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Potential reno-protective and anti-inflammatory effects of rutin in guinea pig

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ABSTRACT

This work was carried out to evaluation the reno-protective effect and anti-inflammatory activity of rutin in guinea pigs. Twenty-four male guinea pigs categorized into 4 groups, six pigs in each group. Group one as a normal control was given (2 ml/kg) distilled water. Second group as non-treated control was given 5% of carboxymethyl cellulose orally. Third group, animals received rutin (20 mg/kg). The fourth group was given aqueous suspensions of indomethacin (10 mg/kg) in 5% polysorbate 80 orally once daily for seven days. For model of chronic inflammation each pig were anesthetized by light ether and then after thirty minutes a sterile cotton pellet weighing (50 ±1 mg) was implanted under the skin on both sides for the scapular region. All pigs were anaesthetized by using chloroform on the 8th day then the blood collected. The kidney were dissected and put it in 10% formalin for histopathological exmaination. Then isolated the cotton pellets from each, dried at 60 °C and estimated the dry weight. Administration of rutin (20 mg/kg body weight) for seven days showed a significant ($P<0.05$) inflammation inhibition. The inflammatory inhibition rate was 33% for (rutin), 37% for (indomethacin) and 0% for control. Also, rutin (20 mg/kg) improved the biochemical related to inflammation. This was also supported by histopathological findings of the examined kidneys. On conclusion, Rutin possess anti-inflammatory activity and protective role against granuloma-induced alterations.

1. INTRODUCTION

Inflammatory diseases are globally identified as the major cause of morbidity across the population (Dewanjee *et al.,* 2013). Inflammation is the reaction of tissues to pathogenic which leads to the restoration of a normal tissue structure and function. Acute inflammation has a limited useful role for the body, whereas chronic inflammation is an undesired persevering phenomenon that can facilitate to the evolution of inflammatory diseases (Kaplanski *et al.*, 2003). Many inflammatory diseases like atherosclerosis (Frostegard, 2010), obesity, cardiovascular disease (Mathieu *et al.,* 2010), rheumatoid arthritis, and cancer have a relation with chronic inflammation (Metsios *et al.,* 2010).

Treatment of the chronic inflammatory diseases like rheumatoid arthritis and inflammatory bowel diseases is still a challenge due to lack of safe and effective medicines (Kulkarni *et al.,* 2006).

Anti-inflammatory drugs both steroids or NSAIDs have many side effects including bronchospasm, gastric injury, ulceration (Tapiero *et al.,* 2002), inhibition of platelet aggregation, kidney, and liver toxicity (Batlouni, 2010) and cardiac abnormalities (Dogne *et al.,* 2006) which ultimately have limited their use. Therefore, it is necessary to investigate for new anti-inflammatory drugs with fewer side effects. Products obtained from the natural sources such as medicinal plants have been used in the advancement of new anti-inflammatory drugs with less undesirable effects (Halliwell and Gutteridge, 1992).

Among many different groups of natural products, flavonoids, are a group of chemical compounds of benzo-pyrone derivatives, widely distributed in the plant kingdom. They have relatively simple chemical structures, but more than 4,000 derivatives have been reported from nature, indicating their chemical diversities. Flavonoids, also known as nature's tender drugs, possess various biological and pharmacological activities including antiviral, anti-inflammatory, anticancer, antimicrobial, and antithrombotic activities (Havsteen,1983).

Many investigations have confirm that varieties of flavonoid molecules possess anti-inflammatory activity on various animal models of inflammation. Thus, it may be valuable to continuously evaluate the anti-inflammatory activity of flavonoids, not only for establishing anti-inflammatory mechanisms, but also for developing a new class of anti-inflammatory agents. Flavonoids modulate the enzyme activities of arachidonic acid (AA) metabolizing enzymes such as phospholipase A2 (PLA2), cyclooxygenase (COX), and lipoxygenase (LOX) and the nitric oxide (NO) producing enzyme, nitric oxide synthase (NOS). An inhibition of these enzymes by flavonoids reduces the production of AA, prostaglandins (PG), leukotrienes (LT). Thus, the inhibition of these enzymes exerted by flavonoids is definitely one of the important cellular mechanisms of anti-inflammation (Hyun *et al.,* 2004).

