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Effect of Curcumin on Helicobacter pylori on Experimental Rats

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Effect of Curcumin on Helicobacter pylori in Experimental Rats

Abstract

Helicobacter pylori (H. pylori) is a spiral shaped bacterium that lives and multiplies in the lining of the stomach, and it is a common cause of many stomach diseases. This study aims to show the effect of curcumin on helicobacter pylori in experimental rats .Sixty adult male albino rats,were randomly into six groups (10 rats each). as follows: **Group (1):** composed of normal rats as negative control groups(-ve), **Group (2):** composed of rats those were subjected to oral administration of at a dose (200 mg/kg/day) CAE. **Group (3):** composed of rats those were subjected to oral administration of at a dose (400 mg/kg/day) CAE.**Group (4):** composed of rats those were subjected to injection of (1mL/rat) twice daily) ***H. pylori*** act as a positive control(+ve)**.Group (5):** comprise of rats infected by *H. pylori* those were treated oral with at a dose (200 mg/kg/day) CAE and **Group (6):** comprise of rats infected by *H. pylori* those were treated oral with at a dose (400 mg/kg/day) CAE, for six weeks . The results revealed that curcuma aqueous extract had 15 Phenolic constituents . Besides results indicated that the rats treated with CAE 200 and 400 mg/kg/day showed significantly decreased in serum cholesterol, triglycerides, glucose and LDL-C matched with a significant increase in serum HDL-C**,** In addition , at *($p \leq 0.05$)* there were decreased ,serum ALT, AST, ALP, and GGT, Also, serum urea, creatinine, and uric acid levels, when compared with group (4), So this study recommended using curcumin in diets for its many benefits.

Keywords:

Helicobacter pylori (H. pylori), curcumin ,Antioxidant, Lipids profile and Liver and Kidney Function

Introduction

Medicinal plants play a crucial role in maintaining human health and contain various phytochemical compounds recognized as therapeutic active agents. The important advantages of medicinal plants include alleviating symptoms of diseases, enhancing the immune system, and even preventing certain illnesses. Additionally, the use of various medicinal plants as an alternative or complementary approach to conventional treatments can contribute to reduce side effects and toxicities associated with chemical drugs **(Farzaneh *et al.*, 2024).**

Curcumin is the main active ingredient in *Curcuma longa L* (Turmeric), a yellow indian spice (native to Southeast Asia) obtained from the ginger family *(Zingiberaceae)*; it is also known as diferuloylmethane. With its dried and powdered form, it is used all over the world as a spice and coloring agent (in textiles, pharmaceuticals, confectionery, and cosmetics**) (Dai *et al* .,2014 and de los Ángeles ., 2018)**

Curcumin is a yellow-orange hydrophobic polyphenol obtained from the rhizome of the turmeric *(Curcuma longa L.)* plant. **(Tripathy *et al.*, 2021)**. It has been used for centuries for culinary and food coloring purposes, and as an ingredient for various medicinal preparations, widely used in Ayurveda and Chinese medicine. In recent decades, their biological activities have been extensively studied **(Sharifi-Rad *et al*. 2020)**. In addition , Curcumin, the yellow polyphenolic pigment and the major component found in turmeric, possesses a wide spectrum of pharmacological and biological properties including antioxidant, anti inflammatory, neuroprotective, anticarcinogenic, antibacterial, antidiabetic, chemoprotective, and immunomodulatory actions. It has beneficial effects on cardiovascular disease, gastrointestinal tract, and skin as well **(Mirhadi *et al.*, 2024)**

Helicobacter pylori (*H. pylori*) is a strict microaerophilic bacterial species that exists in the stomach **(Huang *et al., 2024).***It is a common bacterial infection that can cause various digestive issues such as gastritis, peptic ulcers, and in some cases, stomach cancer. *H. pylori* is typically transmitted through contaminated food, water, or close contact with an infected person (**Agwa *et al.*,2024).** Also ,*H. pylori* infection is chronic and often lifelong if left untreated. It is linked to chronic gastritis, duodenal and gastric ulcers, and tumors such as intestinal type of gastric cancer and mucosa-associated lymphoid tissue (MALT) lymphoma, which led to classifying the species in the group of the strongest (Group I) carcinogens (**Jia *et al.*, 2022)**

This study aims to show the effect of curcumin on Helicobacter pylori in experimental rats

Materials and Methods

Materials

Plant materials

One kilogram of curcuma (*Curcuma longa*) was obtained from a local supplier (Abd El-Rahman Harraz, Bab El-Khalk zone, Cairo,Egypt).

