Protective potential of Mangosteen (*Garcinia mangostana*) powder against immuno-toxicity of Azathioprine in Experimental Rats

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ABESTRACT

Mangosteen powder is a source of many nutrients, such as proteins, sugars, minerals, essential fatty acids, vitamins and fiber. They are also rich in phenolic compounds. Mangosteen powder (MP) has a specific flavor, which has many uses in the nutritional and therapeutic areas. Because of these distinctive characteristics of Mangosteen powder, it became more likely that study was to investigate the possible azathioprine is widely used as an immunosuppressant. In this This study was performed to evaluate the effectiveness of mangostana powder (MP) on azathioprine (AZA)-induced immune deficiency in albino rats. Twenty-eight rats male Sprague-Dawley, weighing 120±10g. Rats were randomly distributed into 4 groups each containing (n=7). The groups are as follows: the first group 1 negative control, The second groups were divided into: group I positive group induced dosages oral of azathioprine AZA (25mg/kg/btw/rats). Protective group II with mangosteen powder at level (100 mg/kg/diet), protective group III with mangosteen powder at level (200 mg/kg/diet).

Azathioprine intake has been shown to lead to significant reductions in serum levels of tumor necrosis factor-alpha, interleukin-6, and immunoglobulin E. Additionally, there was a decrease in hepatic reduced glutathione and hepatic nitric oxide levels, along with a notable increase in hepatic malondialdehyde levels. The administration of Mangosteen powder at varying levels demonstrated a potential protective role against the detrimental effects of azathioprine, affecting blood count, antioxidant enzyme activity, and indices. Over the course of the study, both groups receiving 100 and 200 mg/kg of Mangosteen powder displayed a significant increase in feed intake, body weight gain percentage, and feed efficiency ratio, as well as an increase in white blood cell count coupled with a decrease in lymphocyte count. Furthermore, concentrations of immunoglobulins (IgG and IgM) and interleukins (IL4 & IL6) were notably elevated compared to the positive control group. In conclusion, regular consumption of Mangosteen powder may serve as a protective measure with promising immunomodulatory properties and a potent therapeutic value in enhancing the immune response.

Key words: *Mangostana*, Azathioprine, Cytokines and immunoglobulins

القدرة الوقائية لمسحوق المانجوستين ضد السمية المناعية للآزوثيوبرين في فئران التجارب

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ملخص البحث :

يعد مسحوق مانجوستين مصدرًا للعديد من العناصر الغذائية، مثل البروتينات والسكريات والمعادن والأحماض الدهنية الأساسية والفيتامينات والألياف. كما أنها غنية بالمركبات الفينولية. يتمتع مسحوق المانجوستين بخصائص مميزه ، مما جعل له استخدامات عديدة في المجالات الغذائية والعلاجية. تم إجراء هذه الدراسة لتقييم فعالية مسحوق المانجوستانا على نقص المناعة الناجم عن الآزوثيوبرين في الجرذان البيضاء. ثمانية وعشرون فئران ذكر سبراغ داولي، وزنها ١٢٠ ± ١٠ جرام. تم توزيع الفئران عشوائياً إلى ٤ مجموعات تحتوي كل منها على (العدد = ٧). المجموعات على النحو التالي: المجموعة الأولى مجموعة الضابطة السالبة، وتم تقسيم المجموعات الثانية إلى: المجموعة الأولى المجموعة الإيجابية جرعات محرضة عن طريق الفم من الآزوثيوبرين (25 ملغم / كغم / وزن الجسم / الفئران). المجموعة الوقائية الثانية بمسحوق المانجوستين عند المستوى (١٠٠ مجم/كجم/العلية)، المجموعة الوقائية الثالثة بمسحوق المانجوستين عند المستوى (٢٠٠ مجم/كجم/العلية).أشارت النتائج إلى أن تناول الآزوثيوبرين أظهر انخفاضًا ملحوظًا في عامل نخر الورم في المصل ألفا، إنترلوكين ٦، الغلوبولين .علاوة على ذلك، انخفاض مستويات الجلوتاثيون الكبدي وأكسيد النيتريك الكبدي مع ارتفاع كبير في مستوى المالونديالدهيد الكبدي. إن إعطاء مسحوق المانجوستين عند المستوى (١٠٠ ملجم/كجم/علف) وخاصة عند المستوى (٢٠٠ ملجم/كجم/علف)، له دور محتمل ضد التأثير الضار للآزوثيوبرين. في نهاية الفترة التجريبية، أظهرت مستويات كلا المجموعتين الوقائية ١٠٠ و ٢٠٠ جم من ارتفاعًا ملحوظًا في تناول العلف وزيادة وزن الجسم ، وزيادة في عدد كرات الدم البيضاء المرتبطة بانخفاض عدد الخلايا الليمفاوية. كما أظهرت تركيزات الجلوبولينات المناعية والإنترلوكينات زيادة معنوية مقارنة بمجموعة الضابطة الموجبة يمكن أن نستنتج أن الاستهلاك المنتظم للمانجوستين يمكن أن يحمي الجسم من عامل تعديل المناعة الواعد ذي القيمة العلاجية القوية في تحفيز الاستجابة المناعية.

