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Research Article

Isolation and extraction of gallic acid from *Hylocereus undatus* and a biochemical and histological study on laboratory Wistar albino rats with induced rheumatoid arthritis

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Abstract
The herb *Hylocereus undatus*, which is rich in phytochemicals and thought to contain antioxidants comparable to those in its peel, has a lot of promise for use in the food field. The presented study aimed to study the protective effect of the aqueous extrac and the active compounds (gallic acid) isolated from the dragon fruit (*H. undates*) to lessen the impact of rheumatoid arthritis (RA) induced by Freund's Complete Adjuvant (CFA)) by studying some of the changes in biochemical and histochemical parameters. The Wistar albino rats (male) were divided into four groups viz., Group I : A negative control group, dosed only with plain water orally, Group II : Induced for RA by CFA, a positive control group; Group III: dosed with aqueous extract of the fruit of *H. undatus* (kg/500 mg); and Group IV: dosed with gallic acid isolated from this fruit (kg/mg20). The results showed a significant increase in malondialdehyde (MDA), platelet, interleukin 6 (IL-6), and RF in rats treated with CFA and a significant decrease in Hb compared to the negative control group. There was a significant decrease of MDA, Platelet, IL-6, and RF in the groups treated with aqueous extract and a significant increase in Hb compared with the positive control group while there was a significant decrease in each of MDA, platelets, IL-6, and RF. Based on a histological analysis, the study group's liver and kidneys had smaller lesions and different abnormalities than the control . Thus, the fruit plant's gallic acid extracts were protective in reducing RA damage caused by CFA and improving kidney and liver tissue.

Keywords: Freund's Complete Adjuvant (CFA), Dragon fruit, Gallic acid, Histological, *Hylocereus undatus*, Malondialdehyde, Rheumatoid Arthritis

INTRODUCTION

Rheumatoid Arthritis is one of the chronic autoimmune diseases that severely affects the joints, causing them to become stiff and swollen, and causing the inflammation of the joint to become disabling. It is also one of the main causes of physical disability (Jakobsson *et al*., 2022). The incidence of Rheumatoid Arthritis in adults worldwide is estimated at 1%, and middle age is the most common period for the onset of the disease, although it is not limited to a specific age. In addition, it is estimated that its incidence is higher in females than in males (McInnes and Schett, 2017). Arthritis was discovered by Alfred Baring Garrod in the 1850s (Scott *et al*., 2020). Sixty percent of patients with rheumatoid arthritis suffer from symptoms of joint and muscle pain, swelling of the metatarsophalangeal joints, high body temperatures, loss of appetite, as well as general weakness. Arthritis can develop if it is not treated, and many symptoms begin to appear, such as swelling in the hands and feet due to joint stiffness and difficulty moving in the morning, which may lead to disability (Humphreys *et al*., 2014). The development of severe inflammation causes enlarged blood vessels in the synovial membrane and expansion of the soft tissues inside the joints, which destroys the joint tissues (Aletaha and Smolen, 2018).
Dragon fruit (*Hylocereus undatus*) because the plants

have a distinctive role in medical treatments, in addition to the use of this fruit in folk medicine in many Asian countries as herbal medicines to prevent and treat diseases. (Sofowora *et al*., 2013). The scientific name of dragon fruit is derived from the Greek word (Hyle) (Woody) and the Latin words (Cereus) (Waxen) and (Undatus), which refer to its stems with wavy edges (Eggli and Newton, 2004); addition of this fruit in the daily diet may contribute a lot in fighting arthritis as well as the anti-inflammatory properties of this fruit that work to prevent many diseases such as arthritis (Verma *et al*., 2017). So, this study aimed to extract gallic acid (GA) from *H. undatus* and investigate a biochemical and histological effect on laboratory male rats infected with induced rheumatoid arthritis.

MATERIALS AND METHODS

The dragon fruits, *H. undatus,* were taken from a local market in Nineveh Governorate 36.34076° N, 43.12685° S, collected, cleaned well, kept in bags, and placed in the refrigerator until use. The chemicals that were used in the study were the standard ready-made analysis kits, which are: Rheumatic factor from Abbott company, Interleukin-6 from BIOSORURCE company, glutathione peroxide from Elabscience company, malondialdehyde was estimated through its reaction with thiobarbituric acid in a solution (Tri Chloro Acetic Acid(T.CA.) with (150 microliters) of albino male rats serum.

Animals used

Twenty-four adult albino Wistar male rats weighing 200-250 grams were taken from the animal house in the College of Veterinary Medicine / University of Mosul-pleced in cages prepared for this regard and provided with water and animal food for this purpose. They were divided into four groups, left for one week to accommodate the laboratory conditions, such as light and temperature, and then performed injection and dosing operations.

Ethical approval

The ethical guidelines outlined in the Declaration of Helsinki were followed to conduct this investigation. The local ethics committee of the College of Education at Mosul University in Iraq examined and approved the study protocol,the official form numbered UM.VET.2022.029 .

Method of extraction of dragon fruit

The fruit was cleaned and washed well with water to get rid of dust and impurities. Then it was dried from the water. An electric blender was used to mash and mix it to obtain juice. Gauze papers were used in several layers to filter this juice, and the juice was diluted using 20% distilled water. The mixture was wrapped using aluminum leaves, which were left to stir to stir constantly for 12 hours. Sterile containers were used to put the mixture in it after filtering by using the electric oven to dry at a temperature of (40˚C). The extract (crude) was collected by scraping it and placed in clean, sterile and opaque glass containers in the refrigerator at a temperature (2-4 ˚c) until it was used in the experiment. It was prepared according to the method (Dey *et al*., 2020) and with modifications (Mitscher *et al*., 2013).

Isolation of gallic acid

The quantitative measurement of the identified gallic acid was carried out by Reverse-phase HPLC analysis by using a SYKAMN HPLC chromatographic system equipped with a UV detector (Chemstation) (C18-OSD) with column dimensions (25cm and 4.6mm). The room temperature of the column was 30 °C. By the stepwise separation method, it was separated by taking A (methanol) and B (1% formic acid) in water (vol/vol) (70:30), and the flow rate was 0.9 ml/min. The volume of injected samples was 100 μl, and the standards were 100 μl. This was done automatically using the automatic sampling appliance at the wavelength of 280 nm (Radovanović *et al*., 2015).

Substances and dosages

One gram of fruit extract and gallic acid was dissolved in10 ml of distilled water, and the rats) Laboratory animals(were given these substances at a concentration of 500 mg/kg and 20 mg/kg for 28 days (Akomolafe *e tal*., 2014; Swarup *et al*., 2010). The rats were injected with 0.1 ml of Freund's Complete Adjuvant(CFA) in the toe of the right foot to induce rheumatoid arthritis (Adeneye *et al*., 2014).

Experiment design

Twenty-four Wistar albino male rats were taken and divided into four groups, each with six rats:

Group I: A negative control group dosed with plain water throughout the experiment.

Group II: A positive control group. On the first day of the experiment to induce arthritis, was counted as it was injected with 0.1 ml of (CFA) in the right toe .

Group II: Rats were dosed orally with an aqueous extract of the dragon fruit of plant *H. undatus* at the dose of 250 mg/kg on the first day of the experiment, and after 7 days, they were injected with 0.1 ml of (CFA) in the right sole to cause arthritis.

Group IV: Rats were dosed orally with gallic acid extracted from the same fruit plant at the dose of 20 mg/kg on the first day of the experiment, and after 7 days, they were injected with 0.1 ml of (CFA) in the right sole to cause the disease arthritis.

Blood samples

At a specified period (0, 14, 28 days), blood was drawn from the rats' sinus, definitely in the eye socket, through the method of blood drip by using special capillary tubes (Stillinger *et al*., 1983). This was collected in tubes and then left in the water bath for 10 minutes at a temperature (37 °C), after which the serum was separated by centrifugation for 15 minutes at a speed of (5000 cycles/sec) and kept at a temperature -20°C in special tubes for testing (Abd Al-azem *et al*., 2019).

