



Therapeutic Efficacy of Lotus (*Nelumbo nucifera*) Leaves Extract to Manage Diabetes mellitus

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<i>Article History</i>	<i>Abstract</i>
<p>Received: 11 Feb 2022 Revised: 26 May 2022 Accepted: 10 August 2022</p>	<p>Natural bioactive compounds that target pancreatic cells for the prevention and treatment of diabetes mellitus are gaining popularity. The purpose of this research was to investigate the physiologic effects of Lotus leaves on insulin and sugar levels in order to improve diabetes control. The Soxhelt apparatus was used to extract the Lotus ethanol extract, which was diluted with distilled water at two concentrations: 5 mg/ml (5 percent) and 10 mg/ml (10 percent).. Thirty female white albino rats were randomly separated into three groups: G1(n=10) was given distilled water 1ml, G2(n=10) and G3(n=10) were given a single dosage of 5% and 10% extract solution, respectively, daily for ten days. The results showed significant decrease of glucose level in serum after 10 days of treatment G2= 87.36±1.5 and G3=69.7±1.7 and significant increase of insulin level in serum G2= 78.92±1.03 and G3= 89.36±1.46.</p>
<p>CC License CC-BY-NC-SA 4.0</p>	<p>key words: - <i>physiologic, efficacy, lotus, leaves, diabetes, mellitus.</i></p>

Introduction

A Diabetes mellitus is becoming more common over the world, and it is posing a severe threat to people's health in every country (1). The total number of diabetics is expected to climb from 171 million in 2000 to 366 million in 2030, according to estimates (1). Hyperglycemia is the most common consequence in long-term diabetes patients, which can lead to abnormal insulin release from pancreatic cells and/or insulin action in peripheral tissues, as well as diabetic microvascular diseases (nephropathy, retinopathy, and neuropathy) (2).

Despite significant advances in understanding the pathogenesis and treatment of this pervasive disease, it remains a serious public health concern around the world. The potential of treating it with hypoglycemic medications taken orally has piqued people's interest in recent years. Although several forms of oral hypoglycemic medications and insulin are available for the treatment of diabetes mellitus, patients are increasingly requesting herbal medicines with anti-diabetic activity. Current treatments appear to be ineffective in preventing diabetes complications, with a two- to four-fold increase in the risk of cardiovascular events (2).

Investigations of hypoglycemic medicines of plant origin utilized in traditional medicine are relevant, according to World Health Organization recommendations on diabetes mellitus (3). Herbal items have long been used for medical purposes in practically every culture on the planet (4), and the quest for anti-diabetic drugs will continue to focus on plants and other natural resources for many years. In diabetic animal models, researchers have repeatedly discovered that various plant compounds have distinct hypoglycemic properties (5).

Nelumbo nucifera is a tree native to China, Japan, and India. *Nelumbo nucifera* is an agricultural crop that has been farmed for food and drink in South Korea for thousands of years. The leaves, blossoms, seeds, and rhizomes of the lotus have all been believed to have medical properties, many Korean cooking dishes include lotus as a nutritious ingredient. In addition, lotus tea, noodles, juice, and other products have acquired appeal in South Korea. Huang and colleagues recently showed that a 100 percent methanol extract of lotus leaves cured glucose intolerance in obese mice caused by a high-fat diet (6).

The aim of the study was to focus on the physiologic efficacy of lotus (*Nelumbo nucifera*) leaves on insulin, and sugar level to management of diabetes mellitus.

Materials and methods

This research was carried out on 15 male Albino rats living in the Animal Station of the College of Veterinary Medicine in Iraq, under the supervision of the bioethics committee of the College of Veterinary Medicine/ University of Al-Qadisiyah. The experiment was carried out on 30 female Albino rats obtained from the college's animal house. They were 6 weeks old and were kept in boxes in a typical habitat with a room temperature of 25 °C, 45 % -50 % relative humidity, and ad libitum feed and clean water (12 hours light and 12 hours darkness). These rats were randomly divided into three groups, each with ten rats. G1 served as the control group, G2 served as the second treatment group (5%), and G3 served as the third treatment group (10%). The study only included rats with a blood glucose level of more than 250 mg/dL.

Preparation of Nelumbo nucifera extract:

Nelumbo nucifera leaves were taken from a river in Al-Dewaniah province, washed three times with distilled water, dried in the shade for two weeks, ground using an electrical grinder, and kept in 70% ethanol for 48 hours in a Soxhelt apparatus (Electrothermal, UK). The ethanol leaves extract was then dried in a Rotary Evaporator, weighed, and mixed with distilled water to make final concentrations of 5mg extract/ml and 10 mg extract/ml, respectively, and stored at 4C° until use.

