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Clinicopathological evaluation of anti-inflammatory and hepatoprotective effects of rutin in guinea pigs

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ABSTRACT

This study was carried out to evaluate the anti-inflammatory activity of flavonoid (Rutin 20 mg/kg) on each (carrageenan-induced paw edema acute inflammation and (cotton pellet granuloma) chronic inflammation and, effect of rutin on some hematological, biochemical, and histopathological examination in guinea pigs induced by inflammation. A total of eighteen male guinea pigs were categorized into three groups, six pigs on all group. Group one as non-treated control, they given 5% carboxymethyl cellulose orally for seven days. Group two received single dose orally (20 mg/kg) Rutin for seven days. Group three received (10 mg/kg) Indomethacin orally for seven days. By injecting of 0.1 ml of carrageenan suspension in saline into the sub-planter superficies of the right hind paw, the acute inflammation (edema) was induced in all experiment guinea pigs one hour after compounds taken. Then estimated the volume of paw edema immediately after carrageenan injection through one, two, three and four hours by using a plethysmograph system (VGO Basile, Italy). The results indicated that, rutin (20 mg/kg body weight) showed significant lowered ($P < 0.05$) in the carrageenan induced paw edema and cotton pellet induced granuloma model comparing with control and indomethacin groups. In addition, rutin (20 mg/kg) improved the hematological and biochemical profiles and able of maintain the biochemical varies related to inflammation. This was also supported by histopathological studies of liver sections. On conclusion, rutin possess anti-inflammatory activity. Thus, the rutin showed protective role against acute and chronic inflammation.

1. INTRODUCTION

The inflammation expression derived from the term "inflammare". Inflammatory response is type of the greater significant step related to the protection of body versus infections; While it advanced to hurtful disease require medicine therapy (Laup attarakasem *et al.*, 2003). The disease that cause of inflammation like asthma, hepatitis, colitis and rheumatoid arthritis, cause of lethal and deficiency in the universe (Emery, 2006). Also, the chronic inflammation cause of evolution of different of infection involved tumor, neurodegenerative, cardiovascular defects (Willerson and Ridker, 2004).

Inflammation defense is define as series of various efficient technicality consisting of definitive cellular, humoral, and vascular steps which are qualified by the activity of liquid, inflammatory cells (macrophages, eosinophil, and neutrophils) and plasma to the location of infection (Hou *et al.*, 2004). A variety inflammatory mediators like leukotrienes, serotonin, histamine, oxygen- and nitrogen-derived free radicals and prostaglandins that created by infected and macrophage cells mainly in the series which share in starting of inflammatory response (Safayhi and Sailer, 1997).

Inflammation defense exists by 2 steps: chronic and acute, each is appearing interfered by a variety technique. The essential signs of acute inflammation are heat, redness, pain, swelling and tumor (Nathan, 2002).

The second inflammation is chronic is characterized via aspirate of white blood cells mainly macrophages and lymphocytes also, increase of fibroblasts and collagen fibers (Suleyman *et al.*, 2004).

Flavonoid product is originated from the polyphenolic secondary metabolites or flavus which searched out by the Hungarian scientist Albert Szent-Gyorgi in 1938 from a reaction between fresh vitamin C. It is related to a group of herbal matter that occur in large group of higher herbal species, fundamentally occur in parts of plant as seeds, flowers fruit or bark (Murray, 1989).

Flavonoids have important characteristics in improvement of people health and prevention them from all infections. Also, it has significant role in protect and inhibit pathogen and inhibit the function of inflammation. However newly it has start to be discovered to known the techniques reliable for employ of flavonoids as anti-inflammatory medicine (Nijveldt *et al.*, 2001). It is responsible of pigment of plant leaves, fruit, flowers, also inhibitors for enzyme, harbinger of poison substance, block against blue and UV rays exposure and infection (McGettigan and Henry, 2000).