Helicobacter pylori (*H. pylori*) bacteria

Helicobacter pylorus (H. pylori) bacteria strain was obtained from the microbiology department, National Research Centre, Dokki, Egypt**.**

Chemicals

Kits were used to determine total cholesterol (T.C), triglycerides(T.G), high density lipoprotein cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), glucose, alanine aminotransferases (ALT), aspartate aminotransferases (AST), alkaline phosphatase (ALP), gamma glutamyl transaminase (GGT), urea, creatinine, uric acid, were obtained from Sigma Aldrich (St. Louis, MO, USA**)**

Experimental rats

Sixty adult male Wistar albino rats (*Rattus norvegicus*), weighting(140$\pm$10g) , were obtained from Animal House Colony, National Research Centre, Giza , Egypt.

Methods

Antioxidant of curcuma aqueous extract

Estimation of total extract yield determination according to **(Ashry *et al.*, 2021)**

Determination of total phenolic content (TPC) of curcuma extracts

The content of total phenolic compounds of the extracts was estimated spectrophotometrically by a modified Folin–Ciocalteu colorimetric method of **(Jayaprakasha *et al*.,2003)**

Determination of radical scavenging activity (RSA) of curcuma extracts

The capacity of antioxidants in the extracts to quench DPPH radicals was determined using the method of (**Nogala-Kalucka *et al*. ,2005)**

Determination phenolic content of curcuma extract

HPLC analysis was carried out using an Agilent 1260 series and determined using the method of ***(kujala et al., 2000)***

Experimental design

Sixty adult male Wistar albino rats (*Rattus norvegicus*), weighing (140 ±10g) were obtained from the Animal Colony, National Research Centre, Giza, Egypt; the rats were kept in suitable plastic cages and maintained on free access to food and water for a week before starting the experiment for acclimatization; they received human care in compliance with the standard institution's criteria for the care and use of experimental rats according to the ethical committee of the Faculty of Science, Al-Azhar University, Assuit, Egypt. After the rats were acclimatized to experimental room conditions, they were divided randomly into six groups (10 rats each) as follows: **Group (1):** Comprised of normal rats as negative control groups, **Group (2):** Comprised of rats those were subjected to oral administration of curcuma aqueous extract (CAE) at a dose (200 mg/kg/day), **Group (3):** Comprised of rats those were subjected to oral administration of curcuma aqueous extract (CAE) at a dose (400 mg/kg/day), **Group (4):** Comprised of rats those were subjected to injection of (1mL/rat) twice daliy) ***H. pylori*** act as positive control groups, **Group (5):** Comprise of rat infected by *H. pylori* those were treated with oral of a dose (200 mg/kg/day) CAE and **Group (6):** Comprise of rats infected by *H. pylori* those were treated with oral of a dose (400 mg/kg/day) CAE.

Blood sampling

At the end of the study period, rats were fasted overnight and following diethyl ether anesthesia, about 0.5 ml of blood sample was collected into a heparinized vacutainer tube immediately for the hematological investigations; while non-heparinized blood specimens (3-7 ml) from each rats were drawn from the retro-orbital plexus using sterile glass capillaries (single draw vacutainer needle) into open vacutainer collecting tubes .The non-heparinized blood specimens were left 20 minutes to clot, then centrifuged at 3000 rpm for 10 minutes using a cooling centrifuge (IEC centra-4R, International Equipment

Co., USA). The sera were separated, divided into aliquots and stored at -80°C until biochemical measurements could be carried out as soon as possible.

Body weight Gain

At the beginning and the end of the experimental study, each rat was weighed; and the change in body weight (body gain) was calculated according to **(Ashry *et al.*, 2021)**

Biochemical determination

Lipids profile

Serum total cholesterol (T.C), triglycerides (T.G), high dense lipoprotein-cholesterol (HDL-c) and low dense lipoprotein-cholesterol (LDL-c) were determined according to **Cole *et al.*, (1997); Artiss and Zak,(1997), Lopes-Virella *et al.*, (1977) and Wieland and Seidel, (1983)**;respectively .