Experimental design:

G1 were drenched with 1ml. distilled water, G2 with 5% extract solution at dose 1ml, and G3 with 10% extract solution at dose 1ml. For ten days, all groups were treated on a daily basis. Every day during the treatment period, blood samples were obtained from tail veins in sterile tubes and stored at - 20°. Body weights of rats were estimated every day for ten days. A blood analyzer was used to measure insulin and sugar levels. A spectrophotometer was used to do the nutritional analysis. Measurements of insulin-like growth factor (IGF-1) concentration in serum were done by sandwich ELISA technique. IGF-1 protein levels in the serum were measured using commercially available enzyme linked immunosorbent assay kits specific for rat IGF-1 (catalog number EK0377, Booster Immunoleader, Wuhan, China), with assay sensitivity. The findings were assessed using ANOVA one-way software, version 32, and the variances were deemed significant at $P \leq 0.05$.

Results

Moisture content 8.15 percent, carbohydrate 33.60 percent, crude protein 14.54 percent, crude fat 2.91 percent, crude fiber 24.17 percent, calorific value (kcal) 218.21 percent, and total flavonoid content 7.89 percent were the contents of ethanol extract of *Nelumbo nucifera* gm/100gm, as showed in table-1.

Table-1: Nutritional analysis of ethanol extracts of Nelumbo nucifera leaves.

Contents gm. /100gm	Nelumbo nucifera leaves(%)
Moisture content	8.15
Carbohydrate	33.60
Crude protein	14.54
Crude fat	2.91
Crude fiber	24.17
Calorific value (kcal)	218.21
Total flavonoid content	7.89

The glucose concentrations (mg/100ml.) in serum were significant decrease in G2 during the period of treatment, 1st day=103.7±0.78 and 10th day=87.36±1.5, and in G3 at 1st day= 105.9±0.90 and 10th day=69.7±1.7. While it were nearly stable in G1 at 1s day=104.1±0.84 and at 10th day=103.7±0.57 as showed in table-2.

Table-2: Glucose concentration (mg./100 ml.) values in serum at the period of treatment.

Groups	Period of treatment									
	1 st day	2 nd day	3 rd day	4 th day	5 th day	6 th day	7 th day	8 th day	9 th day	10 th day
G1 (n=10) Aa	104.1 ±0.84	102.9±0. 62ABa	104.02± 0.81Aa	102.9±0 .22Aa	103.8± 0.77Aa	103±0. 58Aa	102.6± 0.64Aa	103.3± 0.75Aa	103.2± 0.52Aa	103.7± 0.57Aa
G2 (n=10) Aa	103.7 ±0.78	101.9±0. 66Aab	100.8±0 .64Bb	99.06±0 .40Bbc	98.5±0. 24Bc	98.2±0. 22Bca	96.04± 0.24Bd	93.8±0. 38Be	91.48± 0.43Bf	87.36± 1.5Bg
G3 (n=10) Aa	105.9 ±0.90	104.1±1. 003Ba	99.38±0 .35Bb	93.56±1 .11Cc	89.1±0. 75Cd	84.2±0. 96Ce	80.74± 0.65Cf	77.1±0. 92Cg	73.78± 0.99Ch	69.7±1. 7Ci

Different letters mean the variances were significant at $p \leq 0.05$. *LSD=2.11*

