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

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ABESTRACT

Mangosteen powder are 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) have a specific flavor, which made them 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 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).

The results indicated that azathioprine intake showed significant decreases in serum tumor necrosis factor-alpha, interleukin-6, immunoglobulin E. Furthermore, hepatic reduced glutathione and hepatic nitric oxide levels were diminished matched with a significant rise in the level of hepatic malondialdehyde. Administration of either Mangosteen powder at level (100 mg/kg/diet) especially at level (200 mg/kg/diet), potential role against damaging impact of azathioprine. blood count and indices and activity of antioxidant enzymes. At the end of the experimental period, both protective groups levels 100& 200 g of MP a significant to rise in feed intake, body weight gain % and FER, increase in the number_of WBCS associated with a decrease in the number of lymphocytes. Also, concentration of immunoglobulins (IgG and IgM) and interleukins (IL4 &IL6) showed a significant increase comped to (+ve) group. It can be concluded that regular consumption of MP can protect the body from the a promising immunomodulatory agent with a potent therapeutic value in stimulating 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 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) (6-1-Methyl-4-nitroimidazol thiopurine) is used as an immunosuppressant usually corticosteroids **Gaston, (2001)**. Azathioprine is used to protect in the rejection of organs transplantion and it is used in treatment of auto-immune diseases. Azathioprine is used to prevent renal graft rejection, and hepatic transplantation **Heneghan and McFarlane, (2002) and Conti *et al.,* (2013)**. Due to its anti-inflammatory activities, azathioprine is used to treat rheumatoid arthritis,

bowel disease, biliary cirrhosis, lupus nephritis and multiple sclerosis **Lin *et al.*, (2000)**.

The therapeutic effect of AZA include treatment of pancreatitis, gastrointestinal disturbances, rashes, muscle and joint pains, fever, chills, tachycardia, hypotension and renal dysfunction **Sweetman and Martindale, (2005).** AZA treatment inhibit infections of bacteria, viral, and inhibition 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) aagainst 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 Kuwait.

Rats: Twenty-eight male albino rats of Sprague Dawley strain were purchased from National research centre, Giza, Egypt. The average weight was (110 ±10 g).

Chemicals and durg: Azathioprine (Azamun) ©: Azathioprine tablets 50 mg manufactured from El-Nasr Pharmaceutical Chemicals Co. "ADWIC" (Egypt). Biochemical kits were purchased from Alkan Co. for Chemicals and Biodignostics , 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:

Mangostana for HPLC analysis was performed using a waters 2487 HPLC system consisting of a dual λ detector and a Waters 1525 binary pump,and equipped with a Waters Symmetry® C18 column (5 mm, 4.6 × 50 mm) with Waters Sentry universalguard column (5 mm, 4.6 × 20 mm) (Waters Corporation,Milford, MA, USA). Phenolic compounds of ashwagandha were studied using the reference HPLC

method by comparing experimental retention times with reported reference values **(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 adaptation period the animals were randomly divided into 4 groups of 7 rats each and one of them was kept as a normal (-ve) control group and treated for 28 consecutive days as follows:

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

And after one day of that, the rats were sacrificed. During the study, the food intake was calculated daily and the body weight gain was recorded daily. 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 end of the experimental period, animals were fasted overnight. They were slightly anesthetized with diethylether. Blood samples were collected. Every blood sample was divided into 2 portions: one portion put into EDTA tubes to hematological parameters determination, while the other was left to clot and then centrifuged then clear serum was separated for determine tumor necrosis factor-alpha (TNF-α), immunoglobin E (IgE) and interleukin-6 (IL-6) . Each animal was rapidly

sacrificed, and the liver was dissected out and then washed with saline, dried, weighted and subjected to homogenization according to **Lin *et al.*, (1998),** centrifuged at 3000 rpm for 20 minutes.

Determination of complete blood count and indices:

Red blood cells (RBCs) count, hematocrite (Hct) value, total haemoglobin (Hb) value, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), platelets (PLT) count and leucocyte parameters (white blood cells (WBCs), lymphocytes, monocytes and granulocytes count according to **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 phenolic compounds as, flavonoids, Polyphenols and Flavones were investigated in mangostana powder (MP). The data in Table (1) indicate that the powder is a rich source of natural antioxidants. Furthermore, the flux of glucose through the polyol pathway may contribute to a loss of antioxidants, which is further aggravated by the glycation and inactivation of lens antioxidant enzymes such as the 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**).

The results in table (2) show the mean value ± SD of feed intake, body weight gain % and FER of the control group and experimental groups. It could be seen that negative control group (-ve) recorded the highest value of feed intake, body weight gain %, and FER65 (15.32 ± 2.14 g, 115.77 ±8.11 g and 0.125±0.01g), respectively. These data are confirmed by **(Tousian et al., 2007 and Hanaa and Madiha 2024)** 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. While it decreased significantly in the remained of the experimental groups. On the other side, the feed intake recorded the lowest value for positive

control group which exposed to medical induction of **immunotoxicity** and without treatment. Polyphenolic compounds are very important constituents, by virtue of their antioxidant activity in activating lipid free radical chains and preventing hydroperoxide. (**Mohamed et al., 2016**)

Antiradical activity of phenolic compounds seen in species depend on their molecular structure; that is, on the availability of phenolic hydrogens, which result in the formation of phenoxyl radicals due to hydrogen donation **Ramarathnam *et al.*, (1997) and Ugwu *et al.*, (2013).**

Table (3) evidenceed 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.

Proticitve 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 owing to free radicals. As they stated, the imbalance of oxidant-antioxidant may be one of the major causes accountable for antimmunotoxicity. Previous studies have well shown the richness of mangostana extracts as well as essential oils in phenolic compounds ***Matosa et al.*, (2009).**

Data presented in Table (4) showed that protective groups of MP powder into rats produced insignificant changes in total white blood cells , lymphocytes, monocytes and granulocytes counts when compared to (-ve) group rats. In the contrary, azathioprine oral administration into rats induced 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.

Data presented in Table (5), showed that protective groups of MP into rats revealed insignificant changes in Hb, RBCs, Hct, MCV, and Plt counts when all

compared with control rats. While, (+ve) group significant decreases in Hb, RBCs count Hct values, MCV values and Plt count. Protective with MP at levels (100 &200g) resulted in significant increases in Hb and RBCs count and significant increase in Hct, MCV and Plt count when compared to (+ve) group rats. These results are consistent with results that attributed that reduction in RBCs, WBCs and platelets counts to bone marrow depression due to the incorporation of 6-TGNs into DNA. Bone marrow served as the major source of all blood cells, including lymphocytes **Ghonime *et al.*, (2011) and Ban *et al.*, (2022)**

Beneficial effect of polyphenols is associated with biological activities 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	15.08±	13.55±	14.5±	14.9±
(g/dl)	2.18 a	1.98 c	3.05 b	2.16 b
RBCs	7.61±	4.94±	6.89±	6.11±
(×106/µL)	3.82 a	4.01 c	1.05 b	2.23 b
Hct	39.61±	33.86±	37.35±	38.78±
(%)	13.51 a	9.65c	8.19 b	13.51 b
MCV	60.6±	50.90±	59.99±	63.71±
(fL)	12.35 a	3.15d	9.91 c	11.5 b
Plt ×103/µL	880.36±	680.19±	750.65±	800.23±
	63.15 a	23.05 d	43.8 c	63.2 b

Values with the same letters indicate insignificant difference and vice versa

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