الكلمات الأساسية: مانجوستانا، الآزاثيوبرين ، السيتوكينات ، الجلوبيولينات المناعية

INTRODUCTION

Functional foods have gained great popularity in the health and therapeutic fields recently, and it has been found that girls use them to a greater extent than men. Some types contain nutritional supplements or other additional ingredients to improve the body's health, examples of which include foods fortified with vitamins and minerals.

Tropical mangosteen (*Garcinia mangostana*) is also known as the queen of fruits or the fruit of kings because the Queen of the Netherlands grew mangosteen in her palace garden and used to give it to kings and princes. It is one of the most delicious tropical fruits. Mangosteen ripens on an exotic tropical tree that is native to Southeast Asia and Thailand. The mangosteen fruit is distinguished by its dark purple color and its exceptional and delicious taste (**Aizat *et al.*, 2019**). It is also known as one of the most famous tropical fruits. Mangosteen has been grown in regions of Southeast Asia since ancient times. It was later grown in the Americas, especially in Guatemala, Panama, Ecuador, and Honduras. One of the largest mangosteen farms is in Asia, with Thailand being the largest producing country. Large quantities are produced in Malaysia, the Philippines, Indonesia, and Puerto Rico (**Yao *et al.*, 2023**).

The benefits of Thai mangosteen are fighting aging, and they have a role in losing excess weight because their role in getting rid of excess weight is because mangosteen contains few calories and does not contain saturated fats, which works to improve good cholesterol levels in the body and lower high blood pressure. It improves memory and prevents Alzheimer's disease. Because it contains vitamins and minerals necessary for human health, as it contains calcium, magnesium, potassium, phosphorus, zinc, vitamins C and A, and a high percentage of fiber, it works to prevent the spread and division of cancer cells in several different types of tumors and cancers. It also works to strengthen the immune system against viruses (**El-Seedi *et al.*, 2009, 2010; Ovalle-Magallanes *et al.*, 2017 and Tousian *et al.*, 2017)**.

Azathioprine (AZA), chemically known as 6-1-Methyl-4-nitroimidazol thiopurine, is commonly used as an immunosuppressant, often in conjunction with corticosteroids. **(Gaston, 2001)**. Its applications include preventing organ transplant rejection and treating various autoimmune diseases such as rheumatoid arthritis, inflammatory bowel disease, biliary cirrhosis, lupus nephritis, and multiple sclerosis.

(Heneghan and McFarlane, 2002 and Conti *et al.*, 2013). The therapeutic benefits of AZA encompass the management of pancreatitis, gastrointestinal disturbances, skin rashes, muscle and joint pain, fever, chills, tachycardia, hypotension, and renal dysfunction. **(Lin *et al.*, 2000)**.

The therapeutic effect of AZA includes treatment of pancreatitis, gastrointestinal disturbances, rashes, muscle and joint pains, fever, chills, tachycardia, hypotension and renal dysfunction **(Sweetman and Martindale, 2005).** AZA treatment inhibits infections of bacteria, viral, and inhibits phagocytosis **(Colombel *et al.*, 2010).** Also, liver toxic appeared in azathioprine treatment patients in the form of idiosyncratic cholesterol, vascular disorders, anemia, lecocytpenia, and thrombocytopenia **(Kirmizibekmez *et al.*, 2021).**

Therefore, the present study aims to hypothesize the potential protective impact of mangosteen powder at levels (100 and 200mg /kg /diet) against azathioprine in combination on changes in the immune system of laboratory rats and susceptibility to AZA induced immunosuppression.