Rutin is a natural flavone derivative with a wide range of pharmacological activities which present in many plants, vegetables, fruits and red wine (Tang *et al.,* 2011).

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Different studies have represented the biological effects of rutin, such as anti-oxidative, anti-inflammatory, anti-platelet, anti-hypertensive, anti-thrombic, anti-carcinogenic, anti-diabetic, anti-adipogenic, anti-apoptotic, neuroprotective and cardioprotective activities (Jayameena *et al.,* 2018).

Increased concerns on the side effects of current therapeutic modalities, plants are being valued because of their efficient curative properties and least or no side effects. Hence an attempt has been made to assess the Reno-protective effect and anti-inflammatory activity of Rutin in guinea pigs.

2. MATERIAL AND METHODS

2.1. Chemicals

Flavonoid (rutin) and indomethacin were purchased from Sigma Aldrich Co. (St Louis, MO, USA). Polysorbates 80 USP (Tween 80) as a suspending agent for indomethacin, carboxymethyl cellulose 5% as a suspending agent for flavonoid were purchased from Himedia Chemical Co. India.

2.2. Experimental animals

Male guinea pigs weighing between 350-650 g were gained from the animal house of Biology Department, Ibb University, Yemen conserved it for one week in environmentally controlled conditions (25±5 °C, 55±5% humidity and 12 h light–dark cycle) to accommodate with free access to food and water *ad libitum*. Pigs were eaten grass. The experiment protocol was accepted by Institutional Animal Ethics Committee of Ibb University-Yemen.

2.3. Cotton pellet-induced granuloma

The effect of rutin on the chronic and proliferative phases of inflammation was assessed using a cotton pellet induced granuloma tissue formation model, as previously described by Swingle and Shideman, (1972). Guinea pigs were anesthetized with light ether and then an autoclave sterilized cotton pellet that weighed 50 mg was implanted subcutaneously on each side of the scapular region. After the implantation of the cotton pellet, each treatment group of guinea pigs(n = 6 per group) were administered : group one was treated orally with distilled water and considered as control, while group two as non-treated control were given 5% orally carboxymethyl cellulose, third group of animals were received with rutin (20 mg/kg). The fourth group were given aqueous suspensions Indomethacin (10 mg/kg) dissolved in 5% Polysorbate 80 orally once daily for seven consecutive days. Eight days after implantation of the cotton pellet the animals were euthanized with chloroform then the blood collected. Kidney tissue specimens were fixed in 10% formalin for histopathological examination. Each implanted cotton pellet was removed and dried at 60 °C for 24 h. afterward, the dry weight was measured. The higher weight of cotton pellet was indicated inflammation as anti-inflammatory response (Sedgwick and Willoughby, 1989).

2.4. Determination of biochemical parameters

Kidney functions (Urea, Uric acid, Albumin, Total protein), Lipid profile (Total Cholesterol, HDL-cholesterol, LDL-cholesterol, and Triglycerides) and Glucose were estimated by using of Roche diagnostic Kits (Germany) according to (Tietz *et al.,* 1995) from the sera of control and experimental groups.

2.5. Histopathological studies

After the blood samples were collected, animals were sacrificed and the kidney from each group were isolated and preserved in 10% formalin solution. After paraffin embedding, tissues were sectioned and stained with hematoxylin and eosin (H&E) for observing microscopic changes in the kidney.

2.6. Statistical analysis

Data were expressed as the mean values ± standard deviation (S.D.) for each measurement. The data were also analyzed by one-way analysis of variance (one-way ANOVA) using SPSS (version 20). The test is significant at α 5%.

3. RESULTS

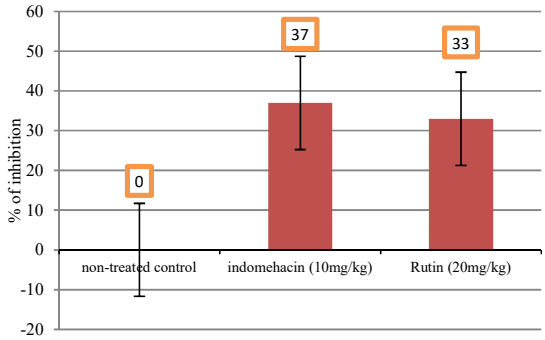
3.1. Cotton pellet induced granuloma.