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Flavonoids play role in contributory in energy carry, appealing pollinators; share to appealing the prey, photosynthesis, breathing process, gene code action and regulate of plant growth hormones (Zaat *et al.*, 1987). The better feature of each class of natural flavonoids have capability to appear anti-inflammatory in vivo and in vitro. So, it organized the inflammatory reply by get out of pro-inflammatory mediators, suppression of synthesis, suppression of immune cell and alteration of eicosanoid production (Kim *et al.*, 2004).

In the plants, greater than 8000 Flavonoids have been discovered as phenyl benzopyrones materials that possess same structure (Nijveldt *et al.*, 2001). Flavonoids are branched into four types according to molecular framework: anthocyanin's, flavanols, flavanones, flavones each have the similar basic flavan base framework replacement on the ring of C (Sugita *et al.*, 2004).

Rutin is a flavonoid with a chemical structure (3,3',4',5,7-pentahydroxyflavone-3-rhamnoglucoside) of the flavonol-kind that is common in the plant kingdom (Hosseinzadeh and Nassiri, 2014). Rutin is synthesized during the phenylpropanoid metabolic pathway, which includes the transformation of the amino acid phenylalanine to 4-coumaroyl-CoA. 4-coumaroyl-CoA can be related to malonyl-CoA to produce the true basis of flavonoids. The biosynthetic pathway continues through a chains of enzymatic changes to produce rutin (Li *et al.*, 2014). Rutin is a better type of the natural antioxidants in the plant. It is formed in some plants and present in *Morus alba*, *Ruta graveolens*, in citrus fruits. Also, Rutin found in vegetables and medicinal herbs like buckwheat. Buckwheat (*Fagopyrum esculentum*) is the origin of rutin. Rutin involve different pharmacological activities like antitumor, antioxidant, antibacterial, antiprotozoal, antiallergic, anti-inflammatory, antiplatelet, antiviral, vasoactive, hypolipidemic, antihypertensive and antispasmodic. Also, it used as an active substance in different plant medicines, the cosmetic, in animal feed, multivitamin preparations and chemical industries (Naif *et al.*, 2015).

NSAIDs are well recognized for cause opposite medicine effects so, there is more study to elevate active medicines treatment for disorder of inflammation. So, the present study was carried to estimate the effective of Rutin as anti-inflammatory agent in the animal model as well as estimate the effect of Rutin on biochemical, hematological parameters and the hepatic parenchyma.

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 and carboxymethyl cellulose 5% as a suspending agent for flavonoid were purchased from Himedia Chemical Co. India. Hematoxylin and Eosin were obtained from commercial sources.

2.2. Pilot Study

In this study 30 guinea pigs were categorized into five groups each consisted of six pigs, and they were treated with rutin by different doses: 10, 20, 40 and 60 mg/kg. Acute inflammation was induced by injecting 0.1 ml of carrageenan into the sub-planter superficies of the right hind paw one hour after Rutin taken. Then determined the volume of paw edema by using Plethysmograph apparatus according to Vogel (1988). The consecutive readings were

taken per hour for a total of 4 hrs. From the study it was revealed that the great dose of plant extract was 20 mg /kg.

2.3. Experimental animals

The experiment male guinea pigs were gained from the animal house of Biology Department, Ibb University-Yemen weighing between (350-650g) and conserved for one week in environmentally controlled conditions (25±5 °C, 55±5 % humidity and 12 hrs 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.

2.4. Carrageenan induced rat paw edema model.

In this experiment 18 male guinea pigs were categorized into three groups, six pigs on each. Group one non-treated control, guinea pigs were given 5% carboxymethyl cellulose. In group two animals were received single dose (20mg/kg) of Rutin orally. Group three received (10mg/kg) of indomethacin orally dissolved in 5% Polysorbate 80 (Tween 80) which prepared directly before use according to Clelia *et al.* (1999).

By injecting of 0.1 ml of carrageenan suspension in saline into the sub-planter superficies of the right hind paw, the acute inflammation (edema) was induced in all experiment pigs one hour after compounds taken. Then estimated the volume of paw edema immediately after carrageenan injection through one, two, three and four hours by using a plethysmograph system (VGO Basile, Italy) then compared with the volume of the right hind paw before edema induced. The percentage inhibition of inflammatory effect of the flavonoids was calculated using the formula:

Anti-inflammation activity (%) = [(Vc-Vt)/Vc] × 100

where:

Vc = The average paw edema volume in the control group.

