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Original Article

Effect of Pangasius Hypophthalmus Fish Extract on Blood Sugar and Uric Acid Levels in Alloxan-Induced *Rattus Norvegicus*

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ABSTRACT

Pangasius hypophthalmus fish, as a dietary source, contains vitamins and minerals that serve as antioxidants, effectively preventing oxidative stress. Diabetes mellitus is rooted in oxidative stress-related pathophysiology. The experimental subjects were categorized into three groups: K1, the control group without treatment; K2, intraperitoneally (i.p) induced with alloxan at a dose of 150 mg/kgBW on the seventh day, with a three-day interval; and K3, the treatment group, which, similar to K2, received alloxan induction while also being administered Pangasius hypophthalmus fish oil extract at a dose of 73 mg/kgBW through intragastric sonde for 14 days. Alloxan induction results in pancreatic cell damage and decreased insulin production, leading to dysregulation of blood glucose levels and consequent hyperglycemia. A significant reduction in blood glucose levels ($p = 0.009$) was observed in the alloxan-induced group and the alloxan-induced treatment group, which received Pangasius hypophthalmus fish oil extract. The presence of Omega-3 in Pangasius hypophthalmus oil stimulates zinc transportation to the cell membrane, promoting insulin stabilization, reducing degradation, and enhancing insulin sensitivity. Comparatively, uric acid levels demonstrated a decline in the alloxan-induced animal group when compared to the untreated group ($p = 0.008$). However, the observed mean increases in uric acid levels within the experimental animal group-induced by alloxan and subsequently administered Pangasius hypophthalmus fish extract-did not attain statistical significance ($p = 0.059$). The Pearson correlation test revealed a robust inverse association of -0.51 between blood glucose levels and uric acid concentrations. Pangasius hypophthalmus fish extract contributes to the reduction of blood glucose levels in experimental animals induced by alloxan. However, no distinct variance in uric acid levels was observed.

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GRAPHICAL ABSTRACT

Introduction

Pangasius hypophthalmus fish holds a prominent position as a widely consumed food source, boasting a plethora of nutrients such as protein, fat, vitamins, and minerals that impart significant benefits to the human body. Vitamins A, B, E, and minerals found in Pangasius function as antioxidants, effectively mitigating oxidative stress [1]. Diabetes mellitus stands as one of the leading contributors to morbidity and mortality, ranking among the top ten prevalent diseases. It is characterized by elevated blood glucose levels that result in complications. The pathophysiology of diabetes mellitus-related complications is often entwined with heightened free radical formation, leading to oxidative stress and subsequent cellular damage [2-6].

Within the context of individuals with diabetes mellitus, the interplay between blood sugar and uric acid levels remains a debatable topic. While certain researchers propose a positive correlation between these two variables, others advocate for an inverse association [6-11].

This experimental study employs an animal model of alloxan-induced diabetes mellitus. Alloxan, a hydrophilic chemical compound, selectively damages pancreatic β cells, culminating in diminished insulin production and consequent hyperglycemia. The administration of Pangasius hypophthalmus fish extract, abundant in antioxidants and other bioactive compounds, is anticipated to enhance antioxidant activity. Administering this extract to animal models of diabetes mellitus aims to lower blood glucose levels and mitigate oxidative stress.

This study aims to prove whether the administration of Pangasius hypophthalmus extract influences blood glucose and uric acid levels in alloxan-induced experimental animals.

Materials and Method

Method

Preparation of experimental animals

A preliminary period of 7 days allowed the experimental subjects to acclimate before embarking on a 17-day treatment regimen. Alloxan induction, at a dose of 150 mg/kgBW, was administered via intraperitoneal (i.p) injection on the seventh day, following its dissolution in a 0.9% NaCl solution [12].

The experimental subjects were categorized into three distinct groups. Group 1 constituted untreated subjects; Group 2 received alloxan induction at 150 mg/kgBW, dissolved in 0.9% NaCl solution via i.p injection on the seventh day, followed by a three-day latency period until the tenth day; and Group 3 mirrored the alloxan induction of Group 2, subsequently receiving Pangasius Fish oil extract at a daily dose of 73 mg/kgBW, dissolved in a 0.5% CMC Na solution and administered via intragastric sonde on the tenth day, continuing daily for 14 days [12-15].

