Study the Effect of Hibiscus *(Hibiscus Sabdariffa)* and Turmeric *(Curcuma Longa, L)* on Immune Indicators in Experimental Rats.

Dr.
Mai Mahmoud Khafagy
Associate Prof. of Nutrition and Food Sciences, Faculty of Home Economics, Menoufia University

Dr.
Amira Hamdy Darweesh
Lecturer of Nutrition & Food Science, Faculty of Home Economics, Menoufia University

Nourhan Salah Elfeky
B.Sc. in Home Economics, Major Nutrition and Food Science, Faculty of Home Economics, Menoufia University (2018)

Abstract

Immunodeficiency is a malfunction of the immune system , leading to frequent and highly contagious infections that last longer than usual. Recently, interest has been paid to natural materials extracted from plants and herbs . Herbs have always been an important source of clinical therapeutics and their active components are beneficial. This research study the potential health effects of hibiscus and turmeric on immune indicators. Forty-two (42) rats, 6 rats in the negative control group and 36 rats in the experimental group. The experimental group was injected with Cyclosporine (50 mg/kg/day) to suppress immunity and then divided into six groups of six rats each. One group was kept as a positive control group, while the other five groups were given different amounts of hibiscus and turmeric powder for a period of 28 days. The amounts given were 2.5%, 5% of hibiscus powder, 0.5%, 1% of turmeric powder, and 3% of a mixture of hibiscus and turmeric powder. Biochemical , biological, the antioxidant activity and immune indicators were determined. The results showed that all treated groups had higher body weight gain, feed efficiency ratio, and feed intake as compared to the positive control group, indicating a significant difference. The results also showed that the addition of hibiscus and turmeric to treated groups, the Immunoglobulin production levels began to return to the normal level, which was close to the level of the

negative control group. The study concluded that hibiscus and turmeric has been found to have important bioactive compounds. they have a vital role to improve immunity, especially turmeric 1% followed by hibiscus 5%.

Keywords: Cyclosporine, Kidney function, Immune globulins, rats, immunity.

دراسة تأثير الكركم و الكركدية على مؤشرات المناعة فى فئران التجارب

المستخلص

نقص المناعة هو خلل في الجهاز المناعي، مما يؤدي إلى التهابات متكررة شديدة العدوى تستمر لفترة أطول من المعتاد. في الآونة الأخيرة، تم الاهتمام بالمواد الطبيعية المستخرجة من النباتات والأعشاب. وكانت الأعشاب الطبية دائما مصدرا هاما للعلاجات السريرية ومكوناتها النشطة مفيدة. يدرس هذا البحث التأثيرات الصحية المحتملة للكركديه والكركم على مؤشرات المناعة. ٤٢ فأرا، ٦ فئران في المجموعة الضابطة السلبية و ٣٦ جرذاً في المجموعة التجريبية. تم حقن المجموعة التجريبية بمادة السيكلوسبورين (٥٠ ملجم/كجم/يوم) لتثبيط المناعة ثم تم تقسيمها إلى ست مجموعات تضم كل مجموعة ستة فئران. من بين المجموعات الست، تم الاحتفاظ بمجموعة واحدة كمجموعة ضابطة موجبة، في حين تم إعطاء المجموعات الخمس الأخرى كميات مختلفة من الكركديه ومسحوق الكركم لمدة ٢٨ يومًا. وكانت المقادير المقدمة هي ٢,٥%، و٥% مسحوق الكركديه، و٠,٥%، و١% مسحوق الكركم، و٣% خليط من مسحوق الكركديه والكركم. تم تحديد المؤشرات البيوكيميائية والبيولوجية والنشاط المضاد للأكسدة والمؤشرات المناعية. أظهرت النتائج أن جميع المجموعات المعاملة كانت لديها زيادة في وزن الجسم، ونسبة كفاءة التغذية، وتناول العلف مقارنة بمجموعة السيطرة الإيجابية، مما يشير إلى وجود فرق كبير. كما أظهرت النتائج أن إضافة الكركديه والكركم للمجموعات المعالجة بدأ مستوى إنتاج الغلوبولين المناعي يعود إلى مستواه الطبيعي الذي كان قريباً من مستوى مجموعة السيطرة السلبية. وخلصت الدراسة إلى أن الكركديه والكركم يحتويان على مركبات

نشطة بيولوجيا مهمة. فلهما دور حيوي في تحسين المناعة، خاصة الكركم ١% يليه الكركديه ٥%.

الكلمات المفتاحية: السيكلوسبورين , جلوبيولينات المناعة, وظائف الكلى ،الفئران ،المناعه.