Estimation of hemoglobin and platelet

Blood samples were collected using tubes containing anticoagulants and were sent to the lab, where an apparatus made by Beckman Coulter (Ac•T 5diff CP, USA) was used to automatically estimate the hemoglobin and platelets for the study groups (Hazzaa *et al*., 2022).

Evaluation of malondialdehyde and interleukin-6 levels

Thiobarbituric acid reactions have been used to assess malondialdehyde (MDA) concentrations using the Halliwell and Chirico approach (1993). For interleukin-6 , whole blood was taken from each rat. The samples were centrifuged at 4°C to extract the serum. The supernatant was then packed and kept at -20 °C. Using the enzyme-linked immunosorbent assay (ELISA), the serum IL-6 concentrations of the rats in each of the five groups were ascertained. The precise procedures followed the instructions provided in the ELISA kit (MyBiosource, USA).

Histopathological study

Histology was studied liver, kidney, and joint specimens were fixed in 10% formalin saline, dehydrated in 70% alcohol, and washed in tap water overnight. It was cleaned in xylene for 20 minutes before being covered in paraffin wax. Transverse serial slices measuring 5 m thick were cut and deposited on an albuminized slide. The slices were stained with hematoxylin and eosin (Hx & E), and then inspected microscopically for histopathological research (Singla *et al*., 2017).

Statistical analysis

The results were analyzed statistically, as the biochemical variables' values were described using the Mean and the Standard Deviation. The Duncan test was used in (ANOVA) to analyze the effect of the studied biochemical variables (Kirkwood, 1988).

RESULTS AND DISCUSSION

The weights of rat members among study groups are summarized in Table 1.

The results of Table 1 showed that the induction of rheumatoid arthritis with CFA in male the laboratory animals resulted in a significant decrease in the probability level (P<0.05) in their weights, reaching (200.83± 2.67g) compared with the negative control group (294.33±6.80 g). in which rheumatoid arthritis did not develop, as this decrease in the weight of the positive control group was agree with the fact that rheumatoid arthritis is associated with a rheumatoid cachexia loss of body mass , which shows the loss of muscle mass and strength due to the development of Rheumatoid arthritis. (Nasuti *et al*., 2019). It is also believed that the development of rheumatoid arthritis leads to a weakness in the absorption of nutrients by the intestine (Cui *et al*., 2019) and that infections cause an abnormal response of the neuroendocrine glands, as this increases hormones such as glucocorticoids and an increase in inflammatory cytokines, and this finally causes hyperemia in metabolism and a significant decrease in weight concerning rheumatoid arthritis rats (Granado *et al*., 2007).

Also, the biological and protective effect of the aqueous extract of dragon fruit (*H. undatus*) (kg/mg 500) showed a significant increase in its weight (3.36650 ± 332 g), P<0.05 when compared with the positive control group (2.67603 ± 200.833g), and this may be because these doses of plant extract may effect on sensory perception, and then effect on appetite (Pansai *e t*

Table 1. Showing relative weights of the rats for the four groups

Weights of rats (Units)	Mean±SD			
	Gallic acid (20mg/kg)	**Extract collection (500mg/kg)**	**Positive control (0.1ml FCA)**	**Negative control (DW)**
Weight at the beginning of the experiment (g)	264.16 ±1.5793A	269.5 ±3.43269A	269.6667±4.68093A	272±6.0991A
Weight at 14 days of the experiment (g)	278±2.8519B	313.33±2.333C	224.3333±4.92386A	289.33±6.5557A
eight at 28 days of the experiment (g)	284.16±3.5158B	332±3.3665D	200.83±2.676A	294.33±6.80B

Similar letters (a, b, c, and d) refer to the significant differences at P<0.05; Different letters (a, b, c, and d) refer to the non-significant differences at P<0.05); FCA: Freund's Complete Adjuvant *al*., 2020), and dragon fruits *H. undatus* extract is a good source of polyphenols that have antioxidant properties (Hernández and Salazar, 2012); And that the pulp and peel of this fruit have many chemical compounds that possess antimicrobial activity that can be used as a natural antioxidant (Patel and Ishnava, 2019), as well as containing many vitamins and minerals which have a significant effect on the body health (Hossain *et al*., 2021).

The protective effect of Gallic acid showed a significant increase in its weight at the probability level (P<0.05) (3.51584 ± 284.1667 g) when compared with the positive control (2.67603 ± 200.833 g) of the body (Reckziegel *et al*., 2016) By influencing the two hormones responsible for feeling full and improving insulin resistance, leptin and adiponectin (Hsu and Yen, 2007). Furthermore, gallic acid has beneficial effects on whole -body metabolism, and Gallic acid significantly stimulates genes responsible for mitochondrial function, including antioxidant genes (Doan *et al*., 2015).

Change in the number of platelets in the blood

The results of Table 2 indicated that the induction of rheumatoid arthritis by CFA in rats caused a significant increase in platelet count, i.e. (1.78 ± 445.06 × 10^3/ ml) at a probability level (P < 0.05) compared to the negative control group that was (1.64 ± 346.6× 10^3/ ml). In which rheumatoid arthritis did not develop, the reason for the high number of platelets is attributed to the stimulation of the immune system against the invasion of pathogenic microorganisms, Whereas, the infiltration of single cells into the joints of mice resulting from arthritis leads to the secretion of tumor necrosis factor (TNF) (Hussain and Khalaf, 2020). Also, different cytokines such as: (interleukin-1 beta) (interleukin-4) (Cytokines IL-6, IL-4) are responsible for pathological thrombocytosis in rheumatoid arthritis because they are positively associated with elevated disease activity (Ertenli *et al*., 2003). The protective effect of the aqueous extract of the dragon fruit plant was 500 kg/mg. It worked on a significant decrease (404±1.31× 10^3/ ml) at the level of probability (P < 0.05) in the number of platelets when compared with the positive control group (445.06±1.78× 10^3/ ml). The attribution of this is because the dragon fruit extract contains phenolic compounds found in many tropical plants, anthocyanins and flavonoids that have an antioxidant effect that can affect the Immune system (Kapcum *etal*., 2021). Flavonoids also have antiviral activity against many viruses by eliminating them and influencing their DNA (Clain et *al*., 2019). The results indicated that the Positive effect for Gallic acid led to a significant decrease (423.6±1.115× 10^3/ ml) in the number of platelets at the level of probability (P < 0.05) when compared with the positive control group (445.06±1.78 × 10^3/ ml), because Gallic acid is one of the most important phenolic compounds that are biologically active and found in plants (Karamac *et al*., 2006; Rasool *et al*., 2010). Gallic acid is an effective antioxidant (Badhani *et al.,* 2015), It is non-toxic to mammals and is generally absorbed in the intestine (Nair and Nair, 2013), as Gallic acid improves the effects of antibodies, which indicates that it facilitates the immune response (Cahyati *et al*., 2021).

Change in the haemoglobin level in the blood

The results of Table 2 showed that the induction of rheumatoid arthritis with CFA in male laboratory rats caused a significant decrease in haemoglobin (9.26± 0.055 g/dL) at a probability level (P < 0.05) compared with the negative control group (13.36±0.076 g/dL) rheumatoid arthritis did not develop, the reason for this is that the decrease in the haemoglobin level in the presence of arthritis is due to a decrease in erythropoietin levels and response, a in the bone marrow , and early destruction of red blood cells (Patil *et al*., 2011), as well as the toxic effects of CFA that may cause disorders or changes in blood components. This, including haemoglobin, is associated with developing arthritis and low iron absorption (Ganna, 2014).