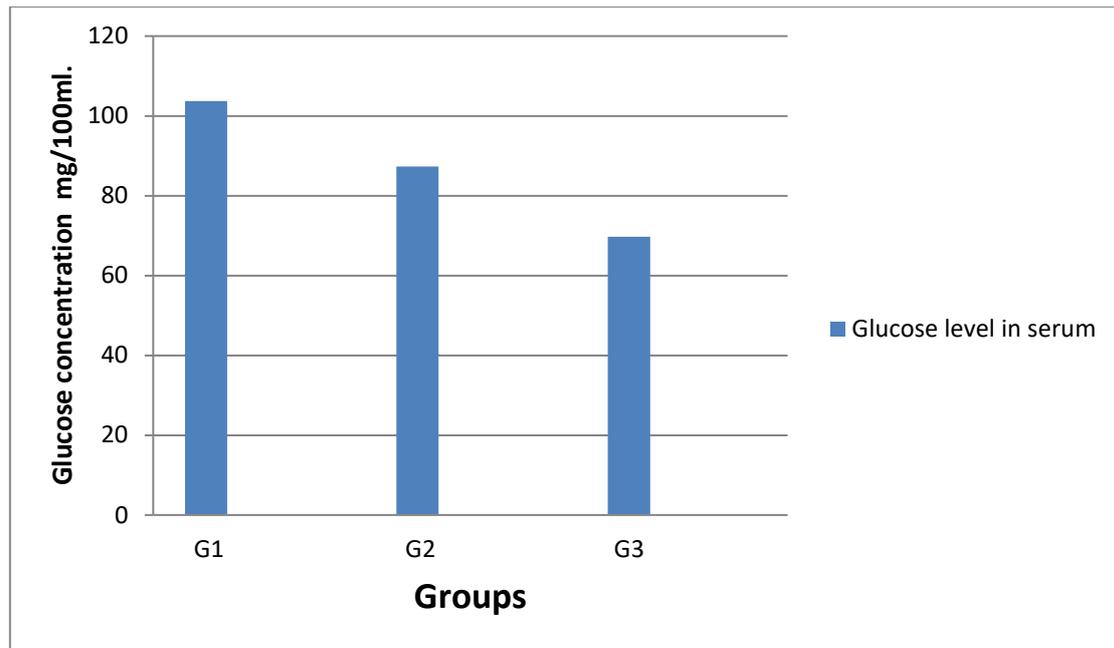


Fig.-1: showed the different levels of glucose in serum between the groups after 10 days of treatment.

The insulin concentrations ($\mu\text{U}/\text{ml.}$) in serum were significant increase in G2 during the period of treatment, 1st day= 54.94 ± 0.61 and 10th day= 78.92 ± 1.03 , and in G3 at 1st day= 56.44 ± 0.74 and 10th day= 89.36 ± 1.46 . While it were nearly stable in G1 at 1s day= 55.1 ± 0.42 and at 10th day= 54.9 ± 0.17 as showed in table-3.

Table-3: Serum insulin concentrations ($\mu\text{U}/\text{ml.}$) values at the period of treatment.

Groups	Period of treatment									
	1 st day	2 nd day	3 rd day	4 th day	5 th day	6 th day	7 th day	8 th day	9 th day	10 th day
G1 (n=10)	55.1 ± 0.42 42Aa	55.2 ± 0.2 4Aa	$54.8\pm 0.$ 18Aa	$55.04\pm$ 0.12Aa	$54.9\pm 0.$ 45Aa	$55.7\pm 0.$ 43Aa	$55.78\pm$ 0.33Aa	$55.36\pm$ 0.33Aa	$55.18\pm$ 0.19Aa	$54.9\pm 0.$ 17Aa
G2 (n=10)	$54.94\pm$ 0.61Aa	$56.16\pm 0.$ 53ABab	58.06 ± 0 .74Bb	$61.12\pm$ 0.98Bc	$64.16\pm$ 1.30Bd	$66.88\pm$ 1.51Be	$68.66\pm$ 1.44Be	$72.44\pm$ 1.09Bf	$75.48\pm$ 0.55Bg	$78.92\pm$ 1.03Bh
G3 (n=10)	$56.44\pm$ 0.74Aa	58.7 ± 0.5 1Ba	63.44 ± 0 .76Cb	$67.3\pm 1.$ 27Cc	$70.86\pm$ 1.15Cd	$73.94\pm$ 1.28Ce	$77.28\pm$ 1.41Cf	$81.22\pm$ 1.19Cg	$85.12\pm$ 1.31Ch	$89.36\pm$ 1.46Ci

*LSD=2.56 *Different letters mean the variances were significant at $p \leq 0.05$.