Vt = The average paw edema volume in the flavonoid-treated, Indomethacin-treated pigs at the same time.

2.5. Cotton Pellet-Induced Granuloma

Twenty-four male guinea pigs which categorized into 4 groups, six pigs in each. Group one as a normal control given (2 ml/kg) distilled water for seven days. Second group as non-treated control were given 5% orally carboxymethyl cellulose. Third group of animals was received with Rutin (20 mg/kg). The fourth group were given aqueous suspensions by Indomethacin (10 mg/kg) dissolved in 5% Polysorbate 80 orally once daily for 7 days from the 1st day of implantation cotton pellet for each animal (Clelia *et al.*, 1999). Then for model of chronic inflammation, each pig were anesthetized by light ether and after thirty minutes each pig was implanted a sterile cotton pellet weighing (50 ± 1 mg) under the skin on both sides for the scapular region according to Swingle and Shideman (1972). All pigs were anaesthetized by using chloroform on the 8th day then the blood collected. Then the blood sample putted in 2 tubes, one containing heparin to stop clotting while second does not contain heparin to allow the blood clot at the temperature of room for thirty minutes. Then to get the serum, blood was centrifuged at 3000 rpm for 15 minutes. The serum samples were collected and saved them at -24 °C to biochemical analysis. Small liver tissue specimens were fixed in 10% formalin for histological examination. Then isolated the cotton pellets from each, dried at 60 °C, and estimated the dry weight. The higher weight of cotton pellet was indicated inflammation as anti-inflammatory response (Sedgwick and Willoughby, 1989).

2.6. Determination of hematological parameters

Packed Cell Volume (PCV), Hemoglobin (Hb), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC), White Blood Cells Count (WBC), Red Blood Cells Count (RBC), Differential Counts of White Blood Cells (Neutrophils, Eosinophil's, Basophils, Lymphocyte, and Monocyte) and Platelets were analyzed by Sysmex GMBH, Bormbarch 1, 22848 Norderstedt, Germany, (2009).

2.7. Determination of biochemical parameters

The following parameters were determined: liver enzymes (AST, ALT, ALP) and Total bilirubin by Roche Kits (Germany) according to Tietz *et al.* (1995).

2.8. Histopathological studies

Experimental animals were put under chloroform anesthesia, they were dissected and then for kidney was isolate. Then S fixed in 10% neutral formalin for 24 hrs, washed by the running tap water, dehydrated in different grades of ethyl alcohols, cleared in xylol, embedded in paraffin, cut by using rotary microtome (Leica, Germany)

in thickness 5 microns. then slides were dried in the incubator at 37 °C and stained with Hematoxylin and Eosin according to Prophet *et al.* (1992).

2.9. Statistical analysis

SPSS (version 20) was used for the statistical analysis and the result data was presented as mean \pm SD. Significant difference between groups was analyzed by student *t* test and One way ANOVA. The test is significant at α 5%.

3. RESULTS

3.1. Anti-Inflammatory studies

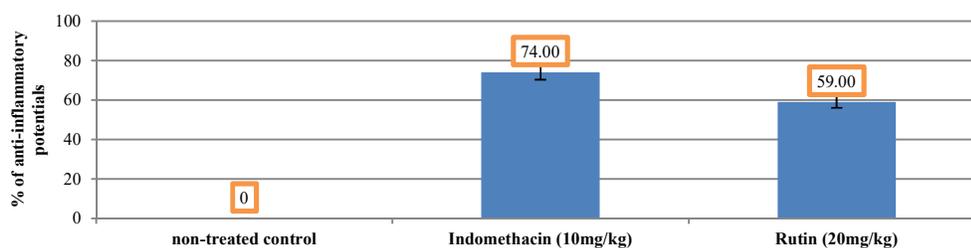
3.1.1. Carrageenan induced rat paw edema.