Introduction

Immune system is a complex integrated network of cells, tissues, organs and soluble mediators, evolved to defend the organism against any foreign insult that threatens the integrity of the organism. **Yatim and Lakkis., (2015).** Optimal nutrition for the best immunological outcomes would be nutrition, which supports the functions of immune cells allowing them to initiate effective responses against pathogens but also to resolve the response rapidly when necessary and to avoid any underlying chronic inflammation. The immune system's demands for energy and nutrients can be met from exogenous sources i.e., the diet, or if dietary sources are inadequate, from endogenous sources such as body stores. Some micronutrients and dietary components have very specific roles in the development and maintenance of an effective immune system throughout the life course or in reducing chronic inflammation. For example, the amino acid arginine is essential for the generation of nitric oxide by macrophages, and the micronutrients vitamin A and zinc regulate cell division and so are essential for a successful proliferative response within the immune system **Lee and Han., (2018**).

Immune deficiency:

Many immune deficiency syndromes, mainly 1- humoral defects, are associated with autoimmune disorders. 2- Hematoloical manifestations, such as thrombocytopenia and hemolytic anemia, are the most common presentation, but many other autoimmune mediated conditions have also been described. 3- Persistent ntigen stimulation, due to an inherenty defective immune system ability to eradicate pathogenesis is the primary immunodeficiency states. One promising strategy is the use of herbal medicines as integrative, complementary and preventive

therapy. The active components in medical plants have always been an important source of clinical therapeutics examples of these plants include hibiscus and turmeric**.(Catanzaro *et al.*,2018).**

Hibiscus plant includes more than 300 species, Among them is *Hibiscus sabdariffa*, .which is a valuable source of traditional medicine. The dried flower contain the flavonoids, gossypetin, sabdaretin, hibiscetin , also, the presence of saponin, tannins, cyanogenic glycoside had been reported . Anthocyanins , flavonols and protocatechoic acid along with other phytochemicals have been identified as contributors to the observed medicinal effect of *Hibiscus sabdariffa* . Hibiscus has great impact due to the diuretic, choleretic, analgesic, antitussive, antihypertensive, antimicrobial, immunomodulatory, hepatoprotective, antioxidant, and anti-cancer effects. These therapeutic properties have been attributed to the bioactive compounds of the plant, mainly phenolic acids, flavonoids, anthocyanins, and organic acids **(Ali *et al.*,(2015).**

Curcumin belong to the family of phytochemicals and have antioxidative and anticarcinogenic properties (**Shehata and ElAreefy, (2016).** Curcuma species (family: Zingiberaceae) are widely utilized in traditional medicine to treat diverse immune-related disorders. There have been many scientific studies on their immune modulating effects to support their ethno pharmacological uses **(Tung *et al.*, (2019)**. Turmeric contains a wide variety of phytochemicals, including but not limited to curcumin, demethoxycurcumin, bisdemethoxy-curcumin, zingiberene, curcumenol, curcumol, eugenol, tetrahydro-curcumin, triethylcurcumin, turmerin, turmerones, and turmeronols .Turmeric contains 2-8% curcumin. (**Sello and Eldemery, (2017**). This study aim to investigate the effect of hibiscus *(Hibiscus sabdariffa)*and turmeric *(Curcuma longa, L.)* on immune indicators in male albino rats .

Material and Methods

Materials

The tested plant in this investigation were hibiscus *(Hibiscus sabdariffa)* and turmeric *(Curcuma longa, L.)*, these plants were purchased from local market in Menoufia , Egypt., and defined by Agriculture Crops Department, Agriculture Faculty , Menoufia University . Using sun to dry hibiscus , then milled to give a powder and kept in dusky stoppered glass bottles in a cool and dry location until use.

Cyclosporine was purchased from pharmaceutical company in Cairo, Egypt . Forty two (42) male albino rats of the Sprague Dawley breed weighting (130 ±10 g) were obtained from the animal house of Research Institute Ophthalmology, El-Giza, Egypt. The work carried out at Faculty of Home Economic, Menoufia University, Egypt.

Rats were fed on basal diet for 7 days as an adaptation period, this diet was formulated according to **(AIN, (1993),** the salt mixtures and vitamin mixtures were prepared according to **(Muller, (1964),** respectively. The rats were kept in wire cages in a typical laboratory setting. To reduce feed loss and contamination, the meals were given to rats in special feed containers. Rats were also given water in special cups. The food and water provided were inspected on a daily and rats weighted weekly.

Methods Induction of Immunity disorder.