Upon completion of the study, the experimental subjects were humanely euthanized under anesthesia induced through intramuscular injection of ketamine at a dosage of 50 mg/kgBW.

Preparation of pangasius fish oil extract

A 750-gram Pangasius fish underwent meticulous cleaning and draining, followed by segmentation into pieces. The fish pieces were were subjected to boiling in 500 ml of distilled water, followed by a 30-minute standing period with gentle agitation. Subsequently, the Pangasius Fish stew was meticulously strained, facilitating the separation of crude oil from solids [1, 16]. The purification process of crude Pangasius oil involved the addition of 2.5% NaCl, followed by a 5-minute heating phase at 50 °C. Separation of the oil and water phases was achieved through the utilization of a separatory funnel, and the extracted oil was stored in an Erlenmeyer flask. Augmentation of the oil with 1% bentonite, relative to its weight, ensued, accompanied by a 30-minute heating episode at 80 °C. Centrifugation, operating at 10,000 rpm for 10 minutes, yielded the isolation of oil from the sediment [17].

Determination of blood glucose levels

To determine the blood glucose levels, the Glucose Oxidase-Phenol 4-Aminoantipyrin (GOD-PAP) method was employed: Enzymatic oxidation in the presence of glucose oxidase produced hydrogen peroxide, which, with peroxidase, reacted with phenol and 4-aminophenazone to form a red-violet quinoneimine complex. Subsequently, a spectrophotometer was employed to assess the absorbance of both standard and sample solutions [18].

Table 1: Results of examination of blood glucose levels and uric acid levels

Group		Blood glucose level (mg/dl)	Uric acid level (mg/dl)
K1 (control group)	1	109	1.0
	2	108	1.2
	3	95	1.6
	4	104	1.1
	5	147	1.4
	6	136	0.6
	7	99	0.6
	8	123	0.5
Mean		115.125	1
K2 (aloxan-induced group)	1	238	0.4
	2	199	0.5
	3	276	0.4
	4	492	0.3
	5	208	0.3
	6	399	0.2
	7	301	0.7
	8	362	0.2
Mean		309.38	0.38
K3 (treatment group)	1	133	1.0
	2	220	1.7
	3	200	0.2
	4	106	1.8
	5	103	0.5
	6	129	1.2
	7	171	0.3
	8	209	1.2
Mean		158.875	0.9875

Principles of uric acid examination via the uricase method

Uricase operates upon uric acid, engendering the production of allantoin, carbon dioxide, and hydrogen peroxide. The resultant hydrogen peroxide, in conjunction with peroxidase, engages with chromogens (amino-antipyrine and dichlorohydroxybenzene sulfonate), thereby engendering a red-hued quinoneimine complex. Quantitative analysis was facilitated by spectrophotometric measurement, performed at 505 nm [9, 19].

Statistical analysis

The acquired data underwent computerized analysis using SPSS, with significance set at 0.05. The results data of blood sugar and uric acid levels in this experiment show in Table 1, the average of blood glucose levels each group in this experiment show in Figure 1, and the average uric acid levels each group in this experiment show in Figure 2.

Results and Discussion

Subsequently, the ANOVA test was conducted, revealing noteworthy distinctions between groups concerning blood glucose ($p < 0.001$) and uric acid levels ($p = 0.013$). The results of the Levene homogeneity test for blood glucose ($p = 0.002$) and uric acid levels ($p = 0.014$) indicated data non-uniformity. As a consequence, the Games-Howell test was employed. The results of the Games-Howel test for blood glucose and uric acid levels can be seen in Table 2.