The results of the present study showed that parentage of inflammatory inhibition were significantly decreased (P<0.05) in Rutin treated group (20 mg/kg) which was 33% when compared with non-treated control (0 %) and indomethacin (10 mg/kg) treated groups 37% (Table 1, Graph 1).

Table 1 Anti-inflammatory activity of Rutin and Indomethacin on cotton pellet granuloma in male guinea pigs

Treatment	Weight of dry granuloma (mg)	% of inhibition
Non-treated control	86.67 ± 18.75	0 %
Indomethacin (10mg/kg)	55.00 ± 6.74*	37 %
Rutin (20 mg/kg)	55.00 ± 1.95*	33 %

Results are Mean ± SD (n=6) *P<0.05 compared to control



Graph 1 Anti-inflammatory activity of rutin and indomethacin on cotton pellet granuloma in male guinea pigs

3.2. Effect of rutin on kidney function biochemical parameter in guinea pigs induced by inflammation.

The kidney profile parameters were mentioned in Table 2. The levels of urea, uric acid, and total protein were significantly lower (P<0.05) comparing with non-treated control group, however in the indomethacin treated group, creatinine and albumin values were higher significantly (P<0.05) comparing with non-treated control group. Also, In rutin administered group, significant decrease (P<0.05) in urea, uric acid, and total protein while significantly (P<0.05) increases in albumin and creatinine values were recorded.

3.3. Effect of rutin on lipid profile biochemical parameter in guinea pigs induced by inflammation.

The lipid profile parameters were mentioned in Table 2. The levels of total cholesterol, triglyceride and LDL were significantly lower (P<0.05) comparing with non-treated control group, however in the Indomethacin treated group, HDL value was higher significantly (P<0.05) comparing with non-treated control group. Rutin administered group

showed non-significant decrease in total Cholesterol and Triglyceride while significant increase in HDL value was observed.

3.4. Effect of rutin on glucose in guinea pigs induced by inflammation.

Glucose level showed significant increase (P<0.05) in the Indomethacin group and Rutin treated group.

Table 2 Effect of Rutin and Indomethacin on biochemical parameters in guinea pigs induced by inflammation.

Parameters	Unit	Normal control	Non-treated control	Indomethacin (10mg/kg)	Rutin (20mg/kg)
Urea	mg/dl	55.50±4.667	68.00±4.487$^{\#}$	57.67±2.011*	58.00±0.258*
Uric acid	mg/dl	2.57±0.316	4.10±0.475$^{\#}$	2.93±0.148*	3.57±0.165*
Creatinin	mg/dl	0.73±0.115	0.30±0.037$^{\#}$	0.64±0.004*	0.63±0.004*
Total protein	g/dl	4.45±0.115	5.40±0.037$^{\#}$	4.63±0.152*	4.83±0.092$^{\#*}$
Albumin	g/dl	2.92±0.187	1.00±0.037$^{\#*}$	2.10±0.037$^{\#*}$	1.88±0.004$^{\#*}$
Triglyceride	mg/dl	62.83±8.129	108.67±1.282$^{\#}$	69.33±15.285*	95.17±0.373
Total Cholesterol	mg/d	19.33±2.319	37.33±9.426$^{\#\#\#}$	23.00±0.730**	28.33±1.838
HDL Cholesterol	mg/d	6.33±1.054	2.17±0.167$^{\#}$	3.88±0.004*	3.89±0.004*
LDL Cholesterol	mg/dl	15.00±1.897	37.67±1.647$^{\#}$	21.67±0.004*	24.67±0.004*
Glucose	mg/kg	181.50±0.365	66.00±5.477$^{\#}$	138.67±0.365*	160.33±8.401*

All value represents mean± SD of six animals. # P < 0.05, ### P < 0.001 compared with normal control value. * P < 0.05, ** P<0.01 compared with non-treated control values respectively.