The protective effect of the aqueous extract of dragon

Table 2. Showing biochemical variables in the serum of rats in the five groups

Biochemical Variables (Units)	Mean±SD			
	Gallic acid (20mg/kg)	**Extract collection (500mg/kg)**	**Positive control (0.1 ml FCA)**	**Negative control (D.W.)**
Platelet count × 10^3 / ml	423.6±1.115C	404±1.31b	445.06±1.78D	346.6±1.64A
Hb g/dL	11.6±0.073C	10.3±0.091b	9.26±0.055D	13.36±0.076A
RF IU/ml	22.04±0.0915C	19.82±0.352b	40.26±0.269D	12.95±0.167A
IL-6 pg/ml	301.7667±1.84475C	287.8667±1.04329b	369.5±1.2463B	48.15±0.6483
MDA µmol/l	3.52±0.0051c	2.9±0.0085b	3.97±0.069D	1.82±0.0076A

Similar letters (a, b, c, and d) refer to the significant differences at (P<0.05); Different letters (a, b, c, and d) refer to the non-significant differences at (P<0.05); FCA: Freund's Complete Adjuvant, RF: Rheumatoid factor; MDA: Malondialdehyde fruit plant (*H. undatus*) (kg/mg 500), led to a significant increase (10.3 ±0.091 g/dL) at the level of probability (P < 0.05) in the haemoglobin level when compared with the positive control group (9.26± 0.055 g/dL) and the reason is attributed to the flavonoids found in the plant are of pharmacological importance against the new inflammation and on the other hand, stimulating the production of red blood cells. One study also confirmed that the plant contains vitamin C, which can change the oxidation state by inhibiting the gene expression of CD40 ligand (CD40L), produced in cases that cause inflammation and clots (Mohammed *et al*., 2017). The Biological effect of Gallic acid led to a significant increase (11.6± 0.073 g/dL) at the level of probability (P < 0.05) in the haemoglobin level when compared with the positive control group (9.26±0.055 g/dL) and this is due to the ability of gallic acid in inhibiting of lipid peroxidation and eliminating the free radicals and its role in lessening oxidative stress (Punithavathi *et al*., 2011). Gallic acid affects haemoglobin by inhibiting the release of free iron from it, binding to the heme molecule in the haemoglobin structure, and maintaining the membranes of red blood cells (Ramkumar *et al*., 2014).

Change in the level of rheumatoid factor

The results of Table 2 showed that the induction of rheumatoid arthritis by CFA in male laboratory rats resulted in a significant increase in the rheumatoid factor (40.26± 0.269 IU/ml) at a probability level (P < 0.05) compared to the negative control group (12.95±0.167 IU/ml) in which rheumatoid arthritis did not develop and the cause of the increase in the rheumatoid factor is attributed to the response of the immune system against the pathogenic microorganism (Patel and Pundarikakshudu, 2016). CFA releases enzymes that alter the connective tissue significantly, leading to an immune response in the groups where rheumatoid arthritis was developed (Vetal *et al*., 2013). Rheumatoid factor is a clear diagnostic marker that indicates the risk of developing rheumatoid arthritis (Nell *et al*., 2005). The protective effect of the aqueous extract of dragon fruit (kg/mg500) showed a decrease in the levels of rheumatoid factor (19.82± 0.352 IU/ml) at a probability level (P < 0.05) when compared with the positive control (0.269 ± 40.26). Dragon fruit extract decreased inflammatory cytokines such as (INF-a and IL-6) (Harahap *et al*., 2018). The protective effect of gallic acid showed a significant decrease (22.04 ±0.0915 IU/ml) at the level of probability (P < 0.05) in the levels of rheumatoid factor when compared with the positive control (40.26±0.269 IU/ml). This is because gallic acid is one of the effective compounds found in plants that lessen inflammatory cytokines, the level of (INF-a) in the blood serum, as well as its role in inhibiting the release of (interleukin-2 and interleukin-4) (IL-2 and IL-4) from lymphocytes (Rathi *et al*., 2013).

Change in interleukin-6 levels

The results of Table 2 showed that the induction of rheumatoid arthritis in the positive control of male laboratory rats through using CFA led to a significant increase (369.503±1.2463 pg/ml) at the level of probability (P < 0.05) in the levels of interleukin-6 when compared with the control negative group (48.15± 0.6483 pg/ml), in which rheumatoid arthritis did not change. Maybe it is the reason for this is attributed to an increase in responsible or pro-inflammatory cytokines such as (TNF-a), as well as (IL-1b and IL-6), which it leads to an increase in the exacerbation of inflammation, as it was observed when the substance (CFA) was used, which led to a significant increase in these cytokines that are responsible for inflammation in rat (Singh and Vinayak, 2015). The protective and Biological effect of the aqueous extract of the dragon fruit plant (kg/mg 500) showed a significant decrease (287.8667± 1.04329 pg/ml) in interleukin-6 levels at a level of probability (P < 0.05) when compared with the positive control group (369.503± 1.2463 pg/ml), and the reason for this is because of some natural compounds found in the plant at a high percentage such as (sesquiterpene) that are present in the peels, which is regarded one of the compounds with antioxidant and anti-inflammatory properties through its effects on COX enzymes, or its effect on the mechanism of activation or deactivation of some inflammatory mediators such as histamine, interleukins, and prostaglandins (Eldeen *et al*., 2016). The protective and biological effect of Gallic acid showed a low significance (301.7667± 1.84475 pg/ml) in the levels of interleukin-6 at the level of probability (P < 0.05) when compared with the positive control (369.503± 1.2463 pg/ml), and the reason for this is attributed to the fact that gallic acid can induces programmed cell death in patients with rheumatoid arthritis it also ,inhibit inflammatory cytokines as IL-6 in fibroblast-like synovial cells, (Yoon *et al.,* 2013).

Change in malondialdehyde (MDA) levels

The results of Table 2 indicated that the induction of rheumatoid arthritis by CFA in rats causes a significant increase (3.97 ±0.069 μmol/l) at a potential level (P < 0.05) in MDA compared with the negative control group (1.82 ±0.0076 μmol/l) Rheumatoid arthritis did not develop, and this is due to the increase in oxidative stress which is chemical reactions that lead to an imbalance between each of the antioxidants and oxidants and thus, what is known as occurs, and (ROS) leads to damage to biological molecules and the occurrence of many inflammatory diseases such as arthritis rheumatoid arthritis and vascular infections (Rochette *et al*., 2014). The protective effect of the aqueous extract of dragon fruit (kg/mg 500) showed a significant decrease

(2.9 ±0.0085 μmol/l) at the level of probability ($P < 0.05$) in malonaldehyde levels when compared with the positive control (3.97± 0.069 μmol/l), and the reason for this is attributed to the extract of the dragon fruit plant is a source of many natural antioxidants such as ascorbic acid (Machha *et al*., 2007), as well as the anthocyanin compound, which is one of the flavonoids found in the plant, which improves the performance of the mitochondrial function by lessening free radicals, as a pigment that binds quickly with metal ions, which It is a stable compound (metallic anthocyanin) that in turn suppresses the toxic effect of free radicals (Machha *et al*., 2007). The effect of gallic acid showed a significant decrease (3.52± 0.0051 μmol/l) at the level of probability ($P < 0.05$) in malonaldehyde levels when compared with the positive control (3.97± 0.069 μmol/l) due to the ability of gallic acid to remove free radicals, and it was also found that gallic acid has significant effects concerning the activity of antioxidant enzymes (Karimi-Khouzani *et al*., 2017), as gallic acid works to eliminate oxidative stress, and changes the balance between oxidants and antioxidants, thus lessening malonaldehyde levels (Nouri *et al*., 2021).

