrat. Effect of Forest Bee Honey (*Apis dorsata*) Supplementation on Expression of HIF-1α, SOD, and TNF-α in Rats (*Rattus norvegicus*) Liver Exposed to Physical Stress. *J. Med. Chem. Sci*., 2023, 6(10) 2348-2356
DOI: https://doi.org/10.26655/JMCHEMSCI.2023.10.10
URL: http://www.jmchemsci.com/article_171404.html