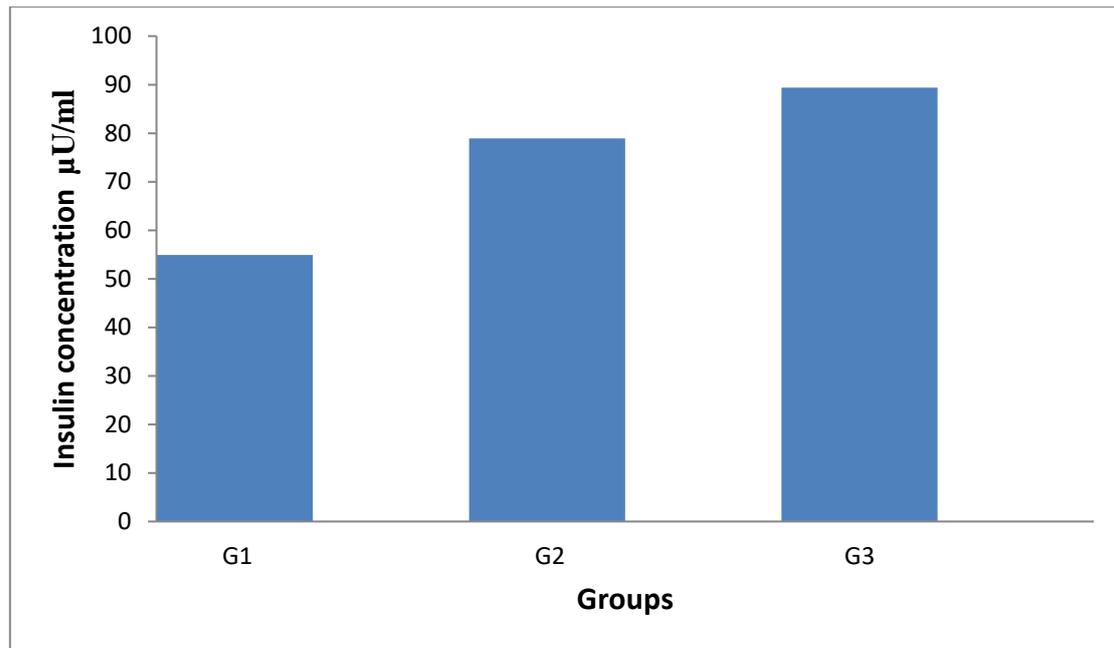


Fig.-2: showed the differences of insulin levels between the groups after 10 days of treatment.

The body weights (gm) were significant decrease in G2 during the period of treatment, 1st day=181.6±0.2 and 10th day=162.9±0.3, and in G3 at 1st day= 182.8±0.7 and 10th day=147.8±0.3. While it were nearly stable in G1 at 1s day=184.1±0.5 and at 10th day=185.7±0.8 as showed in table-3.

Table-4: showed the estimation of body weights (gm).

Groups	Period of treatment									
	1 st day	2 nd day	3 rd day	4 th day	5 th day	6 th day	7 th day	8 th day	9 th day	10 th day
G1 (n=10)	184.1± 0.5Aa	184.3±0. 7Aa	183.9±0 .5Aa	186.5± 0.1Aa	183.2± 0.8Aa	184.2± 0.8Aa	182.7± 0.5Aa	183.6± 0.3Aa	186.2± 0.4Aa	185.7± 0.8Aa
G2 (n=10)	181.6± 0.2Ba	180.4±0. 6Bb	178.9±0 .7Bc	178.2± 0.1Bc	176.8± 0.4Bd	173.8± 0.1Be	171.2± 0.5Bf	167.8± 0.8Bg	164.2± 0.7Bh	162.9± 0.3Bi
G3 (n=10)	182.8± 0.7Ca	178.1±0. 4Cb	175.2±0 .5Cc	172.4± 0.6Cd	168.5± 0.6Ce	163.2± 0.8Cf	160.5± 0.5Cg	157.3± 0.9Ch	152.1± 0.1Ci	147.8± 0.3Cj

*Different letters mean the variances were significant at $p \leq 0.05$. * LSD=2.18