In this study, Indomethacin 10 mg/kg treated group showed significantly ($P < 0.05$) lowered paw edema which were 65%, 71%, 72% and 74% after one, two, three and four hrs. Rutin treated group at 20 mg/kg/ body weight showed significantly ($P < 0.05$) lowered paw volume edema which was 32%, 48%, 57% and 59 % after one, two, three and four hours, respectively when compared with non-treated control group values (Table 1).

Table 1 Effect of Rutin versus Indomethacin on carrageenan induced edema paw volume in male guinea pig.

Groups	Mean paw edema (cm)				% Percent inhibition at 4 hours
	1hr.	2hr.	3hr.	4hr.	
Non-treated control	0.620 \pm 0.008	0.850 \pm 0.005	1.160 \pm 0.009	1.170 \pm 0.006	0.0 %
Indomethacin (10 mg /kg)	0.220 \pm 0.008 ^a	0.250 \pm 0.005 *	0.330 \pm 0.009 *	0.300 \pm 0.006 *	74.0 %
Rutin (20 mg/ kg)	0.420 \pm 0.005 ^a	0.440 \pm 0.005 *	0.500 \pm 0.004 *	0.480 \pm 0.007 *	59.0 %

Values are expressed as mean \pm SD, n=6 animals per group, * $P < 0.05$ as compared to control (One-Way ANOVA).



Graph 1 Percentage of anti-inflammatory potentials (%) with rutin versus indomethacin in the carrageenan induced paw edema.

3.1.2. Cotton pellet induced granuloma:

In the present study, percentage of inhibition were significantly ($P < 0.05$) decreased in rutin (20 mg/kg) which was 33% when compared with non-treated control 0% and (10 mg/kg) indomethacin 37% (Table 2, Graph 2).

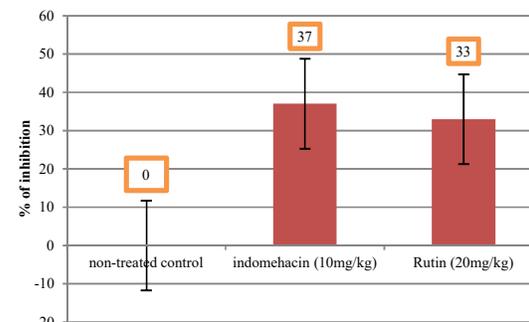
3.2. Effect of rutin on hematological parameters in guinea pigs induced by inflammation.

Table 3 showed that, after indomethacin treatment, neutrophils, eosinophils, monocyte percentages and MCHC were significantly decreased however, platelets counts, PCV, Hb, MCV and lymphocyte percentage showed significant increased. It was found that slightly significantly increased in PCV, RBC, MCH, platelet counts and lymphocyte percentage while the values of MCHC, neutrophils, eosinophils and monocyte percentages were significantly decreased in Rutin treated pigs compared to non-treated control group.

Table 2 Anti-inflammatory activity of Rutin versus Indomethacin on cotton pellet granuloma in normal male guinea pigs

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

Results are Mean \pm SD (n=6) * $P < 0.05$ compared to control.



Graph 2 Anti-inflammatory activity of Rutin versus Indomethacin on cotton pellet granuloma normal male guinea pigs.

3.3. Effect of rutin on hepatic biochemical parameters in guinea pigs induced by inflammation:

After Indomethacin treatment liver enzymes ALT, AST were significantly decreased. However, ALP level showed non-significantly increased compared to non-treated control group. It was found that, ALT, AST were significantly decreased while the values of ALP and Total bilirubin showed non-significantly increased in Rutin treated groups compared to non-treated control group.

Table 3 Effect of rutin and indomethacin on hematological parameters in control and treated groups.