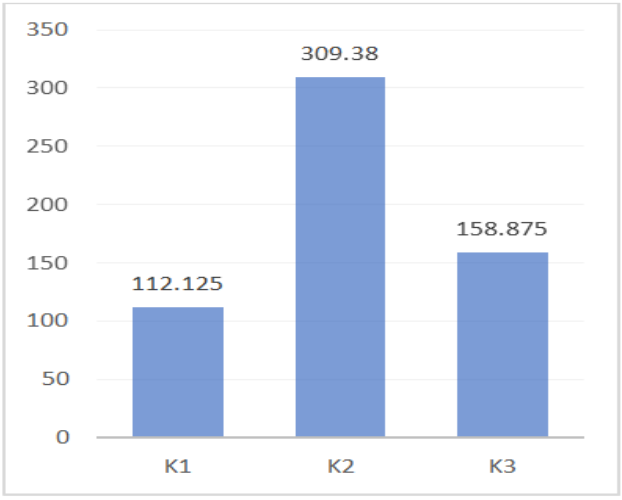

Figure 1: Diagram of average blood glucose levels in each group [K1 (control group); K2 (alloxan-induced group); and K3 (treatment group)]

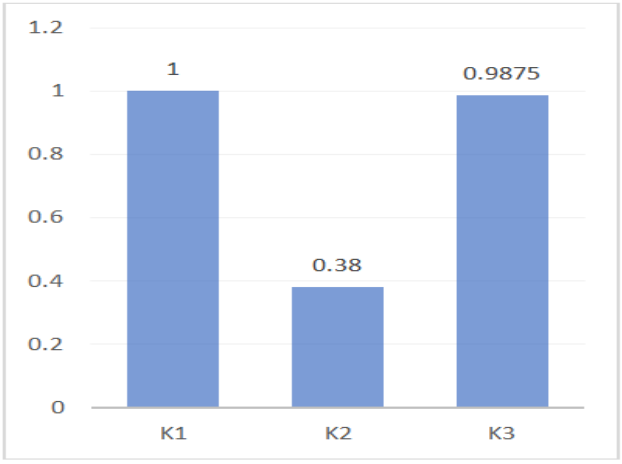

Figure 2: Diagram of the average uric acid levels in each group [K1 (control group); K2 (alloxan-induced group); and K3 (treatment group)]

Table 2: Results of the Games-Howel test for blood glucose and uric acid levels

Variable	Group (I)	Group (II)	Significant level
Blood glucose level	K1 (control group)	K2 (aloxan-induced group)	0.002
		K3 (treatment group)	0.085
	K2 (aloxan-induced group)	K3 (treatment group)	0.009
Uric acid level	K1 (control group)	K2 (aloxan-induced group)	0.008
		K3 (treatment group)	1.000
	K2 (aloxan-induced group)	K3 (treatment group)	0.059

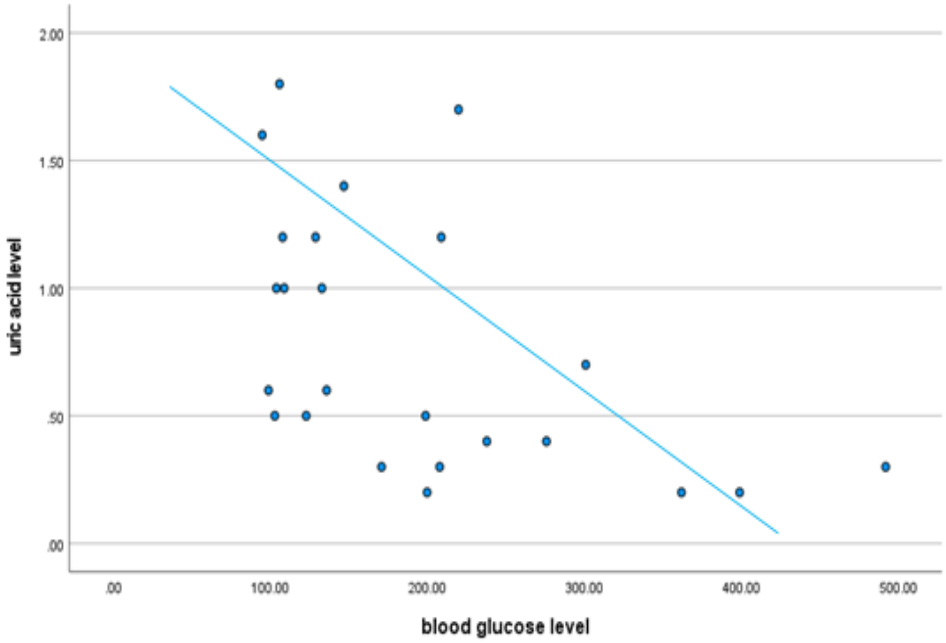

Figure 3: Scatter plot for blood glucose and uric acid levels

The results of the Pearson correlation test exhibited a robust inverse correlation between uric acid levels and blood glucose levels (p = -0.51), indicating a strong contrary relationship. The result of correlation between blood glucose and uric acid levels using Scatter plot can be seen at Figure 3.