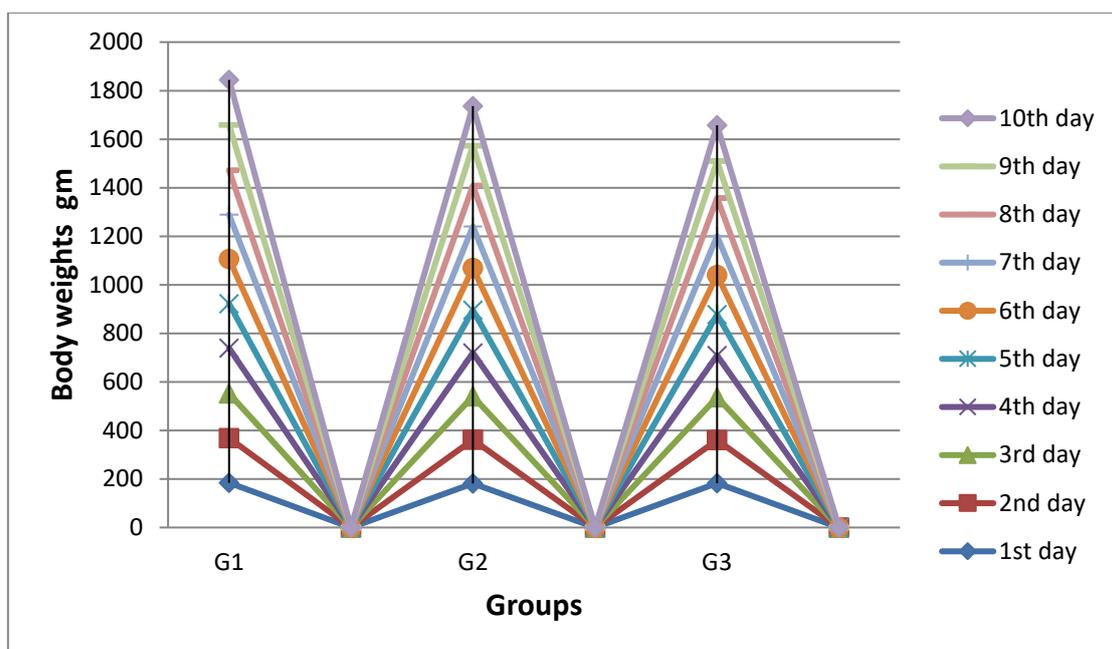


Fig.-3: showed the comparison of body weights (gm) between G2 and G3.

Table-5: showed the circulating IGF-1 concentrations (ng/ml.) in serum

Groups	Period of treatment									
	1 st day	2 nd day	3 rd day	4 th day	5 th day	6 th day	7 th day	8 th day	9 th day	10 th day
G1 (n=10)	329.7	322.7	343.3	335.4	329.7	334.7	330.2	322.2	328.6	319.4
	318.9	314.7	323.7	343.2	338.7	327.9	325.6	346.8	333.7	322.5
	322.5	323.8	318.2	324.6	325.4	328.5	324.9	324.7	321.6	321.7
	330.1	327.8	328.3	332.8	336.8	330.7	329.9	345.1	329.1	327.8
	323.8	326.2	326.1	328.5	329.6	331.6	324.5	322.0	327.7	321.5
G2 (n=10)	325.4	328.7	332.5	335.4	336.4	338.2	341.6	342.2	346.8	352.7
	324.5	328.7	330.1	333.5	335.2	338.3	342.5	346.7	352.2	357.8
	326.0	329.3	332.6	336.5	338.5	339.7	341.3	347.8	353.1	358.5
	332.4	331.0	332.6	336.1	337.8	339.5	341.2	346.7	347.2	359.2
	334.0	334.3	334.2	336.9	338.5	338.9	342.7	345.3	347.6	353.5
G3 (n=10)	325.1	332.5	336.8	341.3	348.6	354.6	361.3	368.7	372.8	382.7
	327.3	337.2	345.5	348.7	353.8	359.4	368.9	373.2	379.0	384.6
	325.7	336.5	339.7	343.5	346.2	354.0	358.6	363.1	375.1	385.8
	328.6	338.5	347.2	354.6	358.5	362.6	370.8	372.3	376.5	382.3
	326.3	341.5	357.3	364.0	373.7	379.2	382.5	384.8	386.2	388.7

