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AMELIORATIVE ROLE OF QUERCETIN ON INTESTINAL HITOMORPOMETRIC, OXIDATIVE STATUS AND PRO-INFLAMMATORY CHANGES IN HYDROGEN PEROXIDE–EXPOSED RATS

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ABSTRACT

This study was aimed to explore the antioxidant and anti-inflammatory role of quercetin (QCT) in hydrogen peroxide (H_2O_2) treated rats. Forty (40) adult male rats were randomly divided into four groups (10 ratseach) and were handled daily using gastric gavage for 30 days: Control group(C) in this group the rats were received ordinary tap water administered the vehicle only (norm al saline), H_2O_2 group(T1) The rats in this group were administered orally0.5 ml of hydrogen peroxide (H_2O_2) and given water containing 1% of H_2O_2 along experiment period (one month); H_2O_2 and Quercetin (T2) group: the animals in this group were administered orally 0.5 ml of hydrogen peroxide (H_2O_2) and given water containing one percent of H_2O_2 for 15 days followed by oral administration of Quercetin (20 mg\kg B.W) for another 15 days; Mixed (T3): group the rats were given QCT plus 1% of H_2O_2 in drinking water in the same previous doses for one month. Blood sample were collected by cardiac puncture technique at the end of the experiment and serum were collected for estimation the concentration of tumor necrosis factor –alpha (TNF- **α**), intrleukin-10 (IL-10) and total antioxidant capacity (TOAC). After animal scarifying, sections from small and large intestine were taken for estimation tissue reduced glutathione (GSH) and malondialdehyde (MDA) concentration. The tissue samples of intestine (Duodenum, jejunum, ileum and colon) were taken for Histomorphometric analysis (The villus height, thicknesses, depth of epithelial crypts and the number of goblet cell). The results showed that the oral intubation of quercetin for 15 days after H_2O_2 (group T2) or combination of quercetin and H_2O_2 for one month (groupT3) caused significant decrease in TNF-**α**, significant increase in the IL-10 at the end of experiment comparing to the value inT1 group and the value tend to normalize that of control group. A case of oxidative stress as explained by elevation in intestinal malondialdehyde (MDA) and depression in reduced glutathione (GSH) accompanied with depression in TAOC concentrations in H_2O_2 treated group comparing to QCT group which caused alleviation of pro-inflammatory and oxidative stress induced by H_2O_2. Histomorphometric analysis of intestine revealed significant elevation in depth, thickness of villi with elevation in villus height to crypt depth ratio that caused improvement in absorptive capacity of intestine, as well as elevation in number of goblet cell in some part of intestine were observed in QCT treatment groups (T2 and T3) comparing to H_2O_2 (T1)treated group. On conclusion, the current study documented, for first time, in vivo damaging effect of H_2O_2 on intestinal (oxidative status, Morphometric), in addition to its effect on anti-inflammatory status. The result also pointed to protective and preventive effect of quercetin.

Keywords **:** Quercetin, intestine, oxidative status, hydrogen peroxide, IL-10.

Introduction

Quercetin (QCT), a plant derived flavonoids is widely found in many food, mainly grains, fruit vegetable and tea (Boots *et al*., 2008) Quercetin have antioxidant, anti-inflammatory and anti-apoptotic properties, facilitated it use as nutritional supplement for animals (Mahdaviniae *et al*., 2019). Some of the beneficial effects of QCT include protection, anticancer and antiviral activities, in addition to its antidiabetic, gastroprotective, antihypertensive and immunomodulatory activity (Lakhanpal *et al*., 2007). Unique biological components of QCT contain potential physiological health benefit that include disease resistances, enhanced mental and physical performances and stimulation of mitochondrial biogenesis (Sun *et al*., 2020). In Addition to its anti-inflammatory and antioxidant activity (Cebecioglu *et al*., 2019). The possible role of QCT in normal intestinal physiology and in sever gastrointestinal (GIT) disorder including enteropathic damage induced by non-steroidal anti-inflammatory drugs (Singh *et al*., 2017a and b), as well as alleviation of increase intestinal permeability (Moura *et al*., 2015) and dextran sodium salts induced colitis (Dong *et al*., 2020). As others flavonoids, the antioxidant andanti-inflammatory properties of QCT is the major contributor to its protective effects (Zaragoza *et al*., 2020). Attributed to its antiviral and antioxidant effect, recent study pointed to the use of QCT as synergetic therapy for privation and treatment of COVID-19 (Manuer *et al.,* 2020).