MATERIALS AND METHODS

- MATERIALS:

Plant materials: Purple mangostana *(Garcinia mangostana L.)* were purchased from local markets at Kuwait.

Rats: For the experimental study, twenty-eight male albino rats of the Sprague Dawley strain were procured from the National Research Centre in Giza, Egypt, with an average weight of 110 ± 10 g.

The chemical and drug utilized in the study was Azathioprine in tablet form (Azamun©) at a dosage of 50 mg, sourced from a reliable manufacturer. El-Nasr Pharmaceutical Chemicals Co. "ADWIC" (Egypt). Biochemical kits were purchased from Alkan Co. for Chemicals and Biodiagnostics , Dokki, Egypt.

- METHODS:

a-Mangostana fruit powder (MP):

Mangostana as all were oven-dried at 45 °C. The dried were ground separately into powder by domestic electrical mill and stored at 4 °C until further use (**Shehata *et al.*, 2021**).

b-Chemical analysis:

HPLC analysis of Mangostana was conducted using a Waters 2487 HPLC system equipped with a dual λ detector, a Waters 1525 binary pump, a Waters Symmetry® C18 column, and a Waters Sentry universal guard column. The identification of phenolic compounds in ashwagandha was carried out by comparing experimental retention times with established reference values using an HPLC method. **(Zhishen *et al.*, 1999).**

c-Induction of immunotoxicity:

Immunotoxicity (IMTX) groups (21 rats) induced with high dosages oral of azathioprine (AZA) 25mg/kg/btw/rats dissolved in 2 mL normal saline (**Matsumoto *et al.*, 1990)**. Blood was extracted from tail vein for white blood cells (WBCs), lymphocytes, monocytes and granulocytes count analysis from each rat to make sure the induction of immunotoxicity in azathioprine group as immunotoxicity rats.

d-Experimental design:

After the adaptation period, the animals were randomly assigned to four groups consisting of seven rats each. One group served as the normal (-ve) control and was treated for 28 consecutive days.

Group (1): Normal control rats (ve-) received basal diet.
Group (2): Immunotoxicity group which the animals were subjected to induction of IMTX through administration of AZA and fed on the basal diet.
Group (3): Immunotoxicity group protected by MP at level 100mg/ /kg/ diet once daily
Group (4): Immunotoxicity group protected by MP at level 200mg/ /kg/ diet once daily

Following a day of acclimatization, the rats were euthanized. Throughout the study, daily food intake was calculated, and daily body weight gain was monitored.. All experimental animals in this study were managed according to the guidelines for the Behavioral Research and were approved by the Research Ethics Committee, Home Economics Department, nutrition and food science, Zagazig University, Egypt, under animal protocol (ZU/FSE/2024/4/No 2).

e-Blood and tissue sampling:

At the study's conclusion, the animals underwent an overnight fasting period, were mildly anesthetized with diethyl ether, and blood samples were collected. The blood samples were divided into two parts: one for hematological parameter analysis in EDTA tubes, and the other to determine levels of tumor necrosis factor-alpha (TNF-α), immunoglobin E (IgE), and interleukin-6 (IL-6) after clotting and centrifugation. Each animal was swiftly sacrificed, and the liver was dissected out washed, dried, weighed, and homogenized following established protocols before centrifugation at 3000 rpm for 20 minutes. **(lin *et al*. (1998)**

Determination of complete blood count and indices:

Red blood cell (RBCs) count hematocrit (Hct) value, total hemoglobin (Hb) value, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet (PLT) count, and white blood cell (WBCs) parameters were analyzed. **Drabkin (1949) and Mc Inory (1954).**

e-Statistical analysis: The gained data were statistically analyzed by SPSS computer software according to **Artimage and Berry (1987)**. The calculation accrued by analysis of variance ANOVA & follow up LSD (SPSS) Computer program variation.