3.4. Histopathological examination of kidney in cotton pellet induced granuloma in adult male guinea pigs.

Figure (1) exhibits the histopathological findings of kidney in control, non-treated group, Indomethacin and Rutin groups:

The microscopic examination of the kidneys of non-treated control cotton pellet induced granuloma showing severe congestion of the glomerular tuft and renal blood vessels as well as interlobular blood capillaries. Perivascular and periglomerular leukocytic infiltration were also seen particularly, mononuclear leukocytic cells. Focal areas of hemorrhages were seen in the renal medulla. The glomeruli showing sever thickening and the Bowman's capsule with periglomerular fibrous connective tissue and Focal intertubular connective tissue proliferation were recorded. Also, renal tubules showed degenerative change in tubular epithelium represented by cloudy swelling. Also, focal areas of tubular necrosis were found (figure 1b & c).

The examined kidneys of guinea pigs treated with indomethacin (10 mg/kg) showed congestion of interlobular blood capillaries and renal blood vessels. The glomeruli showed congestion of the glomerular tuft with vacuolation of the glomerular endothelium. Shrinkage of some of the renal glomeruli was also seen. The renal tubules showed cystic dilatation with flattened lining epithelium. The epithelium of the renal tubules also showed necrotic changes manifested by pyknotic nuclei (figure 1 d & e).

The microscopic examination of the kidneys of guinea pigs treated with rutin (20mg/kg) showed moderate congestion of the renal blood vessels. The renal tubule showed mild degeneration change in the form of cloudy swelling with the mild desquamation of some of the lining epithelium. Lumen of the renal tubules contained eosinophilic debris in their lamina. Perivascular mononuclear infiltrations were also seen (figure 1f).