At normal conditions, the reactive oxygen species (ROS) have been generated in most cells, which have an important role for cell defense, moreover, it has a key role in inflammatory response, involving cell proliferation, cell-fate signaling, expression and transcription of genes (Mittal *et al.,* 2014). In addition, ROS also play important pathologic role in many disease statuses through induction oxidative stress

that caused cell injury hence cell death (Kitiyanant *et al*., 2019). ROS generated in the body can be on several types, involving free radicals (e.g. superoxide and hydroxyl), and non-free radicals (e.g. hydrogen peroxide) (Forrester *et al*., 2018).

Excessive generation of ROS and H_2O_2 participate in many oxidative stress associated–diseased condition including cardiovascular disease (Ali and Khudair, 2019a), diabetes (AL-Lahhom *et al*., 2016), cancer (Okon and Zou, 2015), Alzheimer (Dumout and Flint, 2020), inflammatory disease (Chelomvitko, 2018), as well as hyperlipidemia and DNA damage (Ali and Khudair, 2019b).

Besides H_2O_2 and its related oxidative stress could be the major contributor to tissue injury including in vitro induced IBD (Moura *et al*., 2015; Patlevic *et al*., 2016). According to available literature, several studies concerning beneficial and damaging effect of H_2O_2 have been well studied in vitro. Yet very limited studies have concerned the detrimental effect of H_2O_2 *in vivo* concerning its effect on cardio vascular, hepatic, renal and reproductive system, likewise, it's deleterious effect on intestine and as pro-inflammatory mediator has not been elucidate.

Materials and Methods

This study has been conducted on 40 male adult Wistar albino rats (aged 12-14 weeks and weighted 200±10g). They were adopted after acclimatization (for two weeks) in the animal house of College of Veterinary Medicine- University of Baghdad, during the period extended from November, 2019 to December, 2019. They were housed in a well-ventilated room; feed on standard pellet diet and drinking water and libitum during the experiment. The room temperature was kept at 23±2ºC and 12 hrs. Light/ dark cycle along the experimental period.

After acclimatization twenty eight (28) adult male rat were randomly divided into four equal groups (7 for each) and were handled daily using gastric gavage for 30 days as follow : Control (C) group: The ratsin this group were served as controls and were received ordinary tap water and administered the vehicle (normal saline); H_2O_2(T1) group: Rats in this group were drenched orally 0.5 ml of hydrogen peroxide and given tap water containing 1% of H_2O_2 along experimental period; H_2O_2 and Quercetin (T2) treated group: Rats in this group were handled as in group T1 for15 days followed by oral administration of quercetin (20mg/kg B.W.) for another 15 days. Mixed group (T3): The rats in this group were received quercetin and H_2O_2 for one month in the same previous doses and method of administration.

At the end of experiments, rats were anesthetized by intramuscular injection of xylazine (40mg/kg B.W) and ketamine (90mg/kg B.W), then blood samples were collected via cardiac puncture technique (Para suraman *et al*., 2010) and serum samples were collected for measuring the concentrations of the following criteria using enzymatic kis, tumor necrosis alpha (Sunlong, China), interleukin -10 (Sunlong, China), total antioxidant capacity (Elabscience, USA).

Furthermore, tissue specimens of small and large intestine from the scarified animals were taken for estimation tissue reduced glutathione and malondialdehyde using enzymatic kit (Fine test, China). Besides, sections from whole intestine were taken for histomorphometric analysis according to (Bancroft and Marilyn, 2008). Statistical analysis of data was performed using SAS (Statistical Analysis System - version 9.1). One-way and two way ANOVA and Least significant differences (LSD) post hoc test were performed to assess significant differences among means. P<0.05 is considered statistically significant (Snedecor and Cochran, 1973).