Histological changes in the liver

The liver in the negative control group comprised polygonally shaped hepatocyte cords with a centrally positioned spherical nucleus. These cell cords are arranged around a central vein, with sinusoids separating them (Fig. 1). On the other hand, the liver tissue from the positive control group seemed to have histological abnormalities, such as necrosis and degeneration in some liver cells. Along with the infiltration of certain inflammatory cells close to the central vein, congestion was also observed in the central vein (Fig. 2). The liver texture is nearly normal in the third group, with slight sinus dilatation and congestion in the central vein (Fig. 3). The fourth group's liver tissue revealed more severe histological lesions than the second groups, including increased sinus growth, congestion in the central vein, increased inflammatory cells infiltrating the tissue, and a rise in Kupfer cells (Fig. 4).

Liver dysfunction is caused by hepatotoxicity, and numerous drugs or Hepatocyte death is involved in most types of acute and chronic liver disorders, being an important factor in disease progression. Although it is necessary for the elimination of infected cells as a result of disease, it also occurs as a result of lack of resistance to oxidative stress, liver fibrosis, and hepatocyte necrosis(Conde de. *et al*., 2022). The production of reactive oxygen species after administration of the substance is the cause of RF. It is the primary factor that contributes to liver damage.

Free hydroxyl radicals (OH) are released when many Chemicals (such as those used to induce disease in laboratory rats) is taken. These radicals can break down DNA and other essential components of the cell, including membranes and cell walls (Vincenzi *et al*., 2016). The present study's findings demonstrated several histological alterations, including structural ones, degenerative, necrotic, inflammatory, vascular, and modifications to the constituents of liver tissue in the study groups.

Phenolic components found in plant extracts can strengthen the defense mechanism of the antibody , oxidative action resulting from the accumulation of free radicals is caused by compounds containing groups called aromatic rings and hydroxyl groups, which can donate an electron to free radicals and neutralize them, In addition, the Oxidative action is the process of searching for and suppressing or eliminating free radicals to prevent or reduce stress in large biological molecules, including DNA, lipids, and proteins (Finocchiaro *et al*., 2021). Some studies have shown the effect of natural materials on the liver organ, including an increase in oxygen consumption, a decrease in gluconeogenesis, an increase in carbohydrate metabolism, and a decrease in the metabolism of biologically active substances. Activated inflammatory cells also lead to the production of types of free radicals in rheumatoid arthritis, which leads to tissue damage resulting from increased synthesis of oxidized fats and prostaglandins, and this is evidenced by the results of an increased level of MDA, IL-6, which causes tissue membrane damage through the interaction of oxygen with acids. Polyunsaturated fatty acids (PUFAs) are found in the phospholipids of living cell membranes (Lad and Bhatnagar,2017).Additionally, the research discovered that giving the amino acids glycine and taurine considerably lowers the vital indications of harm caused by oxidative stress in the liver. (Heidari *etal*., 2016). In addition, polyphenolic compounds, including gallic acid and other plant flavonoids, have antioxidant properties and have a strong activity in eliminating radicals, which can interact with free radicals and ultimately end the radical chain reactions. Here, the ability of phenols and flavonoids appears to reduce oxidative stress in the liver of rats suffering from inflammation in rheumatoid joints (Sukketsiri *et al.*2016).

Histological changes in the kidney

The kidney's histological structure in the negative control group is shown in Fig. 5. It shows the typical configuration of kidney tissue, which is made up of proximal and distal convoluted tubules, interstitial tissue that links these parts, and glomeruli encircled by Bowman's capsule. The kidney tissue in the positive control group showed histological abnormalities, including dilatation of Bowman's capsule, necrosis and vacuolar degeneration in the epithelial cells lining the urry tubules, and atrophy in some glomeruli (Fig. 6). Compared to the previous group, the kidney tissue in the third group had

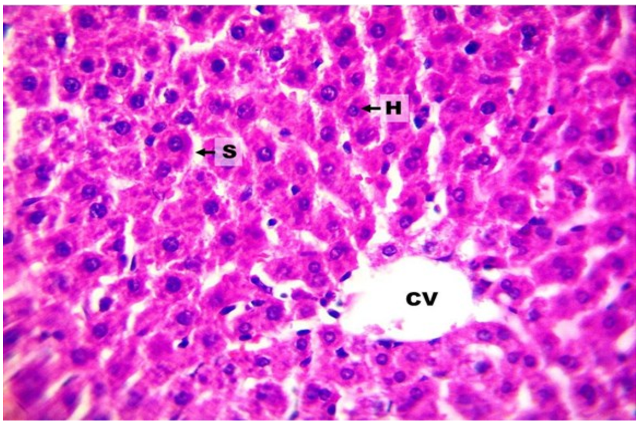

Fig. 1. *Histological section of a rat liver from the negative control group showing the normal histological features of the liver tissue represented by hepatocytes (H), central vein (CV) and sinusoids (S). Hematoxylin and eosin X400 stain*

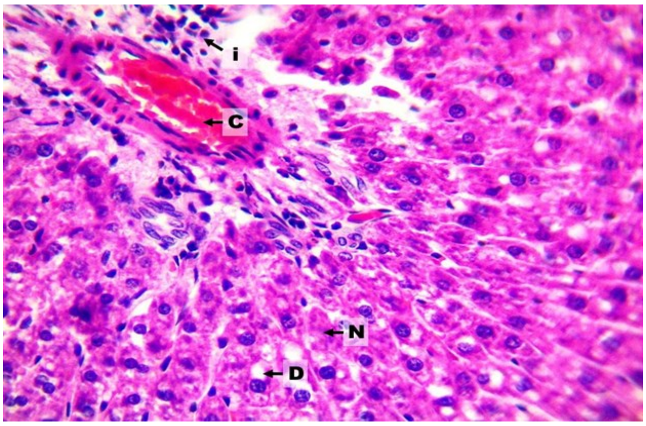

Fig. 2. *Histological section of a rat liver from the positive control group, showing degeneration (D) and necrosis (N) of hepatocytes, congestion of the central vein (C) and infiltration of inflammatory cells (i). Hematoxylin and eosin X400 stain*

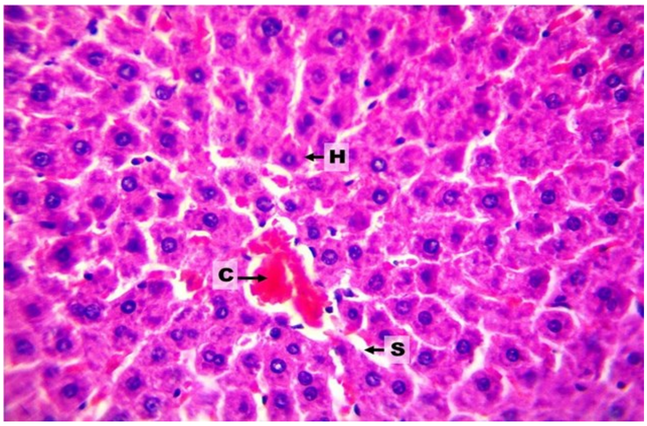

Fig. 3. *Histological section of a rat liver from the group treated with aqueous extract Kg/mg500 showing the normal composition of hepatocytes (H) and sinusoids (S) with the existence of central vein congestion (C) stained by hematoxylin and eosin X400*

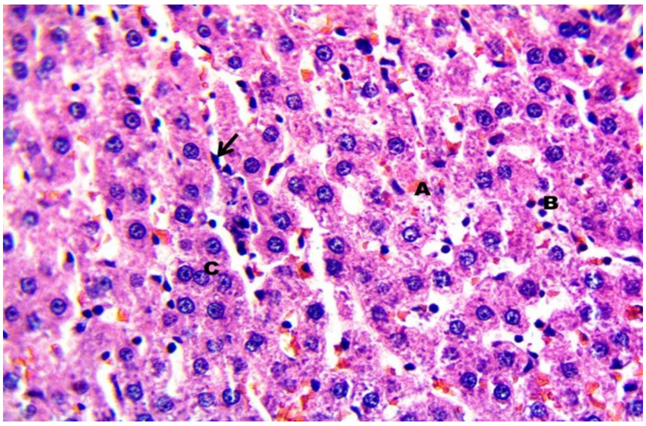

Fig. 4. *Histological section of a rat liver from the group treated with Gallic acid, showing the existing of congestion of hepatic sinusoids (A), infiltration of inflammatory cells (B), an increase in the number of kupfer cells (arrow), and the existing of binuclear hepatocytes (C). Hematoxylin and eosin stain 400X*

smaller severe histopathological lesions. These included Bowman's capsule dilatation, atrophy in some glomeruli, and vacuolar degeneration in the urinary tubule epithelial cells (Fig. 7). Histological lesions in the fourth group included dilation of Bowman's capsule and the appearance of a split in the glomerulus. Some urinary tubules had swollen epithelial cells (Fig. 8).