Thirty six (36) male albino rats weighing (130 ±10 g) were injected with cyclosporine (50 mg/ kg/day subcutaneous in olive oil for ten days . according to the described method by **(Oriji, (2003).**

Experimental Designs and Animal Groups:

After one week of convalescence rats were housed in environmentally controlled The research was conducted and approved in the faculty of Home Economics, Animal House, Department of Nutrition and Food science, University of Menoufia, Egypt, according to Ethical approval of the science Research Ethics committee of faculty of Home Economics cleared the study protocol #19-SREC- 11- 2021

The experiment used Forty two mature albino rats weighing (130 ±10 g), were divided into two main groups .The first group(6 rats each) serving as a negative control group while, the second group were: (36 rats) injected with Cyclosporine to induce suppress immunity, then divided into six groups (6 rats each), one of them kept as positive control group, while the five groups were given 2.5% , 5% of hibiscus powder., 0,5% , 1% of turmeric powder and 3% of mixture of hibiscus and turmeric powder for 28 days.

Biological Indices Calculation

Biological evaluation of the different diets was carried out by determination of feed intake daily, body weight gain g (BWG g /day) and feed efficiency ratio (FER) according to **(Chapman *et al*., 1959)**

using the following equations:

Body Weight Gain = Final weight (g) - Initial Weight (g)

Feed efficiency ratio (FER) = Gain in body weight(g)/Feed intake(g).

Blood sampling

The end of the experimental period each rat separately then, rats are slaughtered. Blood samples were received into clean dry centrifuge tubes and left to clot at room temperature, then centrifuged for 10 minutes at 3000 r.p.m to separate the serum. serum was carefully aspirated and transferred into clean covette tubes and stored frozen at -20°C for analysis according to method described by **(Malhotra, 2003)**.

Biochemical analysis

Blood samples were taken from the portal vein and placed in dry, clean centrifuge tubes for serum separation. Blood samples were centrifuged for 10 minutes at 3000 rpm to separate the serum. Serum samples were kept frozen at -20 °C until chemical analysis according to (**Schermer (1967**).

The serum was used to determine the following**:** Serum immune globulins (IgA and IgM) were estimated according to **(Burlingame and Rubin,(1990).** Serum Tumor necrosis factors (TNF) was determined according to **(Maury,1986).** The activity of aspartate aminotransferases (AST), alanine aminotransferases

(ALT) and alkaline phosphates (ALP) enzymes were assigned by the method of **(Yound , 1975); (Tietz, 1976**) and (**Belfied and Goldberg ., (1971**) respectively.

Statistical Analysis:

The data was performed by using computer program (SPSS) one -way ANOVA when a significant main effect was detected, the means were separated with The Student New man-keuls test. Differences between treatments of ($p \leq 0.05$) were considered significant **(Sendcor and Cochran., (1979).**

Results and Discussion

1.Effect of hibiscus and turmeric on feed intake (FI) , feed efficiency ratio (FER) and body weight gain (BWG) of Cyclosporine injected rates:

Data presented in **Table (1)** showed the effect of hibiscus and turmeric on feed intake (FI) , feed efficiency ratio(FER) and body weight gain (BWG). For body weight gain, it was observed that the highest result value was detected in the group of 1% turmeric followed by 3% mixture. All levels results showed significant changes as compared with both of controls. In case of feed intake, it was showed that the highest effect at level 1% turmeric. Concerning with feed efficiency ratio, it was observed that the mean value of FER of control positive was lower than negative control. Treated groups showed significant increases in mean values as compared with positive control expect 1% turmeric showed non-significant as compared with positive control group.

Results agreed with **Abubakar *et al.*, (2010)** who indicated that lowered immunity was able to reduce weight gain in mice, while at the same time it increase the intake . **(Hussein and Abd El-Maksoud,(2013),** they mentioned that previous studies have reported significantly improvement in body weight and feed intake in rats treated with curcumin compared with untreated rats.

(Umar *et al.*, (2009), Consumption of the *Hibiscus sabdariffa* significantly ($p<0.01$) retarded the rate of weight gain in rats; even though the feed-intake was not significantly affected.

Table (1): Effect of hibiscus and turmeric on feed intake , feed efficiency ratio and body weight gain of cyclosporine injected rates .

Animal Groups	**Feed intake(FI) (g/day)**	**Feed efficiency ratio (FER)**	**Body weight gain (BWG)**
Negative control (G1)	14.32±1.11[a]	0.097±0.001[a]	39.27±1. 51[a]
Positive control (G2)	5.21±0.32[d]	0.074±0.02[d]	10.86±2.55[e]
Rats with 2.5 % hibiscus (G3)	5.43±0.21[d]	0.078±0.007[c]	11.93±1.75[e]
Rats with 5 % hibiscus(G4)	6.39±0.87[c]	0.080±0.008[c]	14.48±1.78[d]
Rats with 0.5 % turmeric (G5)	6.46±0.96[c]	0.081±0.022[c]	14.73±0.23[d]
Rats with 1 % turmeric (G6)	7.55±0.91[b]	0.085 ±0.017[b]	17.96 ±1.03[b]
Rats with 3 %mixture (G7)	7.16 ±1.03[b]	0.080±0.21[c]	16.04±1.01[c]
LSD	0.97	0.003	1.11

***Values presentel as* mean± SD**

Body Weight Gain (BWG),Feed efficiency ratio (FER) ,Feed intake(FI).