Serum glucose

Serum glucose level was determined according to the CHOP-PAP method by the photometric system described by **Young (2001)**

Liver functions

Serum alanine aminotransferases (ALAT) , aspartate aminotransferases (ASAT), alkaline phosphatase (ALP) and gamma glutamyl transaminase (GGT) were determined according to **Schumann & Klauke, (2003); Moss & Henderson, (1999), IFCC, (1983); Trinder, (1969) & Tietz *et al.*, (1983)** , respectively.

Serum proteins and albumin were determined according to the method described by **(Henry, 1964)**. Serum globulin was determined according to the method described by **(Dumas, 1971)** .

Kidney functions

Serum, urea, creatinine and uric acid were determined according to the method described by **Husdan and Rupoport, (1969); andTrinder (1969) ;and Chaney et al.,(1960)** , respectively.

Statistical Analysis

The obtained data were statistically analyzed by the SPSS computer . Software expressed as mean $\pm$ SD. Effects of different treatments were analyzed one-way (ANOVA) followed by Duncan's multiple range tests. Differences were considered significant at($P \leq 0.05$) according to **Snedecor and Cochran(1986)**

Results and discussion

Antioxidant of curcuma aqueous extract

Curcuma aqueous extract yield (%), total phenolic compounds (TPC) and radical scavenging activity (RSA)

The data in **Table(1)** revealed that the yield was 5.1 %, the total phenolic content (TPC) was 8.9% , and radical scavenging activity (RSA) was 51.2% of the curcuma aqueous extract . These results agree with ***(*Sera *et al., 2019)*** they reported that water extract was the most efficient solvent to extract antioxidant contents such as total phenolic compounds (3.65 ± 0.02 mg GAE/g) and flavonoids (4.99 ± 0.17 mg QCE/g) content. Radical-scavenging activity was also higher in water extract compared with others such as DPPH (51.10 ± 2.29%) and 15.58% yield of turmeric .This is due to the turmeric leaf extract showing potential as a functional food source from its antioxidant components such as total phenolic compounds and flavonoids that enhance radical-scavenging activity.

Table (1): curcuma aqueous extract yield (%), (TPC) and (RSA)

Parameters / Extract	Yield (%)	TPC (mg/g)	RSA (%)
Curcuma aqueous extract (CAE)	**5.1**	**8.9**	**51.2**

Phenolic constituents of the aqueous extract of curcuma

As shown in **Table (2)** 15 phenolic constituents of the aqueous extract of curcuma were analyzed by High Performance Liquid Chromatography (HPLC) analysis. The compounds identified were found to include high contents of chlorogenic acid ,gallic acid, syringic acid and ferulic acid while, the lowest values were in methyl gallate, cinnamic acid, vanillin and daidzein . These results agree with ***(Wang, 2003)*** Who reported that the phenolic compounds profile of different turmeric extracts showed that the prepared turmeric had the highest content of different phenolic compounds such as gallic acid, catechin, syringic acid, ellagic acid, coumaric acid, vanillin, ferulic acid, naringenin, and cinnamic acid, in comparison of other turmeric extracts. Also, calebin-A, vanillic acid, vanillin, quercetin, and other phenolic compounds have also previously been identified from turmeric **(Tanvir**

et al.*,2017*; Mean *et al.*, 2001;*and* Gupta et *al.*, 2013*)**. This is due to a linear correlation between the content of total phenolic compounds and their antioxidant capacity. ***(*Katsube *et al.*, 2004; and Katalinic *et al.*, 2006*).

Table (2) Phenolic constituents of the aqueous extract of curcuma (CAE)

Parameters	Conc. (µg/g)
Gallic acid	392.39
Chlorogenic acid	492.34
Catechin	100.01
Methyl gallate	3.15
Coffeic acid	*N.D
Syringic acid	151.87
Rutin	36.40
Ellagic acid	*N.D
Coumaric acid	41.24
Vanillin	11.95
Ferulic acid	150.95
Naringenin	*N.D
Rosmarinic acid	113.98
Daidzein	25.55
Querectin	35.17
Cinnamic acid	7.61
Kaempferol	118.17
Hesperetin	109.97

***N.D. Not detected**

Body weight gain

Effect of *H. pylori* and curcuma aqueous extract on body weight gain in experimental rats(%) .