RESULTS AND DISCUSSION

The antioxidant properties of phenolic compounds such as flavonoids, polyphenols, and flavones in mangostana powder were examined. The data presented in Table 1 highlight the powder's significant natural antioxidant content. Additionally, the flow of glucose through the polyol pathway may impact antioxidant levels, particularly with glycation and inactivation of lens antioxidant enzymes like superoxide dismutases.(**Ortega-García and Peragón 2010 and Xiao *et al*., 2015**). These findings are in parallel with those obtained by **Nazre *et al*.(2018)** who reported that mangosteen fruit contained plenty of phenolic compounds like benzoic acid derivatives are recognized to possess antioxidative and anti-inflammatory properties (**Paull *et al*., 2012**).

Table 2 displays the mean values ± SD for feed intake, body weight gain %, and feed efficiency ratio (FER) of both control and experimental groups. The negative control group exhibited the highest feed intake, body weight gain %, and FER65 (15.32 ±2.14g, 115.77 ±8.11g and 0.125±0.01g), respectively. These data are confirmed by Tousian et al. (2007) and Hanaa and Madiha (2024). whereas the

experimental groups showed a significant decrease. Conversely, the positive control group, subjected to immunotoxicity induction without treatment, had the lowest feed intake. Body weight gain %, negative control group was (19.11 ± 1.34 g), and then there was insignificant increase in the treated groups with level mangostana (5, 10, &15 %) respectively. The reduction was notable within the remaining experimental groups. Conversely, the positive control group, subjected to medical induction of immunotoxicity without treatment, exhibited the lowest feed intake value. Polyphenolic compounds play a crucial role as constituents due to their antioxidant properties in initiating lipid free radical cascades and inhibiting hydroperoxide formation. (**Mohamed *et al.*, 2016**)

The antiradical activity of phenolic compounds in various species is dependent on their molecular structure, specifically the availability of phenolic hydrogens that lead to the formation of phenoxy radicals through hydrogen donation. **(Ramarathnam *et al.*, 1997and Ugwu *et al.*, 2013).**

Table (3) evidenced that protective groups at level 100 and 200 g into rats improved effects in serum tumor necrosis factor-alpha, interleukin-6 and immunoglobulin E in rats' levels comparable to control rats (**Table 3**). On the other hand, oral intake of AZA to rats reinforce decreases significantly in serum of levels tumor necrosis factor-alpha, interleukin-6 and immunoglobulin E.

Protective groups with mangostana powder (MP) groups at levels (100 &200 g) after immunotoxicity of azathioprine in rats resulted in significant increases in serum tumor necrosis factor-alpha, interleukin-6 and immunoglobulin E level when compared to (+ve) group. **Aci and Keskin, (2023)** indicated that the reduction in antioxidant activities is owing to free radicals. As they stated, the imbalance of oxidant-antioxidant may be one of the major causes accountable for ant immunotoxicity. Previous studies have well shown the richness of mangostana extracts as well as essential oils in phenolic compounds **(*Matosa et al.*, 2009).**

Table 4 data indicates insignificant changes in total white blood cells, lymphocytes, monocytes, and granulocytes counts in rats treated with MP powder compared to the (-ve) control group. In contrast, administration of azathioprine to rats led to significant decreases in total White blood cells, lymphocytes, monocytes and granulocytes

counts. protective groups of MP at levels (100 &200g) increased significantly improve total White blood cells and monocytes counts that were reduced by AZA treatment but it significantly improved lymphocyte and granulocytes count $P<0.05$ when compared with (+ve) groups.

Table 5 results show insignificant changes in various blood parameters in rats given MP, while the positive control group exhibited significant decreases. Treatment with MP (100&200g) resulted in notable increases in certain blood parameters compared to the (+ve) control group. These findings align with previous research attributing reduced blood cell counts to bone marrow depression caused by the incorporation of 6-TGNs into DNA, as the bone marrow is a primary source of blood cells, including lymphocytes. **(Ghonime *et al.*, 2011 and Ban *et al.*, 2022).**

Beneficial effects of polyphenols is associated with biological activities such as antioxidant, anti-platelet aggregation, free radical-scavenging properties and inhibition of vascular muscle cell proliferation. These observations explain cardiovascular protective properties **(Fuhrman and Aviram, 2015).**

Conclusion: This investigation showed the potential value of mangosteen powder as a good source of natural antioxidants, which have a protective action against immunotoxicity-induced by AZA development. The regular ingestion of concentrated mangosteen fruit powder reduced tumor necrosis factor-alpha (TNF-α), immunoglobin E (IgE) and interleukin-6 (IL-6), and greatly restored the complete.