Results

At the end of experiment, significant elevation (P < 0.05) in serum TNF- α concentration was observed in group T1 due to H_2O_2 for one month comparing to the value in T2, T3 and control group. On other hand, oral intubation of QCT for 15 days after H_2O_2 (group T2)or combination of QCT and H_2O_2 for one month (group T3) caused significant decrease (P < 0.05) comparing to the value inT1 group and the value tend to normalize that of control group. Within the time significant (P <0.05) decrease (T1) or increase (T2, T3) were observed at the end of experiment comparing to zero time figure (1).

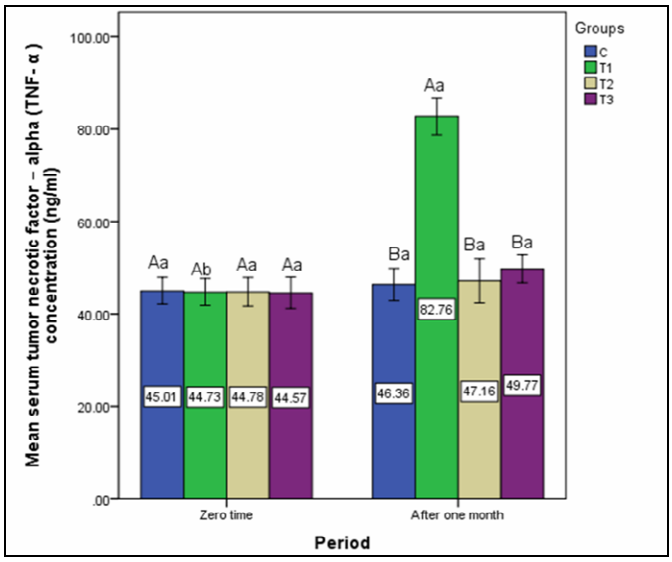

Fig. 1 : Effect of Hydrogenperoxid, Quercetin and \or Their Combination on Serum Tumor Necrotic Factor Alpha(TNF-α) Concentration (ng/ml) in Adult MaleRats

Values are expressed as means ± SE, n=7, C: control group. T1: Rats administered orally 1% H_2O_2 and received water containing 1% H_2O_2 for one month. T2: Rats administered orally 1% H_2O_2 and received water containing 1% H_2O_2 for 15 days, followed by administration of 20 mg/kg B.W. Quercetin for another 15 days.T3 rats were administered orally Quercetin concurrently with 1% H_2O_2 (in same manner and dose) for one month. Different small letters denoted significant differences (P<0.05) between groups. Different capital letters denoted significant differences (P<0.05) between periods

A significant decrease (P<0.05) in serum (IL- 10) concentration were observed in T1 (H_2O_2) group comparing to the values in control and other treated groups. Besides, oral administration of QCT after 15 days of H_2O_2 treatment in groupin (T2) group or combination of QCT and H_2O_2 (T3 group) showed significant (p < 0.05) elevation in this parameter comparing to control and T3 group (figure-2).