The effect of *H. pylori* infected and CAE on the rats' body weight is shown in **table (3).** These results showed that rats treated with *H. pylori* had a significant decrease in body weight; while rats treated with CAE alone showed a significant increase in body weight when compared

with the negative control group.In addition, *H. pylori* infected, treated with CAE low and high doses showed a significant increase in body weight, reflecting the protective potential of these extract. These results agreement with **(Xu *et al.*, 2021)** they reported that the body weight growth curve demonstrated that the body mass of rats in the control group showed a natural growth trend and was higher than that in the other groups. The model group exhibited a significant body weight loss on weeks 8, 10, and 12 compared to the control group ($p < 0.001$), while the body weight of the treatment group rats was gradually increased .**Hemdan and Abdulmaguid(2018)** they observed a significant change in feed intake, body weight gain % and feed efficiency ratio in all treated groups compared with (-ve) control. Also,the best results were recorded by the groups treated with rosemary 2%, followed by Curcuma 2%. In this regards; treated with curcuma and rosemary adapted the appetite for rats, improved biological evaluation it could be due to improve palatability of the trial diet and effect on appetizing promoter according to **Gharejanloo *et al.*, (2017)**

Table (3) effect of *H. pylori* and curcuma aqueous extract on body weight gain in experimental rats(%)

Parameters / Groups	Body weight gain (%)
Group(1) Control(-)	**22. 5±0.55**
Group(2) CAE (200)	**23. 3±0.47**
Group(3) CAE (400)	**23.7±0.65**
Group(4) *H.Pylori* Control(+)	**14.5±0.11***
Group(5) *H.Pylori*+ CAE (200)	**18.5±0.47$^{\#}$**
Group(6) *H.Pylori*+ CAE (400)	**20.4±0.91$^{\#}$**

The same column, means with different superscript letters are significantly different **at** ***(p≤0.05)***

Effect of curcuma aqueous extract on serum (T.C), (T.G), (HDL-c), (LDL-c) and glucose in experimental rats.

The obtained results (**Table 4)** revealed that, the *H.Pylori* infected rats showed a significant increase *(p≤0.05)* in cholesterol, triglycerides, glucose and LDL-C matched with a significant decrease in HDL-C when compared the control group; however the treatment of CAE for six weeks induced non-significant changes in serum cholesterol,

triglycerides, HDL-C and LDL-C levels. Moreover, the *H.Pylori* infected rats treated with CAE showed significant ($p \leq 0.05$) decreases in serum cholesterol, triglycerides, glucose and LDL-C matched with a significant increase in serum HDL-C when compared to the *H.Pylori* infected group. These results agreement with **(Ghobashi *et al.*, 2022)** They showed that the infected rats' serum levels of cholesterol , triglycerides , LDL and VLDL were substantially higher than non-infected rats', but HDL levels were significantly lower. Compared with the control group (GI). This is due to upregulation of the total and low-density lipoprotein-cholesterol (LDL-C) and decreasing of high-density lipoprotein cholesterol (HDL-C), which may be associated with infection, creating an atherogenic lipid profile and promoting atherosclerosis **(Buzás, 2014)**

Table (4): Effect of curcuma aqueous extract on serum (T.C), (T.G), (HDL-c), (LDL-c) and glucose in experimental rats.

Parameters / Groups	T.C (mg/dl)	T.G (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	Glucose (mg/dl)
Group(1) Control(-)	129±8.1	141±1.4	44.6±0.6	55.1±4.8	92.3±2.4
Group(2) CAE (200)	131.5±4.5	132.5±9.1	44±0.33	56.05±3.8	92.4±4.5
Group(3) CAE (400)	124.2±4.4	131.2±5.7	44.2±0.6	53.7±4.6	88.1±5.1
Group(4) *H.Pylori* Control(+)	$166.7\pm5.3^{*}$	$221.5\pm7.5^{*}$	$40.5\pm0.5^{*}$	$84.1\pm5.4^{*}$	$106.6\pm1.6^{*}$
Group(5) *H.Pylori*+ CAE (200)	$131.2\pm6.6^{\#}$	$129.7\pm1.7^{\#}$	$42.7\pm0.6^{\#}$	$62.5\pm7.6^{\#}$	$70.1\pm2.08^{\#}$
Group(6) *H.Pylori*+ CAE (400)	$114.4\pm9.4^{\#}$	$121.8\pm5.6^{\#}$	$44.2\pm0.69^{\#}$	$51.02\pm9.2^{\#}$	$60.8\pm2.6^{\#}$