Table 1: **Phenolic compounds mangostana extract**

Phenolic compounds	λ[a] (nm)	EtR[b] (min)	RtR[c] (min)
Flavonoids	479	66.4	56.6
Flavones	682	45.1	35.3
Polyphenols	355	24.2	10.5

a wavelength for determination, b experimental retention time, c standard retention time.

Table (2): Effect of mangostana (MP) on feed intake and body weight gain in rats of body weight gain, food intake and food efficiency ratio (FER) in rats

Groups / Variables	Control (-ve)	*rats received azathioprine*		
		Control (+ve)	MP **100 g**	MP **200g**
Initial weight(g)	120.31± 3.45 [a]	122.24± 4.55 [a]	124.31± 5.01 [a]	124.17± 4.99 [a]
Feed intake (g/w)	15.32± 2.14 [a]	13.55± 2.55 [a]	15.71± 2.71 [a]	15.81± 2.14 [a]
Final weight (g)	236.08± 27.17 [a]	189.13± 17.37 [b]	230.08± 20.13 [a]	232.31± 22.34 [a]
Weight gain (g)	115.77± 8.11 [a]	66.89± 6.11 [b]	105.77± 9.17 [a]	108.14± 10.15 [a]
FER	0.125± 0.01[a]	0.082± 0.03 [b]	0.112± 0.05 [a]	0.113± 0.01 [a]

Values with the same letters indicate insignificant difference and vice versa.

Table (3): Effect of mangostana (MP) on serum level of tumor necrosis factor-alpha, interleukin-6, immunoglobulin E in rats

Groups / Variables	**Control (-ve)**	*rats received azathioprine*		
		Control (+ve)	**MP 100 g**	**MP 200g**
Tumor necrosis factor-alpha **(** pg/mf **)**	95.25± 2.18 a	72.55± 13.98d	86.00± 9.4bc	90.00± 11.6 b
Interleukin-6 **(** pg/mf **)**	9.61± 3.82a	5.14± 4.01c	7.55± 2.4 ab	8.05± 3.4 a
Immunoglobulin E (IgE) **(**IU/mf**)**	35.56± 13.21 a	29.10± 3.42 c	33.73± 7.84b	34.0± 3.54 ab

Values with the same letters indicate insignificant difference and vice versa.

Table (4): Effect of mangostana (MP) on blood level of white blood cells, lymphocyte, monocyte and granulocyte counts in rats.

Groups / Variables	Control (-ve)	*rats received azathioprine*		
		Control (+ve)	**MP 100 g**	**MP 200g**
White blood cells (x103/µl)	8.61± 3.82 a	4.14± 4.01 d	5.65± 1.13 bc	6.73± 2.72 b
Lymphocyte (x103/µl)	6.23± 2.01 a	3.15± 0.91 c	4.16± 1.15b	4.59± 2.01b
Monocyte (x103/µl)	5.59± 4.67a	3.65± 2.9 bc	4.29± 1.3b	5.18± 2.01a
Granulocyte counts (x103/µl)	1.95± 0.99 a	0.85± 0.04 b	1.19± 2.0a	1.29± 0.9 a

Values with the same letters indicate insignificant difference and vice versa.

Table (5): Effect of mangostana (MP) on blood level of haemoglobin (Hb), Red blood cells (RBCs) haematocrit (Hct)%, mean corpuscular volume (MCV) and platelet (Plt) in rats.

Groups / Variables	Control (-ve)	*rats received azathioprine*		
		Control (+ve)	MP **100 g**	MP **200g**
HB (g/dl)	15.08± 2.18 a	13.55± 1.98 c	14.5± 3.05 b	14.9± 2.16 b
RBCs (×106/µL)	7.61± 3.82 a	4.94± 4.01 c	6.89± 1.05 b	6.11± 2.23 b
Hct (%)	39.61± 13.51 a	33.86± 9.65c	37.35± 8.19 b	38.78± 13.51 b
MCV (fL)	60.6± 12.35 a	50.90± 3.15d	59.99± 9.91 c	63.71± 11.5 b
Plt ×103/µL	880.36± 63.15 a	680.19± 23.05 d	750.65± 43.8 c	800.23± 63.2 b

Values with the same letters indicate insignificant difference and vice versa

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