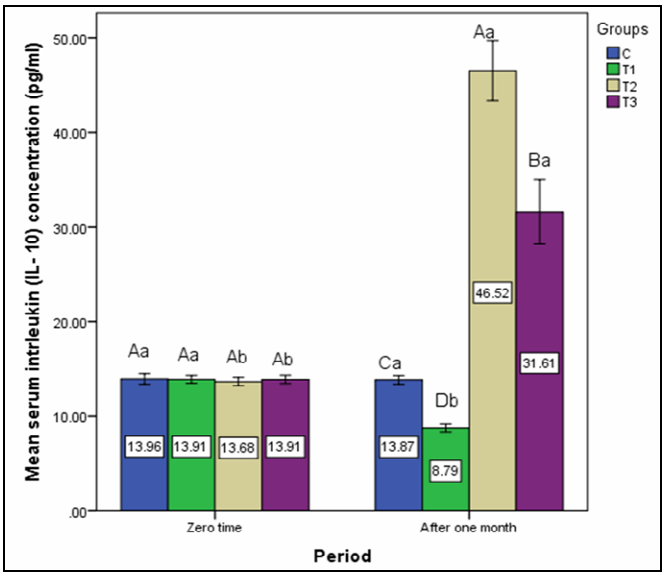

Fig. 2 : Effect of Hydrogen Peroxideand Quercetin and / or their Combination on Serum Intrleukin-10 (IL-10) Concentration (pg/ml) in Adult Male Rats.

Values are expressed as means ± SE, n=7, C: control group. T1: Rats administered orally 1% H_2O_2 and received water containing 1% H_2O_2 for one month. T2: Rats administered orally 1% H_2O_2 and received water containing 1% H_2O_2 for 15 days, followed by administration of 20 mg/kg B.W. Quercetin for another 15 days. T3 rats were administered orally Quercetin concurrently with 1% H_2O_2 (in same manner and dose) for one month. Different small letters denoted significant differences (P<0.05) between groups. Different capital letters denoted significant differences (P<0.05) between periods.

Significant (P< 0.05) decrease in TOAC concentration were observed in H2O2 (T1) treated group which received 1% H_2O_2 for one month comparing to control, T2and T3 treated groups. On the contrary, the protective and preventive role of QCT were observed in T2, T3 after oral intubation of QCT after 15 days of H_2O_2 manipulation (T2 group) or concurrently with H_2O_2 (T3group), where significant (P< 0.05)elevation in TOAC concentration in these groups were observed comparing to H_2O_2 (T1) control group (Figure-3).

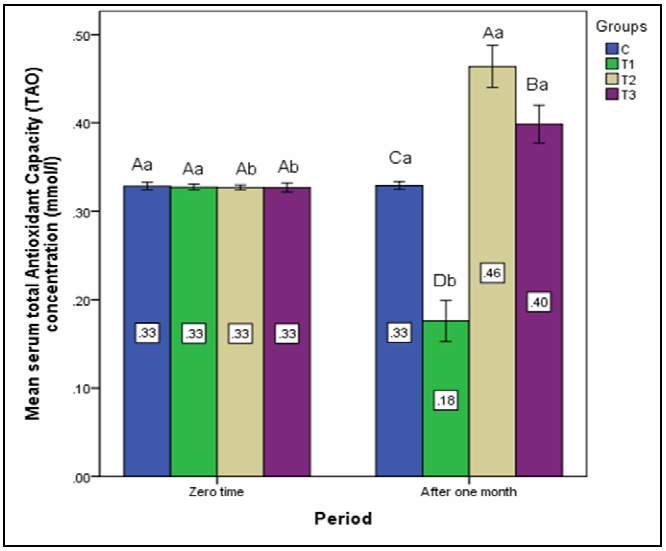

Fig. 3 : Effect of Hydrogen Peroxide and Quercetin and / or their Combination on Serum Total anti-oxidant Capacity (TOAC)concentration (m mol/l) in Adult Male Rats.

Values are expressed as means ± SE, n=7, C: control group. T1: Rats administered orally 1% H_2O_2 and received water containing 1% H_2O_2 for one month. T2: Rats administered orally 1% H_2O_2 and received water containing 1% H_2O_2 for 15 days, followed by administration of 20 mg/kg B.W. Quercetin for another 15 days. T3 rats were administered orally Quercetin concurrently with 1% H_2O_2 (in same manner and dose) for one month. Different small letters denoted significant differences (P<0.05) between groups. Different capital letters denoted significant differences (P<0.05) between periods

Significant decrease (P < 0.05) in intestinal GSH concentration were observed after one month of treatment with 1% hydrogen peroxides figure (4) comparing to the values in control and T3.On the contrary, significant elevation (P< 0.05) in this parameter was observed after oral intubation of QCT for 15 days after H_2O_2 manipulation (T2 group), or concurrently with H_2O_2 for one month (T3 group) comparing to the value of T1 and control groups. Significant differences (P < 0.05) between T2 and T3 were also observed.