Means in the same column with different litters are significantly different (P ≤0.05).

2. Effect of hibiscus and turmeric on serum glucose of rats:

The obtained **Table (2)** showed that the mean value of serum glucose levels of positive control was higher than that of negative control, which were142.08 and 81.05 (mg/dl), respectively, and the value of groups 3,4,5,6,7,were significantly lower ($p<0.05$) when compared to positive control. Group fed on 5% hibiscus was recorded as the nearest mean value for negative control group (healthy rats group).

These results agree in accordauce with **(Zhou *et al.*, 2013)** who showed that during the 28-day study, an increase in serum BGL was observed in Group 2 (diabetic control) in comparison to Group 1 (normal control). Similarly, oral administration of *C. longa* aqueous, ethanol, and hexane extracts (0.5% in diet) as well as curcumin (60–300 mg/kg of body weight) was able to reduce blood glucose levels and in different diabetic rats models.

Results agree with (**Zahid *et al.*, 2014**) who reported that a significant ($p<0.001$) reduction in blood glucose level in normal fasting rats.

Table (2):Effect of hibiscus and turmeric on serum glucose of Cyclosporine injected rats.

Groups	**Glucose (mg/dl)**
Negative control (G 1)	81.05±2.09 [e]
Positive control (G2)	142.08±3.78 [a]
Rats with 2.5 % hibiscus (G3)	135.45±4.52 [b]
Rats with 5 % hibiscus (G4)	121.44±9.02 [c]
Rats with 0.5 % turmeric (G5)	131.53±5.76 [b]
Rats with 1 % turmeric (G6)	118.21±6.88 [d]
Rats with 3 % mixture (G7)	123.05±7.01 [c]
LSD	4.03

Values presentel as mean± SD
Means in the same column with different litters are significantly different (P ≤0.05).

3. Effect of hibiscus and turmeric on kidney function of Cyclosporine injected rats :

Results of the effect of hibiscus and turmeric on urea, creatinine and uric acid of rats of experimental rats are presented in **Table (3).** It was found that positive control group recorded the highest value of serum Creatinine , Urea and Uric acid comparing to negative control group. Treated groups with the different hibiscus and turmeric levels caused siginificant reductions in Creatinine , Urea and Uric acid levels when compared with positive control group. Group 5% hibiscus had

the highest effect on kidney function.

Study by **Cai *et al.*, 2022**) have shown that curcumin was able to affect NF-κB signaling pathway and reduce the expression of IL-1β, IL-6, IL-8 and TNF-α, thus preventing renal inflammatory injury. In the prevention of renal tubular oxidative damage, curcumin reduced ROS (Reactive oxygen species) production by activating the activity of Nrf2, HO-1 and PGC-1α. In addition, curcumin restored mitochondrial homeostasis by upregulating OPA1 and downregulating DRP1 expresion, while reducing apoptosis by inhibiting the caspase-3 apoptotic pathway. (**Ademiluyi *et al.*, 2013**). Furthermore the modulatory effect of the *H.* sabdariffa calyx red dye supplemented diets on kidney functions as exemplified by plasma creatinine, uric acid, urea and blood urea nitrogen (BUN) levels was also studied. And the finding revealed significant (P<0.05) elevation in plasma creatinine (2.2 mg/dL), uric acid (39.3 mgdL), urea (81.4 mg/dL) and blood urea nitrogen (BUN) (38.0 mg/dL) levels.

Table (3): Effect of hibiscus and turmeric on kidney function of Cyclosporine injected rats .