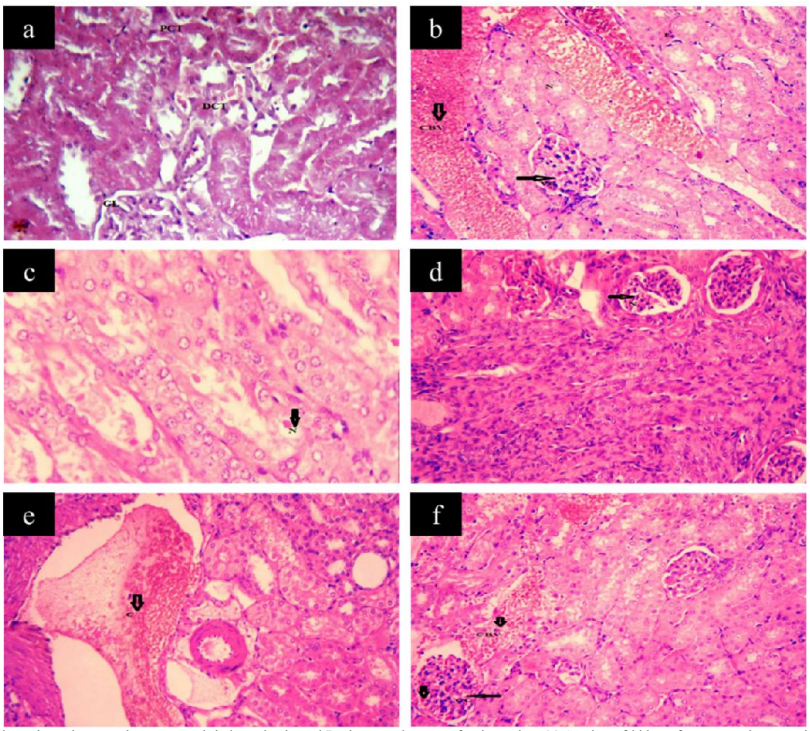


Fig 1 Histopathology of kidney tissue in control, non-treated, indomethacin and Rutin treated group of guinea pigs. (a) Section of kidney from control group given (2ml/kg)distilled water for seven days showed normal kidney architecture, showing the normal appearance of glomerulus (GL) and renal tubules (T) including proximal convoluted tubules (PCT) and distal convoluted tubules (DCT) (H&E stain x 200); (b, c) Non-treated group showing congestion of renal blood vessels(CBV). Note also vacuolation of endothelial lining the glomerular tuft(VG), necrosis of the lining epithelium of some renal tubules(N) also note eosinophilic casts in the lumen of some renal tubules(E)(H&E stain x 400); (d, e) Indomethacin (10mg/kg) treated group for seven days showed congestion of renal blood vessels(C), extensive fibrous connective tissue proliferation of the renal cortex also, necrosis of endothelial lining of glomerular tuft(N) (H&E stain x 200); (f) Rutin treated group (20mg/kg) for seven days showed mild congestion of renal blood vessels (CBV)with inter-tubular hemorrhage. Also note congestion of the capillary of glomerular tuff(CC) (H&E stain x 200).

4. DISCUSSION

In the previous few years, much active new anti-inflammatory drugs (Celecoxib, Rofeoxib and Valdecoxib etc.) have been introduced in the market but unfortunately most of them were responsible for various side effects, especially in highly sensitive patients like newborn babies, pregnant and elderly people (Shaikh, 2012). Therefore, besides excellent anti-inflammatory activity the Coxib forms have been withdrawn from the pharmaceutical market owing to their severe toxicity. Hence, there is an increase in thrust for developing new and novel anti-inflammatory remedies, not only in effectiveness but also regarding their safety. Therefore, scientists turn their attention to find alternatives to present day NSAIDs and to explore the material medical of unexplored potential traditional medicinal plants for safe and novel herbal remedy (Shaikh, 2012).

The induction of granulomas by subcutaneous insertion of a cotton pellet is widely used to study the proliferative phases of chronic inflammation. The results of present study showed significant improvement by lowered of weight of cotton pellet implant compared with non-treated control which percent of inhibition for indomethacin (10 mg/kg) and rutin (20 mg/kg) were 37% and 33% respectively. This reduction in inflammatory granuloma tissue may be due to both the ability of rutin to reduce the number of fibroblasts and the synthesis of collagen and mucopolysaccharides, which are natural proliferative agents involved in the formation of granuloma tissue. Some studies have already demonstrated the presence of various flavonoids like quercetin, hesperidin, luteolin and tannins are responsible for significant antinociceptive, and/or anti-inflammatory activities (Moniruzzaman and Imam, 2014).

Measurements of the effective of urea, uric acid and total protein have indicated a significant test for the measurement of kidney function (Vicentini *et al.,* 2011). The present study showed significant decrease in urea, uric acid, and total protein by administration of flavonoid (Rutin). Our pathological studies confirmed these results; the kidneys of pigs treated with Indomethacin and selected flavonoids showed moderate congestion of the renal blood vessels and intertubular blood capillaries. The glomeruli showed congestion of the glomerular tuft with vacuolation of the glomerular endothelium was seen.

The results of present study showed that administration of Indomethacin 10mg/kg lowered the levels of triglycerides, total cholesterol and LDL levels. Also, administration of rutin 20mg/kg lowered triglyceride and total cholesterol levels compared to control. The decrease in total triglyceride, cholesterol and LDL levels of pig treated with flavonoid may be due to the protective role of flavonoids to prevent oxidation of the hormone sensitive lipase which regulate lipid and cholesterol metabolism (Wan *et al.,* 2014).

Histopathological examination exhibit to be a very important parameter and is definitive in determining cellular alteration that may happen in target organs (Vicentini *et al.,* 2011). Our histopathological examination showed congestion of the renal blood vessels in indomethacin (10 mg/kg) treated group, while with rutin (20 mg/kg) showed mild congestion of renal blood vessels with inter-tubular hemorrhage also congestion of the capillary of glomerular tuff. Rats treated with quercetin detect whole protection of histopathological changes. There showed apoptosis, focal degenerative changes in proximal tubules without vacuoles (Ilic *et al.,* 2014).

5. CONCLUSION

Traditional medicines as natural therapeutic remedies have been used in all over the world for thousands of years, and its widely accepted that multiple constituents are responsible for their efficacy. This experimental result indicated that Rutin has anti-inflammatory effects, and usage is safe**.**

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