Parameters	Units	Normal control	Non-treated control	Indomethacin (10mg/kg)	Rutin (20mg/kg)
Packed Cell Volume (PCV)	%	45.90 ± 0.009	39.77 ± 0.009	45.43 ± 0.009*	43.27±0.004*
Red Blood cells Count (R.B.C.)	x10 ⁶ mm ³	5.38 ± 0.355	3.95 ± 0.009 #	5.37 ± 0.009*	5.27±0.334*
Hemoglobin (Hb)	g./dl	15.40 ± 0.009	12.13 ± 0.009 #	14.99 ± 0.005*	14.23±0.590
Mean Corpuscular Volume (MCV)	fl.	83.93 ± 2.37	79.57 ± 0.009 #	82.50 ± 0.881*	82.27±0.004
MCH	pg	25.82 ± 0.508	24.20 ± 0.009 #	25.33 ± 0.273*	25.01±0.004***
MCHC	g/l	30.75 ± 0.596	37.73 ± 0.009 #	30.80 ± 0.009*	31.79±0.004*
Platelets Count	g./dl	399.83±59.189	214.33 ± 93.904 #	372.67 ± 0.009*	352.67±0.004***
White Blood Cells (W.B.C.)	x10 ³ mm ³	10.82 ± 2.46	6.37 ± 3.28 ####	8.40 ± 2.79	7.97±1.729
Differential count:					
Neutrophil	%	43.00 ± 0.894	51.00 ± 0.894 #	44.33 ± 1.211*	43.00±0.365*
Eosinophil's	%	1.00 ± 1.265	8.00 ± 0.894#	1.00 ± 1.265*	2.00±0.365*
Basophils	%	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00
Lymphocyte	%	55.00 ± 0.894	34.00 ± 0.894	54.00 ± 0.894*	53.00±0.365*
Monocyte	%	1.00 ± 1.265	7.00 ± 0.894 #	1.00 ± 1.265*	2.00±0.365*

Each value represents mean± SD of 6 animals. # P < 0.05, #### P < 0.001 compared with normal control value. * P < 0.05, *** P < 0.001 compared with non-treated control values respectively.

Table 4 Effect of Rutin and Indomethacin on biochemical parameters in control and treated groups.

Parameters	Unit	Normal control	Non-treated control	Indomethacin (10mg/kg)	Rutin (20mg/kg)
AST	U/L	173.67±32.167	269.17±0.401 [#]	190.00±0.365*	188.00±0.365*
ALT	U/L	108.17±19.884	143.67±0.211 ^{###}	120.00±0.365*	115.00±0.365*
ALP	U/L	128.67±20.786	57.00±2.408 [#]	105.67±7.121 ^{**}	75.00±1.461
Total Bilirubin	mg/dl	0.17±0.048	0.04±0.007 [#]	0.09±0.004	0.08±0.007

Each value represents mean± SD of 6 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 liver in cotton pellet induced granuloma in adult male guinea pigs:

The microscopic examination of the liver of guinea pigs in control group showed microscopic changes. The hepatocytes appeared as polygonal cells with rounded nuclei and there are a few spaced hepatic sinusoids with fine arrangement of Kupffer cells. Also, it appears normal lobular architect with hepatocyte arranged in cords encircling the central canal (Figure 1a).

After seven days the microscopical findings in non-treated control "cotton pellet induced granuloma" were represented by sever congestion of the central veins and sinusoids. Moreover, the portal blood vessels showed severe congestion with perivascular mononuclear cellular infiltration. Hyperplasia of bile ductal epithelium with the presence of eosinophilic granular substances in the ductal lumen was seen. Periductal fibrous connective tissue was

also found. Hepatocellular degenerative changes in the form of vacuolar and hydropic degeneration or even fatty changes were detected (Figures 1b and c).

The microscopic examination of the liver in groups treated with indomethacin (10 mg/kg) revealed severe congestion of the portal blood vessels and dilatation of the hepatic sinusoids. The hepatocytes showed degenerative changes in the form of vacuolar or fatty changes. The portal areas showed hyperplasia of the bile duct. The lumen of the affected bile ducts showing degeneration alteration of the lining epithelium with the presence of few amounts of eosinophilic substances in the ductal lumens (Figure 1d).

The examined liver of guinea pig treated with rutin (20 mg/kg) showed mild congestion and dilatation of the central veins with mild hyperplasic of the epithelial cells lining the bile ducts (Figures 1e & f).