The changes in kidney tissue (Fig.6) may be because rheumatoid arthritis is considered a chronic inflammatory disease and is characterized by tissue changes during the disease. It is a systemic disorder that primarily affects the joints but also affects the kidneys in a way that is of clinical importance because it leads to a worsening of the symptoms of the disease and its progression to the occurrence of death, including kidney infections when rheumatoid arthritis occurs, including secondary amyloidosis, and rheumatoid nephropathy as a type of spread of the disease outside the joint when compared (Fig.5). Balendran *et al.* (2017) also found that rheumatoid arthritis patients who did not begin drug treatment excreted six times more of the enzyme β-N-acetyl glycosaminidase in the urine when compared to healthy controls. It is demonstrated that the lysosomal enzyme is found in clear concentration in mammalian cells and higher concentrations in renal parenchymal cells, indicating that rheumatoid arthritis damages the kidneys (Keiteh and John , 2021). The effect of the disease on the joints of induced laboratory rats is shown in Fig. 6. On the other hand, the results of histological sections of rats treated with plant extracts indicate the role of the plant extracts containing phenolic compounds also show an effect on affected kidney tissue (Fig.7), which may be attributed to their anti-inflammatory and immune-regulating effect. One of the previous experiments indicated the role of phenolic compounds, which may prevent the formation of blood vessels and reduce inflammation in animal tissue affected by arthritis (Rosa *et al.*, 2021). As shown in Fig.

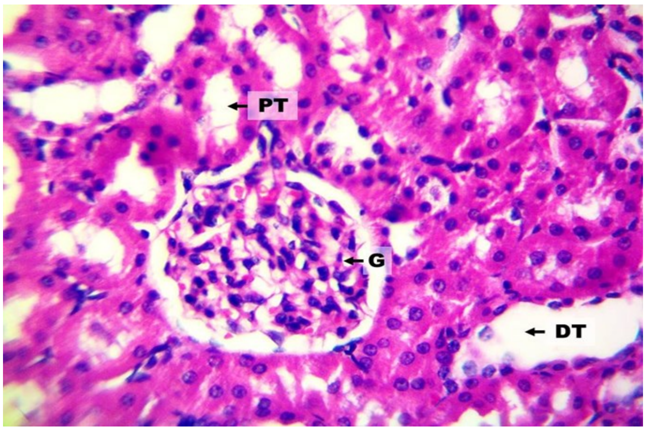

Fig. 5. *Histological section of a rat kidney from the negative control group showing normal histological features represented by renal glomeruli (G) surrounded regularly by renal proximal tubules (PT) and distal renal tubules (DT). Hematoxylin and eosin 400X stain*

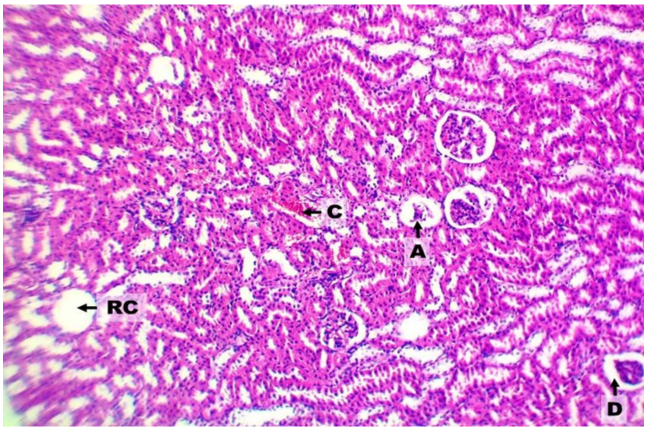

Fig. 6. *Histological section of a rat kidney from the positive control group, showing the presence of atrophy of the renal glomeruli (A), expansion of Bowman's capsule (D), vacuolar degeneration (VD), and necrosis (N) of the epithelial cells lining the renal tubules. Hematoxylin and eosin X400 stain*

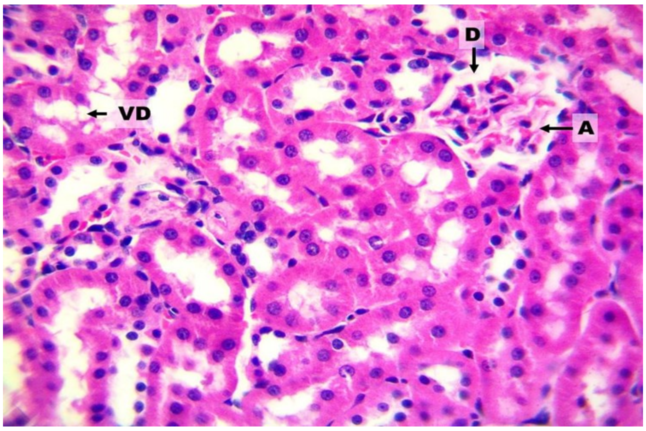

Fig.7. *Histological section of a rat kidney from the group treated with aqueous extract Kg/mg 500, showing the atrophy in the glomeruli (A), dilatation in the Bowman's capsule (D), and vacuolar* degeneration *in the epithelium cell of the renal tubular (VD). Hematoxylin and eosin X100 stain*

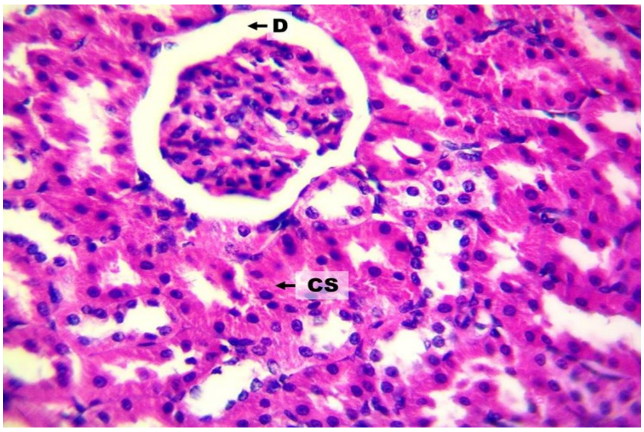

Fig. 8. *Histological section of a rat kidney from the group treated with Gallic acid, showing the fragmentation in the glomeruli and dilatation in the Bowman's capsule (D), cellular swelling in the epithelium cell of the renal tubular (VD). Hematoxylin and eosin stain, 400X*

8, the effect of callic acid on kidney tissue may be because callic acid is one of the phenolic compounds that is rapidly and globally distributed in all tissues. Perhaps the kidney is the main metabolic organ, with the highest percentage mechanisms of callic acid metabolism and excretion, followed by the heart, liver, spleen, and lung. Callic acid was demonstrated to reduce inflammatory responses mighty therapeutic pro-inflammatory such as nitrite, NO, PGE2 and IL-6. Moreover, it also helps induce the apoptotic process by enhancing the expression of Caspase-3, Bax, and p53 proteins and improves the inflammatory response by reducing the release of IL-6 and COX-2 and the regulation metabolism and energy balance of intracellular pro-inflammatory such as nitrite, NO, PGE2 and IL-6. Moreover, it helps to induce the inflammatory response, promote programmed cell death, reduce inflammation, balance energy levels and regulate nutritional metabolism (Jinrong, 2021). This demonstrates the effect of gallic acid treatment in laboratory mice. Finally, gamma acid comes from most common fruits and widespread plants. Calic acid is considered one of the metabolites of natural plants and has a vital anti-inflammatory effect on cancerous tumors, neurological diseases, and diseases of the digestive and skeletal systems, including joints, vertebrae, and others that can be studied in the future; in addition to studying the pharmacodynamic mechanisms of GA and compare it with medical drugs.