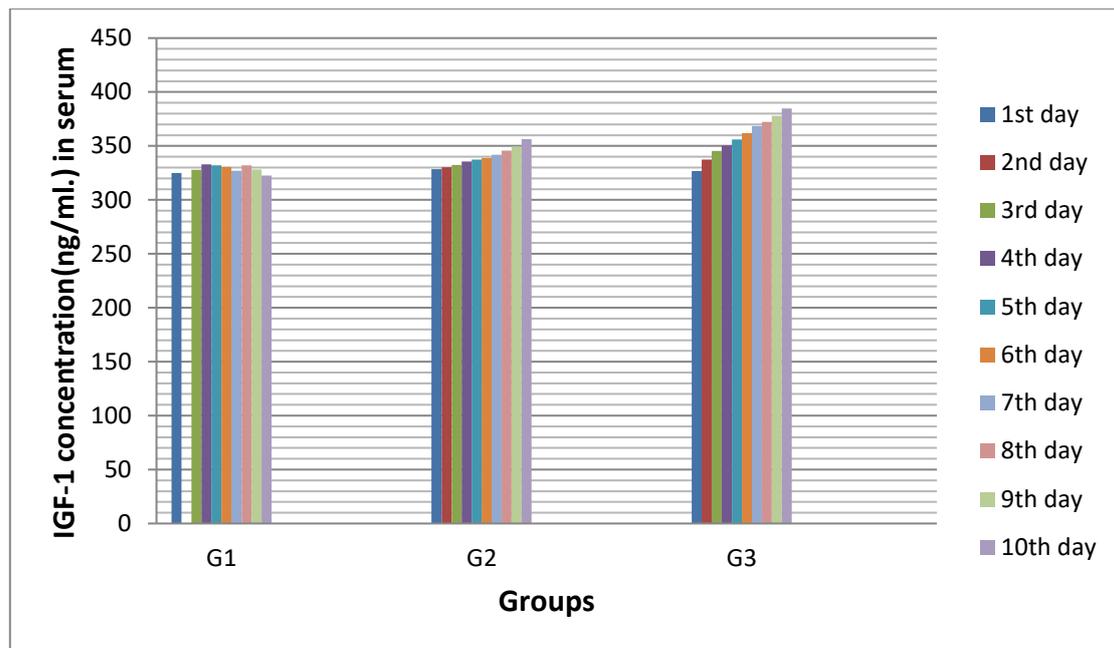


Fig.-4: showed the differences between G2 and G3 of circulating IGF-1 concentration (ng/ml.) in serum

Discussion

It is well known that *Nelumbo nucifera* plant is worldwide distributed in many countries and may cause real problem for agriculture work ship and has harmful effect on fish by consume the water soluble oxygen in addition that these countries have a series general health with management of diabetes. (7-9). The results of the current study showed significant gradual decrease of glucose concentration in serum due to single dose 1 ml. daily during the 10 days of treatment to G2, the results also showed significant gradual increase of insulin concentration in serum during the period of treatment of G3 when compared with control group G1(10-12).

References

- [1] Wild, S.; Roglic, G.; Green, A.; Sicree, R.; King, H. Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. *Diabetes Care* 2004, 27, 1047–1053.
- [2] Nair, M. Diabetes mellitus, part 1: Physiology and complications. *Br. J. Nurs.* 2007, 16, 184–188.
- [3] Haffner SM, Lehto S, Ronnema T, Pyorala K, Laakso M : Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl J Med*,1998, 339:229-234.
- [4] World Health Organization : The WHO Committee on Diabetes Mellitus: Second Report. Technical Report Series 646. World Health Organization, Geneva,1980, p 61.
- [5] Kusano S, Abe H : Antidiabetic Activity of White Skinned Sweet Potato (*Ipomoea batatas* L.) In Obese Zucker Fatty Rats. *Biol Pharm Bull*,2000, 23:23-26.
- [6] Huang CF, Chen YW, Yang CY, et al. Extract of lotus leaf (*Nelumbo Nucifera*) and its active constituent catechin with insulin secretagogue activity. *J Agric Food Chem* 2011; 59(4): 1087-1094.
- [7] ZADEH, Firoozeh Abolhasani, et al. Cytotoxicity evaluation of environmentally friendly synthesis Copper/Zinc bimetallic nanoparticles on MCF-7 cancer cells. *Rendiconti Lincei. Scienze Fisiche e Naturali*, 2022, 1-7.
- [8] ROHMAH, Martina Kurnia, et al. Modulatory role of dietary curcumin and resveratrol on growth performance, serum immunity responses, mucus enzymes activity, antioxidant

- capacity and serum and mucus biochemicals in the common carp, *Cyprinus carpio* exposed to abamectin. *Fish & Shellfish Immunology*, 2022, 129: 221-230.
- [9] ARIF, Anam, et al. The functions and molecular mechanisms of Tribbles homolog 3 (TRIB3) implicated in the pathophysiology of cancer. *International Immunopharmacology*, 2023, 114: 109581.
- [10] MARGIANA, Ria, et al. Functions and therapeutic interventions of non-coding RNAs associated with TLR signaling pathway in atherosclerosis. *Cellular Signalling*, 2022, 100: 110471.
- [11] LEI, Zimeng, et al. Detection of abemaciclib, an anti-breast cancer agent, using a new electrochemical DNA biosensor. *Frontiers in Chemistry*, 2022, 10.
- [12] BASHAR, Bashar S., et al. Application of novel Fe₃O₄/Zn-metal organic framework magnetic nanostructures as an antimicrobial agent and magnetic nanocatalyst in the synthesis of heterocyclic compounds. *Frontiers in Chemistry*, 2022, 10.