Groups	**Creatinine mg/dl**	**Urea mg/dl**	**Uric acid mg/dl**
Negative control (G 1)	0.89 ± 0.01^{d}	17.7 ± 4.19^{d}	0.99 ± 0.11^{d}
Positive control (G2)	1.37 ± 0.12^{a}	30.6 ± 2.72^{a}	2.38 ± 0.62^{a}
Rats with 2.5 % hibiscus (G3)	1.21 ± 0.02^{b}	24.11 ± 3.66^{c}	2.07 ± 0.06^{b}
Rats with 5 % hibiscus (G4)	1.05 ± 0.42^{c}	19.12 ± 0.58^{d}	1.62 ± 0.01^{c}
Rats with 0.5 % turmeric (G5)	1.31 ± 0.80^{a}	27.63 ± 1.44^{b}	2.22 ± 4.95^{a}
Rats with 1 % turmeric (G6)	1.20 ± 0.33^{b}	23.07 ± 1.05^{c}	2.04 ± 0.32^{b}
Rats with 3 %mixture (G7)	1.24 ± 0.52^{b}	26.53 ± 2.90^{b}	1.87 ± 0.77^{b}
LSD	0.12	2.16	0.24

Values presental as means ± SD
Means in the same column with different litters are significantly different (P ≤0.05).

4. Effect of hibiscus and turmeric on liver enzymes of Cyclosporine injected rats :

The data presented in **Table** (**4**) showed the effect of hibiscus and turmeric on liver enzymes Aspartate aminotransferases, Alanine aminotransferases and Alkaline phosphates (AST,ALT and ALP) of rats. The highest ALT level occurred in both positive control group and rats fed on 5% hibiscus and the negative control group showed the lowest value . Rats fed on basal diet with 1% turmeric had high effect on ALT level.As for AST level, it was observed that control positive group which is significantly higher than all other groups. Regarding ALP level, The control group which fed basal diet only showed significant changes with the others. The highest value was recorded for positive control group and the lowest value was recorded for negative control group followed by rats fed on basal diet with 1% turmeric which recorded no significant changes between rats fed on basal diet with 3% mixture.

The obtained results are agree with those reported **by** (**Serairi *et al.,* 2018**) who showed that This study was conducted to investigate potentially protective and curative effects of *Curcuma longa* root (turmeric) powder on liver enzymes. Turmeric was administered ., Using HPLC analysis, turmeric powder was rich in curcumin (62.97%), demethoxycurcumin (20.86%) and bisdemethoxycurcumin (16.17%). *Curcuma longa* powder showed important in vitro antioxidant activities. Results showed that the activities of aspartate aminotransaminase and alanine aminotransaminase, and the levels of bilirubin and serum lipids were increased in rats.

Table (4): Effect of hibiscus and turmeric on liver enzymes of Cyclosporine injected rats .

Groups	ALT(U/L)	AST(U/L)	ALP(U/L)
Negative control (G 1)	39.76±6.07^{d}	38.61±7.94^{d}	89.3±5.32^{f}
Positive control (G2)	70.66±4.76^{a}	65.10±1.10^{a}	195.7±9.47^{a}
Rats with 2.5 % hibiscus (G3)	67.25±3.82^{a}	60.59±4.26^{a}	187.3±7.86^{b}
Rats with 5 % hibiscus (G4)	54.87±7.42^{b}	51.09±2.48^{b}	162.4±8.13^{d}
Rats with 0.5 % turmeric (G5)	60.31±3.80^{b}	55.04±6.79^{b}	172.3±4.95^{c}
Rats with 1 % turmeric (G6)	46.52±4.82^{c}	45.13±2.75^{c}	144.67±8.15^{e}
Rats with 3 %mixture (G7)	56.25±2.92^{b}	50.19±5.16^{b}	150.7±5.07^{e}
LSD	6.89	4.86	6.04

Values presentel as mean± SD
ALT: Alanine aminotransferase. AST: Aspartate aminotransferase. ALP: Alkaline phosphatase.
Means in the same column with different litters are significantly different (P ≤0.05).

5. Effect of hibiscus and turmeric on immunoglobulin production of cyclosporine injected rats

Data presented in **Table** (**5**) showed the effect of hibiscus and turmeric on serum immune globulins (IGM, IGA,IGG) and Serum Tumor necrosis factors (TNF) levels of experiment rats. In case of IGA, IGG and IGM, positive control group recorded the lowest value as a result of injection by cyclosporine (50mg / kg /day) with significant(P≤0.05) differences, while the highest levels was recorded for negative control group. By adding curcum longa and to treated groups, the IGA, IGG and IGM levels began to return to the normal level, which was close to the level of the negative control group. The best result was recorded for 1% turmeric group. Followed by 3% mixture, 2.5% hibiscus

and 5% hibiscus which recorded the lowest value. In case of TNF, it was found that the highest value was recorded for positive control group, while the lowest value recorded for negative control group with significant (P≤0.05) differences. The mean values were 145.76 and 75.03 mg/dl, respectively. Treating induced-cyclosporine rats by germinated and roselle and turmeric improved TNF levels compared to positive control group. The highest value of treated groups recorded for 2.5% hibiscus group, while the lowest value recorded for 1% turmeric group with significant (P≤0.05) differences. The mean values were 140.02 and 105.37 mg/dl, respectively. The similar results were obtained by **Simanjuntak *et al.*, (2019**) who found that Anti-Toxoplasma IgG - IgM antibody levels increased significantly 3 days after feed rats turmeric. Anti-Toxoplasma IgG- IgM antibody levels increased significantly (*P*< 0.05) 3 days, 6 days, and 10 days after injections of tachyzoites on G4.