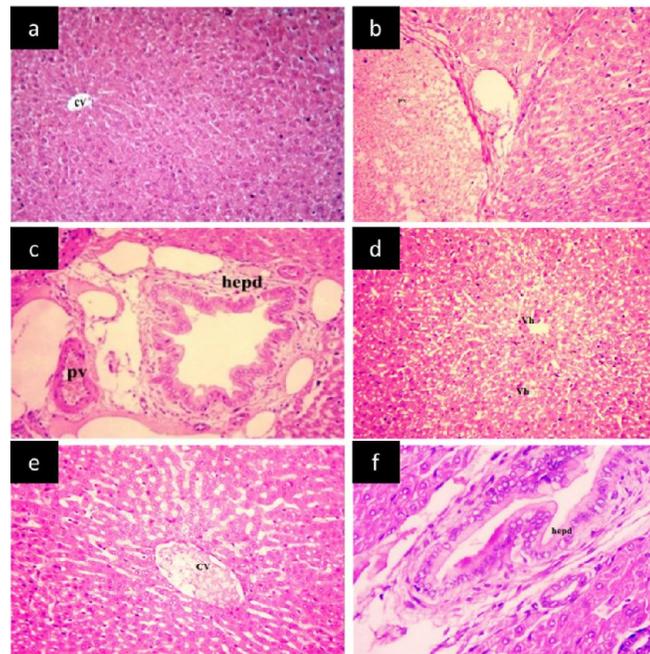


Fig. 1 Histopathological study of liver tissue in control, non-treated, Indomethacin and Rutin treated group of guinea pigs. (a) Control group given (2 ml/kg)distilled water for seven days showed normal liver architecture, the hepatocytes appeared as polygonal cells with rounded nuclei and there is a few spaced hepatic sinusoids with fine arrangement of Kupffer cells (H&E ×200). (b, c) Non-treated group showed congestion, dilatation of portal vessel (pv) and hyperplasia of the lining epithelium of the bile duct (hepd). (d) Indomethacin (10 mg/kg) treated group for seven days showed vacuolation of hepatocytes (Vh) (H&E stain ×100). (c, f) Rutin treated group (20 mg/kg) for seven days showed congestion, dilatation of central vein (cv), hyperplasia of the lining epithelium of the bile ducts in association with newly formed bile duct (hepd) (H&E stain ×200, 400).

4. DISCUSSION

Carrageenan-induced inflammation is common method to assess the anti-inflammatory activity of natural materials (Winter *et al.*, 1962). The possible technique of carrageenan to induced inflammation via two steps; one step is distinguished by the synthesis of serotonin, kinins and histamine; the two step is distinguished by formation of prostaglandins, lysosome enzymes (Brooks and Day, 2014). The second step is effective to varies anti-inflammatory chemicals (Vinegar *et al.*, 1969). The data of current experiment showed that Flavanol (Rutin) has good $P < 0.05$ anti-inflammatory effect compared to Indomethacin 10 mg/kg. The mechanism Flavonoids as anti-inflammatory activity is the inhibit prostaglandins formation and the enzymes in the arachidonic acid pathway (Havsteen, 2002). Rutin showed good anti-inflammatory effect (Okwu and Omodamino, 2005). Indomethacin inhibit the enzymes I and II cyclooxygenase which are require in the formation of inflammation mediating chemicals like prostaglandin E2 (PGE2) from arachidonic acid (Moody *et al.*, 2006).

To estimate the proliferative, exudative and transudate of chronic inflammation cotton pellet is great used to induce granuloma. The wet weight of the granuloma was influenced by the absorption of the surrounding fluid by the cotton pellet (Raju *et al.*, 2005). Natural compound as Flavonoids have ability to reduce the improvement of cotton pellet induced granuloma that used as a model of proliferative agent of inflammation. The results of present study showed significant improvement by lowered of weight of implant compared with control non-treated cotton pellet implanted animals which percent of inhibition for indomethacin (10 mg/kg) and rutin (20 mg/kg) were 37% and 33% respectively. The result of the present work indicated that the flavonoid (Rutin) in the chronic inflammation phase inhibit the formation of mucopolysaccharides, collagen and increase of fibroblasts via granuloma tissue formation. The flavonoids possess anti-inflammatory activity due to of COX-2 enzyme suppression, these effect because of the cellular immigration to infection location and aggregation of collagen (Guardia *et al.*, 2001).