The same column, means with different superscript letters are significantly different at *($p \leq 0.05$)*

Effect of curcuma aqueous extract on serum ALAT, ASAT, ALP and GGT (U/L) in experimental rats

The obtained results in **table(5)** revealed that, the **Group(4)** infected rats showed a significantly increased *($p \leq 0.05$)* ,in ALAT, ASAT, ALP and GGT activities when compared with control negative group**(1)**; however the treatment of CAE for six weeks induced non-significant changes in serum ALAT, ASAT, ALP and GGT activities respectively. Moreover, the H.Pylpri infected rats treated with CAE showed

significant *(p≤0.05)* decreased serum ALAT, ASAT, ALP and GGT activities when compared to infected group**(4)**. These results are in agreement with ***(*White and Lee , *2019* & Dehzad *et al.*, 2023*)*** they reported that the turmeric/ curcumin supplementation significantly reduced blood levels of ALT and AST. This is due to curcumin being a potent antioxidant; it could be presumed that its effect on improving liver function tests is derived from its free radical-scavenging properties. Various molecular mechanisms have been postulated to link the antioxidant properties of curcumin with improvement of liver function tests **(Bardallo *et al.*, 2022).**These results agreement with **(Salehi *et al.*, 2014)** they stated that shown patients' serum levels of liver enzymes alanine transaminase (ALT) and aspartate transaminase (AST) decreased after receiving an eradication regimen of H. pylori. This is due to the an association between H. pylori infection and liver dysfunction and hepatitis

Table (5): Effect of curcuma aqueous extract on serum ALT, AST, GGT and ALP (U/L) in experimental rats

Parameters / Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)
Group(1) Control(-)	56.3±6.9	85.3±7.23	185.7±5.4	3.2±0.40
Group(2) CAE (200)	47.02±6.0	83.8±5.7	181.7±8.8	3.2±0.33
Group(3) CAE (400)	48.2±2.9	85.4±9.9	177.5±10.1	3.1±0.49
Group(4) *H.Pylori* Control(+)	76.4±6.8*	147.8±16.1*	241.7±8.3*	8±0.51*
Group(5) *H.Pylori*+ CAE (200)	33.9±4.7$^{\#}$	77.6±4.8$^{\#}$	125.8±7.03$^{\#}$	3±0.74$^{\#}$
Group(6) *H.Pylori*+ CAE (400)	31.1±0.91$^{\#}$	62.08±7.01$^{\#}$	118.2±4.4$^{\#}$	3±0.69$^{\#}$

The same column, means with different superscript letters are significantly different at *(p≤0.05)*

Effect of curcuma aqueous extract on serum urea, creatinine and uric acid levels in experimental rats

Data in **Table(6)** revealed that, group**(4)** infected rats showed a significant at *(p≤0.05)* increase in creatinine, blood urea and uric acid when compared with group1 control**(-)**; however the treatment of CAE for six weeks induced non-significant changes in serum creatinine, urea, and uric acid respectively. Moreover, the *H.Pylori* infected rats treated with CAE showed significant decreased *(p≤0.05)* serum creatinine, blood urea and uric acid levels when compared to the infected group**(4)**. These results agree with **(Saidi *et al.*, 2019)** they indicated that curcuma administration causes a significant reduction in uremia and serum creatinine. Additionally, it provides nephron protective effect against free radicals through motivation of antioxidant enzymes. This results agree with (**Xu *et al.*, 2021)** they reported that the serum level of uric acid in the rat model group was high compared to that of control group,the treatment group of curcumin (200 mg/kg)showed decreased serum uric acid, also reduced the levels of serum creatinine. This is due to curcumin is the main active constituent of turmeric, and it is characterized by containing phenolic groups which confer antioxidant and anti-inflammatory effects **(Alvarenga *et al.*, 2020)**