Table (5): Effect of hibiscus and turmeric on immunoglobulin production of Cyclosporine injected rats .

Groups	IGA(mg/dL)	IGG(mg/dL)	IGM(mg/dL)	Tumor necrosis factors (TNF) (Pg/ml)
Negative control (G 1)	172.18±10.2^{a}	600.9±5.22^{a}	156.63±6.88^{a}	75.03±0.76^{e}
Positive control (G2)	56.93±3.22^{f}	405.44±2.66^{f}	43.47±3.89^{f}	145.76±10.44^{a}
Rats with 2.5 % hibiscus (G3)	70.55±4.82^{e}	435.43±9.34^{e}	50.05±5.11^{f}	140.02±8.22^{a}
Rats with 5	83.55±3.99^{d}	466.95±10.04^{d}	62.01±3.97^{e}	129.65±11.49^{b}

% hibiscus (G4)				
Rats with 0.5 % turmeric (G5)	90.68±6.45 c	486.67±11.14 c	72.27±7.08 d	131.32±7.75 b
Rats with 1 % turmeric (G6)	117.85±4.09 b	529.04±8.65 b	108.65±4.02 b	105.37±12.03 d
Rats with 3 %mixture (G7)	113.87±7.05 b	518.53±10.38 b	99.43±4.89 c	114.22±6.29 c
LSD	7.03	10.77	8.61	8.33

Values presentel as mean± SD
IGA: Immunoglobulin A. IGG: Immunoglobulin. IGM: Immunoglobulin M. TNF: Tumor Necrosis Factor.
Means in the same column with different litters are significantly different (P ≤0.05).

6. Effect of hibiscus and turmeric on antioxidant enzyems of cyclosporine injected rats

Table (6) The result showed that the lowest (catalase) CAT of treated groups (rats) were recorded for the group 3 fed on 2.5% hibiscus, while the highest value was recorded for the group 6 fed on 1% turmeric with a significant difference (P≤0.05). The mean values were 1.61 and 2.19 (ng/ml), respectively .With reference to SOD, results revealed that the mean value of SOD of positive control group was significantly lower than negative control group. on the other side the result detected that the mean values of groups 3, 4, 5,6 and 7 showed significant higher than positive control group.

The similar results were obtained by (**Ademiluyi *et al.*,**

2013) who found that The modulatory effect of the *H. sabdariffa* calyx red dye supplemented diets on kidney non-enzymatic antioxidant indices such as, GSH and ascorbic acid contents revealed a significant (P<0.05) depletion/reduction in these kidney antioxidant indices. However, supplementation of the diets with *H. sabdariffa* calyx dye protected against alteration in the kidney non-enzymatic antioxidant indices . In addition, the effect of the red dye diet supplementation on kidney enzymatic antioxidant indices such as superoxide dismutase (SOD), glutathione-*s* - transferase (GST) and catalase (CAT) activities was also studied. This study revealed significant reductions in the kidney activities of SOD, GST and catalase in cisplatin administered control group. However, supplementation of the diets with *H. sabdariffa* calyx red dye protect against depletion of these kidney antioxidant enzymes in rat groups fed diets supplemented with the red dye.

This ruslts agreed **Ghasemi et al.,(2022**), who reported that these infected mice treated with curcumin, showed Total and differential WBC counts level were significantly increased but total thiol content and the activities of catalase (CAT) and superoxide dismutase (SOD) were reduced in both the BALF and blood of the PQ group in comparison with the control group (p<0.05 to p<0.001). Both doses of C. longa and curcumin diminished total and differential WBC counts in the blood and BALF but increased CAT and SOD activities in both of them compared to PQ group (p<0.05 to p<0.001).

Table (6): Effect of hibiscus and turmeric on antioxidant enzyems of Cyclosporine injected rats .