The hematological parameters act as an important factor in the estimate of harmful influence of chemicals and medicinal plants (Yakubu *et al.*, 2007). Flavonoids can inhibit the formation of superoxide ions and hydroxy radicals, which are both strong peroxidation factors that are formed in the body in typical situation and caused devastation of cells (Esmaili and Sonboli, 2010). As well, flavonoids protect both the stem cells and the hematopoietic committed produced blood cells during the attack of the reactive free radicals. Moreover, the anti-inflammatory potent of flavonoids may keep the haeme iron in its ferrous case and this could promote erythropoiesis process. The increase in platelet may be due to stimulatory effect on thrombopoietin (Xia *et al.*, 1999).

The present work showed non-significant increase in white blood cells count in rutin 20 mg/kg and indomethacin 10mg/kg treated groups comparing with control group. Neutrophils originate free radical is known to due to cytokines produced by neutrophils also responsible for inflammation. The decrease in neutrophil returned to the presence of neutrophils in the area of inflammation for defense mechanisms. Eosinophil stimulate factor contribute to pro inflammatory functions (John and Shobana, 2012). In the present investigation, there was a significant decrease in the eosinophil's that indicated the significant recovery from the inflammatory process. Indomethacin administration

normalized cotton pellet induced in monocytes count probably due to its role in promotion of macrophage migration (Furst and Munster, 2001). The above-mentioned result was in agree with the result of Selvakumar *et al.*, (2013), who reported that, the values of PCV, RBC, Hb, MCH, PLT, WBC and lymphocyte, were significantly higher after administration of Quercetin compared with control group. Ekeanyanwu and Njoku, (2014) observed that, the flavonoid in the seed of *Monodora tenuifolia* extract were elevated ($P < 0.05$) significantly the hematocrit and Hb level. Also, there was non-significantly increases in RBC, mean corpuscular volume (MCV), MCH concentrations, total WBC, lymphocyte counts, and ALP level. However, it produced non-significant ($P > 0.05$) decrease in (MCHC), monocyte count, neutrophil count, AST, ALT levels. There was no ($P > 0.05$) significant effect on basophils in rutin groups.

Estimation of liver enzymes as serum AST, ALP and ALT have indicated a significant test for the measurement of liver function (Aliyu *et al.*, 2006). The present study showed significantly decreased in AST, ALT by flavonoid (Rutin) is a reference of stabilization of the safety of the cell envelope of the liver cells, preservation the envelope normal. These findings were represented by a mild histopathological change in the liver of pigs after seven days from infection, as a mild inflammation process together with mild congestion of portal blood vessels and mild desquamation of epithelial cells lining the bile ducts. Increase in serum bilirubin concentration was also recorded in the present investigation is a result of bile ductal obstruction that recorded in the microscopic examination of the liver of guinea pig.

In the present study, the histopathological examination showed severe congestion of the portal blood vessels in Indomethacin (10 mg/kg) treated group, while treated group with Rutin (20 mg/kg) showed mild congestion of the portal blood vessels and mild hyperplasia of the epithelial cell lining the bile ducts. The hepatocytes showed degenerative changes in the form of vacuolar or fatty changes in the cytoplasm of hepatocytes. The lumen of the bile ducts showed mild disintegration alteration of the lining epithelium with the presence of few amounts of eosinophilic substances in the ductal lumens. Treatment with Rutin considerably reduced these pathologic changes (Wan *et al.*, 2014).

5. CONCLUSION

Rutin (20 mg/kg) reported high significantly ($P < 0.05$) anti-inflammatory protective against acute and chronic inflammation by improved the biochemical and hematological disorders related to inflammation. The present work showed that Rutin is great safe hence, it can used as a ready reference for the standardization of flavonoids for transformed it to curative types.

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