Conclusion

The present study concluded that CFA caused chronic arthritis, like human rheumatoid arthritis. The aqueous extract of the dragon fruit plant, *H. undatus* comprised many active compounds. The development of rheumatoid arthritis caused changes in the histological and biochemical parameters of Swiss albino male rats. The isolated active compounds of Gallic acid improved rheumatoid arthritis by the rheumatoid factor. Also, The presence of phenolic compounds and flavonoids in the plant extract had a positive relationship with antioxidant activities, Significantly reducing the effects of oxidative stress on the liver and kidneys in favour of reducing oxidative stress resulting from arthritis caused by FCA by enhancing the antioxidant defense within cells, This is useful in developing of pharmaceuticals to treat arthritis, plant extracts can also be used medically due to their ease of availability, lack of side effects, and low cost.

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Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Abd Al-azem, D., Al-Derawi, K. H. & Al-Saadi, S. A.A. M. (2019). The Protective Effects of Syzygium aromaticum Essential Oil Extract against Methotrexate Induced Hepatic and Renal Toxicity in Rats. *J Pure Appl Microbiol*, 13 (1):505-515 https://dx.doi.org/10.22207/JPAM.13.1.57
2. Adeneye, A. A., Oreagba, A. I., Ishola, I. O. & Kalejaiye, H. A. (2014). Evaluation of the anti-arthritic activity of the hydroethanolic leaf extract of Alchornea cordifolia in rats. *African Journal of Traditional, Complementary and Alternative Medicines*, vol. 11, pp. 402-410. https://doi.org/10.4314/ajtcam.v11i2.26
3. . Akomolafe, S. F., Akinyemi, A. J. & Anadozie, S. O. (2014). Phenolic acids (gallic and tannic acids) modulate antioxidant status and cisplatin induced nephrotoxicity in rats. *International Scholarly Research Notices*, vol. 2014. https://doi.org/10.1155/2014/984709
4. Aletaha, D. & Smolen, J. S. (2018). Diagnosis and management of rheumatoid arthritis: a review, *Jama,* vol. 320, pp. 1360-1372. https://doi.org/doi:10.1001/jama.2018.13 103
5. Badhani, B., Sharma, N. & Kakkar, R. (2015). Gallic acid: A versatile antioxidant with promising therapeutic and industrial applications. *Rsc Advances*, vol. 5, pp. 27540-27557. https://doi.org/10.1039/C5RA01911G
6. Cahyati, W. H., Siyam, N., & Putriningtyas, N. D. (2021). The potential of red dragon fruit peel yogurt to improve platelet levels in heparin-induced thrombocytopenia in Wistar rats. *Slovak Journal of Food Sciences*, 15. https://doi.org/10.5219/1497
7. Clain, E., Haddad, J. G., Koishi, A. C., Sinigaglia, L., Rachidi, W., Desprès, P. *etal*. (2019). The polyphenol-rich extract from psiloxylon mauritianum, an endemic medicinal plant from Reunion Island, inhibits the early stages of dengue and Zika virus infection. *International Journal of Molecular Sciences*, vol. 20, p. 1860. , https://doi.org/10.3390/ijms20081860
8. Cui, X., Wang, R., Bian, P., Wu, Q., Seshadri, V. D. D. & Liu, L. (2019). Evaluation of antiarthritic activity of nimbolide against Freund's adjuvant induced arthritis in rats. *Artificial cells, Nanomedicine, and Biotechnology*, 47, 3391-3398. https://doi.org/10.1080/21691401.2019.1649 269
9. Conde de la Rosa, L.; Goicoechea, L.; Torres, S.; Garcia-Ruiz, C.; Fernandez-Checa, J.C. (2022). Role of Oxidative Stress in Liver Disorders. *Liver*, 2, 283-314. https://doi.org/10.3390/livers2040023
10. Dey, P., Kundu, A., Kumar, A., Gupta, M., Lee, BM., Bhakta, T. & Dash, Kim HS S. (2020). Analysis of alkaloids (indole alkaloids, isoquinoline alkaloids, tropane alkaloids). *Recent Advances in Natural Products Analysis*, 2020, 505–67. https://doi.org/10.1016/B978-0-12-816455-6.00015-9
11. Doan, K. V., Ko, C. M., Kinyua, A. W., Yang, D. J., Choi, Y.-H., Oh, I. Y. *etal*., (2015). Gallic acid regulates body weight and glucose homeostasis through AMPK activation. *Endocrinology*, 156, 157-168. https://doi.org/10.1210/en.2014-1354
12. Eggli, U. & Newton, L. E. (2004). Etymological dictionary of succulent plant names. *Springer Science & Business Media,* http://dx.doi.org/10.1007/978-3-662-07125-0
13. Eldeen, I., Mohamed, H., Tan, W., Siong, J., Andriani, Y. & Tengku-Muhammad, T. (2016). Cyclooxygenase, 5-lipoxygenase and acetylcholinesterase inhibitory effects of fractions containing, α-guaiene and oil isolated from the root of Xylocarpus moluccensis. *Res J Med Plant*.10,86-294. https://scialert.net/abstract/?doi=rjmp.2016.286.294
14. Ertenli, I., Kiraz, S., Öztürk, M. A., Haznedaroğlu, I., Çelik, İ. & Çalgüneri, M. (2003). Pathologic thrombopoiesis of rheumatoid arthritis. *Rheumatology international*, v 23, 49-60.https://doi.org/10.1007/s00296-003-0289-0
15. Finocchiaro, C., Fadda, M., D'Onofrio, V., Ippolito, M., Pira, C. & Devecchi, A. (2021). Oxidative stress and cancer: Role of n-3 PUFAs. In Cancer (pp. 245-253). Academic Press. https://doi.org/10.1016/B978-0-12-819547-5.00022-5
16. Ganna, S.(2014). The relationship between haemoglobin level and disease activity in patients with rheumatoid arthritis. *Revista Brasileira de Reumatologia (English Edition)*, 54, 437-440. https://doi.org/10.1016/j.rbre.2014.0 6.003
17. Granado, M., Martín, A. I., Villanúa, M. Á. & López-Calderón, A. (2007). Experimental arthritis inhibits the insulin-like growth factor-I axis and induces muscle wasting through cyclooxygenase-2 activation. *American Journal of Physiology-Endocrinology and Metabolism*, 292, E1656-E1665. https://doi.org/10.1152/ajpendo.00502.20 06
18. Halliwell, B. and Chirico, S. (1993). Lipid peroxidation: its mechanism, measurement and significance. *Am J Clin Nutr*. 57,715-724. https://doi.org/10.1093/ajcn/57.5.715S
19. Harahap, N., Sinaga, F. & Nailuvar, R. (2019). Effect of red-fleshed pitaya (Hylocereus polyrhizus) to increase gluthatione peroxidase levels in male rats (Rattus norvegicus): The Induced Oxidative Stress, In Proceedings of The 5th Annual International Seminar on Trends in Science and Science Education, AISTSSE 2018, 18-19 October 2018, Medan, Indonesia. http://dx.doi.org/10.4108/