Groups	SOD U/mL Mean± SD	CATng-ml Mean± SD
Negative control (G 1)	74.64 ± 2.56^{a}	3.65 ± 0.55^{a}
Positive control (G2)	31.02 ± 5.04^{f}	1.56 ± 0.54^{d}
Rats with 2.5 % hibiscus (G3)	36.77 ± 2.33^{e}	1.61 ± 0.89^{c}
Rats with 5 % hibiscus (G4)	44.44 ± 1.07^{d}	1.97 ± 0.44^{b}

Rats with 0.5 % turmeric (G5)	41.62±2.59^{d}	1.87±0.07^{c}
Rats with 1 % turmeric (G6)	59.97±5.32^{c}	2.19±1.05^{b}
Rats with 3 %mixture (G7)	51.26±3.01^{b}	1.96±0.02^{b}
LSD	4.03	0.26

Values presentel as mean± SD
SOD: Super oxide dismutase. CAT: Catalase.
Means in the same column with different litters are significantly different (P ≤0.05).

Effect of hibiscus and turmeric indicated increase on body weight increase, feed intake, feed efficiency ratio. Serum glucose, liver enzymes (AST, ALT& ALP) and kidney parameters (urea, creatinine, uric acid) . Antioxidant enzymes (CAT&SOT) were increased . The immunoglobulin production (IGA, IGG, IGM) were improved . The best treatment were group 6 fed on 1% curcum powder and group 7 fed on 3% mixture from hibiscus and turmeric powder.

Conclusion

It can be concluded from this study that immunity can be increased when using hibiscus by 5% and turmeric by 1%, as these percentages led to improving biochemical indicators and increasing the production of immune globulins.

References.

Abubakar, M. G., Lawal, A., Suleiman, B. and Abdullahi, K. (2010) : Hepatorenal toxicity studies of sub-chronic administration of calyx aqueous extracts of Hibiscus sabdariffa in albion rats. Bayero Journal of Pure and Applied Sciences., 3(1): 16- 19.

Ademiluyi AO, Oboh G, Agbebi OJ, Akinyemi AJ. (2013) : Anthocyanin - Rich Red Dye of Hibiscus Sabdariffa Calyx Modulates Cisplatin-induced Nephrotoxicity and Oxidative

Stress in Rats. Int J Biomed Sci. Dec;9 (4):243-8. PMID: 24711761; PMCID: PMC 3884795.

Ademiluyi AO, Oboh G, Agbebi OJ, Akinyemi AJ.(2013) : Anthocyanin - Rich Red Dye of Hibiscus Sabdariffa Calyx Modulates Cisplatin-induced Nephrotoxicity and Oxidative Stress in Rats. Int J Biomed Sci , Dec;9(4):243-8. PMID: 24711761; PMCID: PMC3884795.

Ali, M.M. E ., El-Mosselhy, S. M. A., and El-kholey, E. S.M. .(2015): The Effect of Aqueous and Alcoholic Extracts of Roselle, Tamarind and their Mixture on Liver Function and Lipid Profile in Rats Treated by Potassium Bromate. Journal of Home Economics, 25, (3).

American Institute of Nutrition (AIN) . (1939) : Purified diet for laboratory Rodent, Final report. J. Nutrition, 123:1951.

Belfied, A. and Goldberg,D . (1971) : Alkaline phosphatase colorimetric method. J. Enzyme.,4: 561-570.

Burlingame, R. W.and Rubin, R. L. (1990): Subnucleosome structures as substrates in enzyme-linked immunosorbent assays. Journal of Immunological Methods, 134(2):187-199 **.**

Cai Y, Huang C, Zhou M, Xu S, Xie Y, Gao S, Yang Y, Deng Z, Zhang L, Shu J, Yan T, Wan CC. (2022) **:** Role of curcumin in the treatment of acute kidney injury: research challenges and opportunities. Phytomedicine. Sep;104:154306. doi: 10.1016/j.phymed.2022.154306. Epub 2022 Jul 3. PMID: 35809376

Chapman, D.G.; Castilla, R. and Champbell, J.A. (1959): Evaluation of protein in food. I.A. Method for the determination of protein efficiency ratio-Can. *Journal of Biochemistry. Physiology*.**;** 37: 679-686.

Catanzaro, M., Corsini, E., Rosini, M., Racchi, M. and Lanni, C.(2018): Immunomodulators Inspired by Nature: A Review on Curcumin and Echinacea. Molecules. 2018 Oct 26:23(11):2778.

Ghasemi SZ, Memarzia A, Behrouz S, Gholamnezhad Z, Boskabady MH. .(2022) : Comparative effects of *Curcuma longa* and curcumin on paraquat-induced systemic and lung oxidative stress and inflammation in rats. Avicenna J Phytomed . Jul-Aug;12(4):414-424.

Hussein, M.A and Abd El-Maksoud, H. (2013): Biochemical effects of resveratrol and Curcumin combination on obese diabetic rats, Molecul.& Clinic. Pharmac., 4: 1-10.