Table (6): Effect of curcuma aqueous extract on serum urea, creatinine and uric acid levels on Kidney functions in experimental rats

Parameters / Groups	Urea (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
Group(1) Control(-)	33.9±1.3	0.70±0.16	2.9±0.15
Group(2) CAE (200)	32.9±3.0	0.76±0.05	2.6±0.26
Group(3) CAE (400)	30.2±2.5	0.66±0.03	2.7±0.17
Group(4)H.Pylori Control(+)	61.2±1.3*	1.2±0.09*	4.1±0.20*
Group(5)H.Pylori+CAE (200)	19.5±1.5$^{\#}$	0.63±0.016$^{\#}$	2.9±0.38$^{\#}$
Group(6)H.Pylori+CAE (400)	21.7±1.4$^{\#}$	0.63±0.015$^{\#}$	2.7±0.38$^{\#}$

The same column, means with different superscript letters are significantly different at *($p \leq 0.05$)*

Conclusion

In conclusion, we found that Curcumin induced a marked amelioration in serum cholesterol, triglycerides, HDL-C, LDL-C and glucose levels, induced a marked decreased serum ALT, AST, ALP and GGT activities and induced a marked decreased serum urea ,creatinine and uric acid levels. So this study recommended to curcumin in diets for it is many benefits.

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تأثير الكركمين علي جرثومة المعدة في فئران التجارب

المستخلص

جرثومة المعدة هي بكتريا حلزونية الشكل تعيش وتتكاثر في بطانة المعدة، وهى سبب شائع للعديد من امراض المعدة بما فى ذلك قرحة المعدة ،أجريت الد ارسة لتقييم تأثير الكركمين على جرثومة المعدة في فئران التجارب .ستون من ذكور الفئران الألبينوالبالغة ، والتي تم تقسيمها إلى ستون من ذكور فئران الالبينو البالغة (٦) مجموعات كل مجموعة (١٠) فئران. المجموعة (١) مجموعة الكنترول وتتغذي على الوجبة الاساسية كمجموعة ضابطة سالبة . المجموعة (٢) مجموعة تتغذي على مستخلص الكركم بجرعة (٢٠٠ملجم / كجم) بالفم.المجموعة (٣) مجموعة تتغذي على مستخلص الكركم بجرعة (٤٠٠ملجم/ كجم) بالفم .المجموعة (٤) مجموعة الفئران التى حقنت ب (*H.pylori*) بجرعة١مل لكل فأر كمجموعة ضابطة موجبة.المجموعة (٥) مجموعة مصابة ب (*H.pylori*) ومعالجة بمستخلص الكركم بجرعة(٢٠٠ملجم / كجم) .المجموعة (٦) مجموعة مصابة ب (*H.pylori*) ومعالجة بمستخلص الكركم بجرعة (٤٠٠ملجم / كجم) واستمرت التجربة لمدة ستة اسابيع لجميع المجموعات،فى نهاية التجربة تم ذبح الفئران وجمع السيرم للتحليل الكيميائي . أظهرت النتائج ان مستخلص الكركم يحتوي علي ١٥مركب فينولى. أظهرت النتائج أن الفئران المصابة بجؤثومة المعدة المعالجة بالكركمين٢٠٠ و ٤٠٠ ملجم/كجم *($p<0.05$)* انخفاض في سيرم الكوليسترول والدهون الثلاثية والجلوكوز والكولسترول الضار في مصل الدم وزيادة في مستويات الكولسترول الجيد في مصل الدم ، وبالإضافة إلى ان هناك انخفاض نشاط انزيميALP، ALT،AST، GGT،فى مصل الدم عند*($p<0.05$)* وانخفاض فى مستويات اليوريا والكرياتينين وحمض البوليك ، عند مقارنتها بالمجموعة رقم (٤) المصابة بجرثومة المعدة كمجموعة ضابطة موجبة، لذا أوصت هذه الدراسة باستخدام الكركمين في الوجبات الغذائية لفوائدها العديدة.

الكلمات المفتاحية :

جرثومة المعدة ، الكركمين ، مضادات الاكسدة ،دهون الدم ، وظائف الكبد والكلي .