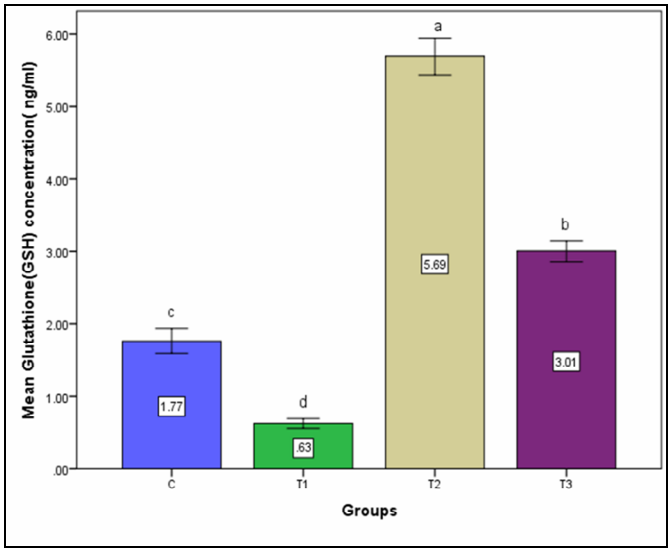

Fig. 4 : Effect of Hydrogen Peroxideand Quercetin and / or their Combinationon Intestinal tissue Reduced Glutathione (GSH). Concentration (ng/ml) In Adult Male Rats.

Values are expressed as means ± SE, n=7, C: control group. T1: Rats administered orally 1% H_2O_2 and received water containing 1% H_2O_2 for one month. T2: Rats administered orally 1% H_2O_2 and received water containing 1% H_2O_2 for 15 days, followed by administration of 20 mg/kg B.W. Quercetin for another 15 days. T3 rats were administered orally Quercetin concurrently with 1% H_2O_2 (in same manner and dose) for one month. Different small letters denoted significant differences (P<0.05) between groups.

The data in figure (5) showed a significant (P <0.05) increase in MDA concentration in H_2O_2 (T1) treated group comparing to the values in T2, T3 and control. Significant decrease (P <0.05) in MDA concentration were observed in groups T2, after intubation of QCT for 15 days comparing to the valuein other treated groups.

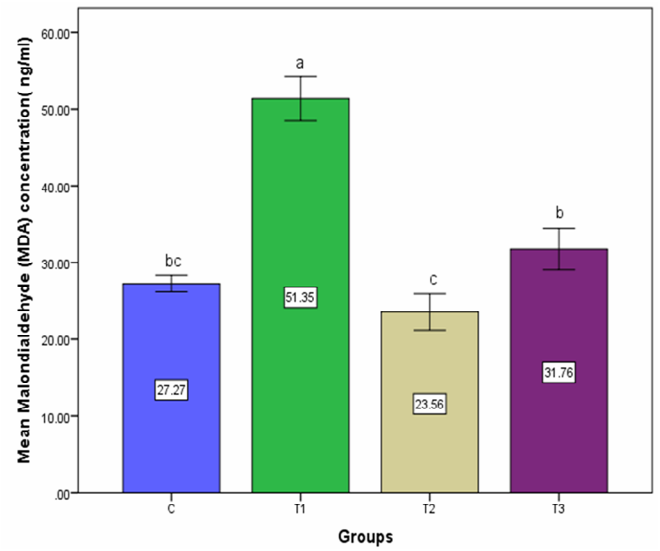

Fig. 5 : Effect of Hydrogen Peroxide and Quercetin and / or their Combination on Intestinal tissue Malondialdeyde (MDA) concentration (ng/ml) In Adult Male Rats.

Values are expressed as means ± SE, n=7, C: control group. T1: Rats administered orally 1% H_2O_2 and received water containing 1% H_2O_2 for one month. T2: Rats administered orally 1% H_2O_2 and received water containing 1% H_2O_2 for 15 days, followed by administration of 20 mg/kg B.W. Quercetin for another 15 days. T3 rats were administered orally Quercetin concurrently with 1% H_2O_2 (in same manner and dose) for one month. Different small letters denoted significant differences (P<0.05) between groups.