Ivanova, J. I., Birnbaum, H. G., Kidolezi, Y., Subramanian, G., Khan, S. A., & Jacobs, S., Oxley,K. and Demott,W. (2001): Laboratory Test Handbook.Lexi-Comp, Inc. The Journal of Urology.,4:405-405.

Lee, G.Y. and Han, S.N. (2018): The Role of Vitamin E in Immunity. Nutrients 10, 1614.

Malhotra, V.K. (2003): Practical Biochemistry for Students. Fourth Edition, Jaypee Brothers Medical Publishers (P) LTD.New Delhi.

Maury, C. (1986): Serum TNF determination using ELISA kits.Acta.Med. Scan.,3:220 – 387.

Muller, A. (1964) : Vitamin mixture. J. Biol. Chem., 150:305.

Oriji, GK. .(2003) : Role of metoprolol, B1-adrenoceptor antagonist, thromboxane A2 and nitric oxide in CsA-induced hypertension. Prostaglandins Leukot Essent Fatty Acids.68(3):233-8.

Russo, P. (2001) : Localized renal cell carcinoma. Curr Treat Options Oncol. Oct;2(5):447-55.

Schermer,S. (1967) : The Blood Morphology of Laboratory Animal. langmans printed in great britain, green and co ltd.,3:320- 350.

Sello, A .A., and Eldemery, M . E. (2017): The potential preventive effects of turmeric (Curcuma longa) rhizomes against

hepatotoxicity in rats induced by benzo[a]pyrene **. (2017),** 27, (2) Journal of Home Economics.

Sendcor, G. and Cochran, W. (1979): "Statistical method 6th Ed". Iowa State College Pub, USA.; 841.

Serairi Beji R, Ben Mansour R, Bettaieb Rebey I, Aidi Wannes W, Jameleddine S, Hammami M, Megdiche W, Ksouri R. (2018) : Does Curcuma longa root powder have an effect against CCl4-induced hepatotoxicity in rats: a protective and curative approach. Food Sci Biotechnol. Aug 19;28(1):181-189. doi: 10.1007/s10068-018-0449-3. PMID: 30815309; PMCID: PMC6365324.

Shehata, N. and ElAreefy, A. A. (2016): Protective effects of curcumin during benzo(a)pyrene induced liver toxicity and carcinogenicity in rats .26, (4), Journal of Home Economics.

Simanjuntak TP, Hatta M, Tahir AM, Sirait RH, Karo MB, Tambaib T, Dwiyanti R, Noviyanthi RA, Junita AR. (2019) : Analysis of Anti-toxoplasma Immunoglobulin G and Immunoglobulin M Antibody Levels after Intervention with *Curcuma Longa* Extract on Early Pregnant Mice with Acute Toxoplasmosis. J Glob Infect Dis, Jan-Mar;11(1):25-29. doi: 10.4103/jgid.jgid_28_18. PMID: 30814832; PMCID: PMC6380095.

Tietz,N. (1976) : Fundamentals of Clinical Chemistry. Philadelphia. B.W. Standers, P.243.

Tung, B. T.; Nham, D. T.; Hai N. T. and Thu, D. K. (2019): *Curcuma longa L* the Polyphenolic Curcumin Compound and Pharmacological Effects on Liver. Dietary Interventions Liver Dis. 125–134. 10.1016/b978-0-12-814466-4.00010-0

Umar IA, Maryoms NG, Daikwo E, Gidado A, Buratai LB, Igbokwe IO, Ibrahim MA. **(2009**) **:** The effect of aqueous extracts of Hibiscus sabdariffa (Sorrel) calyces on heamatological profile and organ pathological changes in

Trypanasoma congolense - infected rats. Afr J Tradit Complement Altern Med. Jul 3;6(4):585-91.

Yatim, K.M. and Lakkis, F.G. A (2015) **:** brief journey through the immune system. Clin. J. Am. Soc. Nephrol. CJASN. **;** 10:1274–1281. doi: 10.2215/CJN.10031014.

Yound, D. S. (1975) : Determination of GOT. Clin. Chem., 22 (5): 21.

Zahid H, Rizwani GH, Shareef H, Khursheed R, Huma A, Hasan SM. .(**2014) :** Antihyperglycemic and hypolipidemic effects of Hibiscus schizopetalus (Mast) Hook in alloxan-induced diabetic rats. Pak J Pharm Sci Jan;27(1):83-9. PMID: 24374457.

Zhou L., Zhang K., Li J., Cui X., Wang A., Huang S., et al. (2013) : Inhibition of Vascular Endothelial Growth Factor-Mediated Angiogenesis Involved in Reproductive Toxicity Induced by Sesquiterpenoids of Curcuma zedoaria in Rats. Reprod. Toxicol., 37, 62–69. 10.1016/j.reprotox.2013.02.001