eai.18-10-2018.2287360

20. Hazzaa, S. A., Al-lehebe, N. I., Yaseen, AT. and Al-Hamadany, AYM. (2022). Evaluation of Biochemical and Hematological Parameters in Glucose-6-Phosphate Dehydrogenase Deficiency Patients Associated Covid19 infection. *Egyptian Journal of Chemistry*. 65 (4), 221 – 229. https://doi.org/10.21608/ejchem.2021.88082.4240
21. Heidari, R., Jamshidzadeh, A., Niknahad, H., Safari, F., Azizi, H., Abdoli, N. & Najibi, A. (2016). The hepatoprotection provided by taurine and glycine against antineoplastic drugs induced liver injury in an ex vivo model of normothermic recirculating isolated perfused rat liver. *Trends in Pharmaceutical Sciences*, 2(1), 59-76.
22. Hernández, Y. D. O. & Salazar, J. A. C. (2012). Pitahaya (Hylocereus spp.): a short review. *Comunicata Scientiae*,3, 20-237, . https://www.researchgate.net/publication/288047198_Pitahaya_Hylocereus_spp_A_sh ort_review
23. Hossain, F. M., Numan, S. M. N. & Akhtar, S. (2021). Cultivation, nutritional value, and health benefits of Dragon Fruit (Hylocereus spp.): A Review. *International Journal of Horticultural Science and Technology*, 8,259-269. https://doi.org/10.22059/ijhst.2021.311550.400
24. Hsu C.L. & Yen, G.C. (2007). Effect of gallic acid on high fat diet-induced dyslipidaemia, hepatosteatosis and oxidative stress in rats. *British Journal of Nutrition*, 98, 727-735. https://doi.org/10.1017/S000711450774686X
25. Humphreys, J., Warner, A., Chipping, J., Marshall, T., Lunt, M., Symmons*,* D. *et al.* (2014). Mortality trends in patients with early rheumatoid arthritis over 20 years: results from the Norfolk Arthritis Register. *Arthritis care & Research,* 66, 1296 -1301. https://doi.org/10.1002/acr.22 296
26. Hussain K. A. M. & Khalaf, A. A. (2020). Preparation and diagnosis of Xerogel nanocomposites And Studying Their Effect on TNF-α Level before and after Loading Dexamethason in Male White Rats Induced Rheumatoid Arthritis. *Indian Journal of Forensic Medicine & Toxicology*, vol. 14. https://doi.org/10.37506/ijfmt.v14i4.11972
27. Jakobsson, P. J., Robertson, L., Welzel, J., Zhang, M., Zhihua, Y., Kaixin*,* G. *etal.* (2022). Where traditional Chinese medicine meets Western medicine in the prevention of rheumatoid arthritis. *Journal of Internal Medicine,* 292, 745-763. https://doi.org/10.1111/joim.13537
28. Jiang, M., Cui, B. W., Wu, Y. L., Nan, J. X., & Lian, L. H. (2021). Genus Gentiana: A review on phytochemistry, pharmacology and molecular mechanism. *Journal of Ethnopharmacology*, 264, 113391. http://dx.doi.org/10.1016/j.jep.2020.113391
29. Jinrong B., Yunsen Z., Ce T., Ya H., Xiaopeng A., Xiaorui C., Yi Z., Xiaobo W., X.(2021) .Gallic acid: Pharmacological activities and molecular mechanisms involved in inflammation-related diseases. Biomedicine & Pharmacotherapy, 133, https://doi.org/10.1016/j.biopha.2020.110985
30. Kapcum, C., Uriyapongson, S. & Uriyapongson, J. (2021). Phenolics, anthocyanins and antioxidant activities in waste products from different parts of purple waxy corn (Zea mays L.). *Songklanakarin Journal of Science & Technology*, 43, https://doi.org/10.3390/plants12030603
31. Karamac, M., Kosiñska, A. & Pegg, R. B. (2006). Content of gallic acid in selected plant extracts. *Polish Journal of Food and Nutrition Sciences*, , Vol. 15/56, No 1, pp. 55–58 https://www.researchgate.net/publication/285726711_Content_of_Gallic_aci d_in_selected_plant_extracts
32. Karimi-Khouzani, O., Heidarian, E. & Amini, S. A. (2017). Anti-inflammatory and ameliorative effects of gallic acid on fluoxetine-induced oxidative stress and liver damage in rats. *Pharmacological Reports*, 69, 830-835. https://doi.org/10.1016/j.pharep.2017.03.011
33. Kirkwood, B.R. (1988). Essentials of Medical Statistics. Boston, Mass: Black-well Scientific Publications.
34. Machha, A., Achike, F. I., Mustafa, A. M. & Mustafa, M. R. (2007). Quercetin, a flavonoid antioxidant, modulates endothelium-derived nitric oxide bioavailability in diabetic rat aortas. Nitric Oxide, 16(4), 442-447. https://doi.org/10.1016/j.niox.2007.04.001
35. McInnes, B. & Schett, G. (2017). Pathogenetic insights from the treatment of rheumatoid arthritis. *The Lancet,* 389,2328-2337. https://doi.org/10.1016/s0140-6736(17)31472-1
36. Mitscher, L.A., Drake, S., Gollapudi, S.R., Harris, J.A. & Shankel, D.M. (1986). Isolation and Identification of Higher Plant Agents Active in Antimutagenic Assay Systems: *Glycyrrhiza glabra* . In: Shankel, D.M., Hartman, P.E., Kada, T., Hollaender, A., Wilson, C.M., Kuny, G. (eds) Antimutagenesis and Anticarcinogenesis Mechanisms. *Basic Life Sciences,* vol 39. Springer, Boston, MA. https://doi.org/10.1007/978-1-4684-5182-5_13
37. Mohammed, B. M., Sanford, K. W., Fisher, B. J., Martin, E. J., Contaifer Jr, D., Warncke, U. O. *etal*. (2017). Impact of high dose vitamin C on platelet function. *World journal of critical care medicine*, 6, 37. https://dx.doi.org/10.5492/wjccm.v6.i1.37
38. Nair G. G. & Nair, C. K. K. (2013). Radioprotective effects of gallic acid in mice. *BioMed Research International*, 2013. https://doi.org/10.1155/2013/953079
39. Nasuti, C., Bordoni, L., Fedeli, D. & Gabbianelli, R. (2019). Effect of Nigella sativa oil in a rat model of adjuvant-induced arthritis. *Multidisciplinary Digital Publishing Institute Proceedings*, 11, 16. https://doi.org/10.3390/proceedings2019011016
40. Nell, V., Machold, K. P., Stamm, T. A., Eberl, G., Heinzl, H., Uffmann, M. *et al*. (2005). Autoantibody profiling as early diagnostic and prognostic tool for rheumatoid arthritis, *Annals of the Rheumatic Diseases*, 64, 1731-1736. http://dx.doi.org/10.1136/ard.2005.035691
41. Nouri, A., Salehi-Vanani, N. & Heidarian, E. (2021). Antioxidant, anti-inflammatory and protective potential of gallic acid against paraquat-induced liver toxicity in male rats. *Avicenna Journal of Phytomedicine*, 11,633. https://doi.org/10.22038%2FAJP.2021.18581
42. Pansai, N., Chakree, K., Takahashi Yupanqui, C., Raungrut, P., Yanyiam, N. & Wichienchot, S. (2020). Gut microbiota modulation and immune boosting properties of prebiotic dragon fruit oligosaccharides, *International Journal of Food Science & Technology*, 55, 55-64. https://doi.org/10.1111/ijfs.14230
43. Patel M. G. & Pundarikakshudu, K. (2016). Anti-arthritic activity of a classical Ayurvedic formulation Vatari Guggulu in rats," *Journal of Traditional and Complementary Medicine*, 6, 389-394. https://doi.org/10.1016/j.jtcme.2015.08.007
44. Patel, S. & Ishnava, K. (2019). In-vitro Antioxidant and Antimicrobial activity of Fruit Pulp and Peel of Hylocereu sundatus (Haworth) Britton and Rose. *Asian Journal of*