Effect of Hydrogen Peroxide and Quercetin and / or their Combination on Intestinal Morphometric Analysis in Adult Male Rats:

Table (1) illustrates different morphometric alterations in small and large intestine as following:

Duodenum

Significant elevation (P<0.05)in depth of crypt were observed in T2 AND T3 comparing to T1 (H_2O_2) group, and the value in T2 is near that of control, while highest significant elevation (P<0.05) in villi thickness (VT) were observed in T2 and T3 comparing to T1 and control, while significant (P<0.05) elevation in villus height (VH) were observed in T3 group comparing to the value in other groups. Besides, the value in T2 andT3 exceed significantly (P<0.05) that of T1(H_2O_2) group. Highest significant elevation in VH/CD ratio was observed in T2 and control comparing to T1and T3.

Jejunum

Significant (P<0.05) elevation in depth of crypt were observed in T2 group, comparing to other treated group, where T1(H_2O_2) treated group showed Significant (P<0.05) depression comparing to other groups. Thickness of villi (VT) showed Significant (P<0.05) elevation in T2 and T3 comparing toT1 group. Significant differences (P<0.05) between T2and T3 were also observed, highest significant elevation were observed in t2 and the mean value were near that of the control. Concerning VH, Significant (P<0.05) elevation in T2 and T3 were observed comparing to T1.Highest Significant (P<0.05) elevation were observed in T3 (H_2O_2 and Quercetin) group. Significant (P<0.05) elevation in VH\CD ratio were observed in T1 and T3 comparing to other groups.

Ileum

Significant elevation (P<0.05) in CD were observed in T2 and control group comparing to T3 and T1 group, where QCT intubation were given after 15 days of H_2O_2 exposure (T2), normalize the value to that of control. Significant elevation (P<0.05) in VT were observed in T2 group comparing value in T1 and T3 group. The result also showed that highest (P<0.05) Significant elevation in VH were observed in group T 2 and T3 (QCT groups).Comparing to H_2O_2 (T1) group. Comparing to control, T1 and T2 group, significant elevation in VH\CD ratio were observed in T3 group.

Colon

Significant (P<0.05) elevation in depth of crypt were observed in T2 and T3 comparing to T1 and control. Best result was clarified in T2 group. Besides, significant elevation (P<0.05) in crypt diameter were observed in T2 and T3 group comparing to T1.

Number of goblet cell indifferent parts of small and large intestine

In duodenum significant (P<0.05) elevation in goblet cell, were observed in T2 and control groups comparing to T1 and T3 group. While in jejunum, number of goblet cell showed significant elevation in groups of control, T2 and T3comparing to H_2O_2 (T1) group. The number of goblet cell in ileum showed non-Significant (P >0.05) differences in groups T1, T2 and T3 when compared to each other, and they showed significant decrease (P<0.05) as compared to control group. In colon, significant elevation in number of goblet cell were observed in T2 and T3 groups comparing to T1 and the values of these groups were near that of control (Table-1).

Table 1: Effect of Hydrogen Peroxide and Quercetin and / or their Combination on Intestinal Morphometric Analysis in Adult Male Rats:

Groups	Depth of crypt (CD) (µm)	Thickness of villi (TH) (µm)	Villus High (VH) (µm)	VH/CD ratio (%)
Duodenum				
C	89.02±4.50b	53.48±2.48b	262.35±6.59b	2.97±0.10a
T1	72.37±2.37c	35.94±1.15c	175.82±7.52d	2.44±0.13b
T2	89.51±2.59b	62.31±2.58a	231.07±5.27c	2.59±0.11b
T3	100.57±2.46a	63.08±3.41a	312.19±4.39a	3.11±0.05a
LSD	9.1766	7.4954	17.91	0.3068
Jejunum				
C	75.86±2.43b	55.38±3.22a	188.99±20.34bc	2.53±0.32bc
T1	55.73±3.99c	38.00±2.36c	170.06±5.28c	3.15±0.29ab
T2	101.66±3.03a	59.50±2.73a	213.61±6.95b	2.11±0.11c