The scatter plot test for linearity underscored an inverse association between blood glucose levels and uric acid levels. Alloxan serves as an inducing agent for diabetes mellitus in experimental animals, where varying doses and administration methods yield distinct diabetes mellitus types. In this study, a single intraperitoneal administration of 150 mg/kgBW alloxan was employed to induce pancreatic impairment, resulting in diminished insulin synthesis and subsequent diabetes mellitus development within the experimental subjects. Such conditions lead to fluctuations in blood glucose and uric acid levels within the experimental animals' bodies [20-23].

Effect of pangasius fish extract on blood glucose levels in alloxan-induced experimental animals

Alloxan induction inflicts damage upon pancreatic cells, as evidenced by previous studies [24, 25]. This damage precipitates a reduction in insulin production, subsequently disrupting the regulation of blood glucose levels and culminating in hyperglycemia [26, 27]. In this study, the mean blood glucose levels of alloxan-induced experimental animals experienced a significant increase compared to the control group (p = 0.002). These findings underscore the potential of alloxan to act as a trigger for the development of diabetes mellitus.

Pangasius fish, replete with unsaturated fatty acids, vitamins, minerals, and antioxidants, possesses noteworthy potential. Omega-3 within Pangasius oil stimulates zinc movement to cell membranes, culminating in insulin stabilization. Consequently, insulin degradation is inhibited, optimizing its efficacy in reducing blood glucose

levels and increasing insulin sensitivity [6, 11, 28-29]. In this study, a comparison of blood glucose levels between the alloxan-induced group and the alloxan-induced treatment group, administered Pangasius oil extract, underscored a substantial decrease in blood glucose levels ($p = 0.009$).

Effect of pangasius fish extract on uric acid levels in alloxan-induced experimental animals

The interplay between serum uric acid and diabetes mellitus remains an area of contention. Certain researchers propose an inverse association between uric acid levels and diabetes mellitus, attributed to hindered uric acid reabsorption within the proximal tubule due to heightened blood glucose levels among diabetic patients [9, 10, 19]. Notably, Aktas *et al.* (2021) found increased uric acid levels in individuals with type 2 diabetes mellitus, obesity, and hyperlipidemia. Hyperuricemia can cause joint and kidney disorders and is closely related to metabolic diseases [10, 30, 31]. In this study, uric acid levels within the alloxan-induced animal group demonstrated a decrease in contrast to the control group ($p = 0.008$).

Remarkably, the observed mean increases in uric acid levels within the treatment group-induced by alloxan and subsequently administered Pangasius hypophthalmus fish extract-did not attain statistical significance ($p = 0.059$). The recorded increase in uric acid levels did not surpass that of the control group, signifying no significant divergence between the groups ($p = 1$).

Correlation of blood glucose levels and uric acid levels

The Pearson correlation analysis yielded a coefficient of -0.51, highlighting a robust and inverse correlation. The scatter plot between blood glucose and uric acid levels accentuated this inverse relationship. Specifically, an elevation in blood glucose levels was accompanied by a subsequent reduction in uric acid levels.

Conclusion

In conclusion, this study demonstrates that the consistent daily administration of Pangasius fish extract specifically at a dose of 73 mg/kg BW via intragastric sonde for a span of 14 days, led to a substantial decrease in blood glucose levels among the alloxan-induced *Rattus norvegicus* subjects. However, intriguingly, no statistically significant variance was observed in the uric acid levels of the experimental subjects.

Disclosure Statement

No potential conflict of interest was reported by the authors.

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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.

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