Ethnopharmacology and Medicinal Foods, 5, 30-34. https://www.researchgate.net/publication/333966156_Hylocereus_undatus_Ha worth_Britto n_and _Rose
45. Patil, K. R., Patil, C. R., Jadhav, R. B., Mahajan, V. K., Patil, P. R. & Gaikwad, P. S. (2011). Anti-arthritic activity of bartogenic acid isolated from fruits of Barringtonia racemosa Roxb.(Lecythidaceae). *Evidence-Based Complementary and Alternative Medicine*, 2011. https://doi.org/10.1093/ecam/nep148
46. Punithavathi, V. R., Prince, P. S. M., Kumar, R. & Selvakumari, J. (2011). Antihyperglycaemic, antilipid peroxidative and antioxidant effects of gallic acid on streptozotocin induced diabetic Wistar rats, *European Journal of Pharmacology,* 650, 465-471. https://doi.org/10.1016/j.ejphar.2010.08.059
47. Radovanović, B. , Mladenović, J. , Radovanović, A. , Pavlović, R. & Nikolić, V. (2015). Phenolic composition, antioxidant, antimicrobial and cytotoxic activites of *Allium porrum* L. (Serbia) extracts. *Journal of Food and Nutrition Research*, 3(9), 564-569. https://doi: 10.12691/jfnr-3-9-1
48. Ramkumar, K., Vijayakumar, R., Vanitha, P., Suganya, N., Manjula, C., Rajaguru, P. *et al*., (2014). Protective effect of gallic acid on alloxan-induced oxidative stress and osmotic fragility in rats. *Human & Experimental Toxicology*, 33, 638-649. https://doi.org/10.1177/0960327113504792
49. Rasool, M. K., Sabina, E. P., Ramya, S. R., Preety, P., Patel, S., Mandal, N. *et al*., (20100. Hepatoprotective and antioxidant effects of gallic acid in paracetamol-induced liver damage in mice. *Journal of Pharmacy and Pharmacology*, . 62,638-643. https://doi: 10.1211/jpp.62.05.0012.
50. Rathi, B., Bodhankar, S., Mohan, V. & Thakurdesai, P. (2013). Ameliorative effects of a polyphenolic fraction of Cinnamomum zeylanicum L. bark in animal models of inflammation and arthritis. *Scientia Pharmaceutica*, 81, 567-590. https://doi.org/10.3797/scipharm.1301-16
51. Reckziegel, P., Dias, V. T., Benvegnú, D. M., Boufleur, N., Barcelos, R. C. S., Segat, H. J. *etal*. (2016). Antioxidant protection of gallic acid against toxicity induced by Pb in blood, liver and kidney of rats. *Toxicology Reports*, 3, 351-356. https://doi.org/10.1016/j.toxrep.2016.02.005
52. Rochette, L., Zeller, M., Cottin, Y. & Vergely, C. (2014). Diabetes, oxidative stress and therapeutic strategies. *Biochimica et Biophysica Acta (BBA)-General Subjects*, 1840(9), 2709-2729. https://doi.org/10.1016/j.bbagen.2014.05.017
53. Scott, D. L., Galloway, J., Cope, A., Pratt, A. & Strand, V. (2020). *Oxford Textbook of Rheumatoid Arthritis*: Oxford University Press. https://doi.org/10.1093/med/9780198831433.003.0026
54. Singh, A. K. and Vinayak, M. (2015). Curcumin attenuates CFA induced thermal hyperalgesia by modulation of antioxidant enzymes and down regulation of TNF-α, IL-1β and IL-6. *Neurochemical Research*, 40, 463-472. https://doi.org/10.1007/s11064-014-1489-6
55. Singla, K., Sandhu, SV., Pal, K., Bansal, H., Bhullar, RK., Kaur, P. (2017). Comparative evaluation of different histoprocessing methods. *Int J Health Sci. (Qassim)*,11(2),28-34. PMID: 28539860; PMCID:
56. Sofowora, A., Ogunbodede, E. & Onayade, A. (2013). The role and place of medicinal plants in the strategies for disease prevention," *African Journal of Traditional, Complementary and Alternative Medicines,* 10, 210-229. https://doi.org/10.4314/ajtcam.v10i5.2
57. Stillinger, D., Helland, K. & Van Atta, C. (1983). Experiments on the transition of homogeneous turbulence to internal waves in a stratified fluid. *Journal of Fluid Mechanics*, 131, 91-122. https://doi.org/10.1017/S0022112083001251
58. Swarup, K. R. A., Sattar, M. A., Abdullah, N. A., Abdulla, M. H., Salman, I. M., Rathore, H. A. *etal*. (2010). Effect of dragon fruit extract on oxidative stress and aortic stiffness in streptozotocin-induced diabetes in rats. *Pharmacognosy Research*, 2, 31. http://dx.doi.org/10.4103/0974-8490.60582
59. Verma, D., Yadav, R., Rani, M., Punar, S., Sharma, A. & Maheshwari, R. (2017). Miraculous health benefits of exotic dragon fruit," *Research Journal of Chemical and Environmental Sciences,* vol. 5, pp. 94-96.
60. Vetal, S., Bodhankar, S. L., Mohan, V. & Thakurdesai, P. A. (2013). Anti-inflammatory and anti-arthritic activity of type-A procyanidine polyphenols from bark of Cinnamomum zeylanicum in rats. *Food Science and Human Wellness*, vol. 2, pp. 59-67, 2013. https://doi.org/10.1016/j.fshw.2013.03.003
61. Vincenzi, B., Armento, G., Spalato Ceruso, M., Catania, G., Leakos, M., Santini, D. and Tonini, G. (2016). Drug-induced hepatotoxicity in cancer patients-implication for treatment. *Expert Opinion on Drug Safety*, 15(9), 1219-1238. https://doi.org/10.1080/14740338.2016.1194824
62. Lad, H., Bhatnagar, D., (2017),Modulation of oxidative stress mediators in the liver of adjuvant induced arthritic rats by Nyctanthes arbor tristis . *Clin. Phytosci.* 3, 1. https://doi.org/10.1186/s40816-016-0041-4
63. Yoon, C.H., Chung, S. J., Lee, S.W., Park, Y.B., Lee, S.K. & Park, M.C. (2013). Gallic acid, a natural polyphenolic acid, induces apoptosis and inhibits proinflammatory gene expressions in rheumatoid arthritis fibroblast-like synoviocytes. *Joint Bone Spine*, 80, 274-279. DOI: 10.1016/j.jbspin.2012.08.010
64. Sukketsiri, W., Chonpathompikunlert, P., Tanasawet, S., Choosri, N, & Wongtawatchai, T. (2016). Effects of Apium graveolens Extract on the Oxidative Stress in the Liver of Adjuvant-Induced Arthritic Rats. Preventive nutrition and food science, 21(2), 79–84. https://doi.org/10.3746/pnf.2016.21.2.79
65. Balendran, K., Senarathne, L.D.S.U. & Lanerolle, R.D. (2017), Crescentic glomerular nephritis associated with rheumatoid arthritis: a case report. J Med Case Reports 11, 197. https://doi.org/10.1186/s13256-017-1346-8
66. Keiteh W., John W., (2021), 7 - Liver and Kidney Disease in Rheumatoid Arthritis, Clinics in Rheumatic Diseases, Volume 3, Issue 3, , Pages 527-547, https://doi.org/10.1016/S0307-742X(21)00040-0
67. Rosa D., João R., Bruno S. & Maria E. (2021), Phenolic Compounds Impact on Rheumatoid Arthritis, Inflammatory Bowel Disease and Microbiota Modulation, Pharmaceutics, 13(2), 145., https://doi.org/10.3390%2Fpharmaceutics13020145