T3	67.67±1.19b	46.92±1.59b	253.75±15.53a	3.75±0.22a
LSD	8.4088	7.5264	39.898	0.7483
Ileum				
C	94.03±1.42a	52.67±1.17a	189.41±3.74bc	2.01±0.04b
T1	81.04±3.77b	36.83±2.05c	179.18±3.29c	2.24±0.13b
T2	92.67±1.54a	45.25±2.41b	207.41±6.38ab	2.23±0.06b
T3	82.87±1.71b	38.66±2.10c	215.49±10.96a	2.60±0.13a
LSD	6.8627	5.8677	20.113	0.3023

Colon		
Groups	**Depth of crypt**	**Diameter of crypt**
C	118.59±0.61c	54.85±1.29a
T1	119.56±2.84c	36.50±1.75c
T2	167.59±4.64a	50.36±2.35ab
T3	153.00±6.44b	49.32±1.56b
LSD	12.485	5.2657

Number of goblet cell / cells/2000µm^2				
Groups	Duodenum	Jejunum	Ileum	Colon
C	3.50±0.34a	3.16±0.16a	3.58±0.20a	4.91±0.27a
T1	2.00±0.25b	2.08±0.27b	2.08±0.27b	3.41±0.20b
T2	3.66±0.21a	3.08±0.32a	2.66±0.21b	4.50±0.76a
T3	2.58±0.20b	3.08±0.27a	2.50±0.34b	4.50±0.76a
LSD	0.7637	0.7832	0.7735	0.6691

Values are expressed as means ± SE, n=7, C: control group. T1: Rats administered orally 1% H_2O_2 and received water containing 1% H_2O_2 for one month. T2: Rats administered orally 1% H_2O_2 and received water containing 1% H_2O_2 for 15 days, followed by administration of 20 mg/kg B.W. Quercetin for another 15 days. T3 rats were administered orally Quercetin concurrently with 1% H_2O_2 (in same manner and dose) for one month. Different small letters denoted significant differences ($P<0.05$) between groups.

Discussion

Antioxidant status

The result in current study revealed that oral administration of QCT for thirty days caused significant decrease in intestinal MDA and elevation in intestinal GSH concentration comparing to H_2O_2 and mixed groups. Quercetin is regarded as powerful free radical scavenger and prevention of lipid peroxidation in *vitro* (Chen *et al*., 2018), as well as Quercetin antioxidant effects in serum and different organs has been reported (Degrooteetal, 2019). Ren and his colleagues (2018) recorded that QCT protected andameliorate against oxidative stress and injury induced by H_2O_2 in colonic epithelium and QCT was found toal leviate the depression of intracellular GSH concentration caused by H_2O_2 (Donget al., 2020).

Dietary Quercetin supplementation has protective effect on intestine manifested by elevation in GSH -PX, SOD and decrease level of MDA, which indicated improvement in intestinal oxidative stress (Van Le Thanh *et al*., 2016). Experimentally induced colitis model, showed decreased in GSH level and this can be re stored to normal level by antioxidant (Vargas-Robles *et al*., 2019). Quercetin as all flavonoids promote translocation of Nrf2 from cytoplasm to the nucleus and activating Nrf2 signaling pathway (Baharetal, 2017) and then prevent mitochondria dysfunction and oxidative stress. A study reported that QCT ameliorate oxidative stress biomarkers such as myeloperoxidase and MAD (Hong and Piao, 2018), and the mechanism could be amelioration of T-cell mediated colitis by modulating the(HO-1) formation (Juetal, 2017). Besides, improved expression of glutamate cysteine ligase (GLC) catalytic subunit, a firstrate limiting enzyme of GSH synthesis, and elevation of intracellular GSH concentration by QCT (Patlevic *et al*., 2016) treatment accompanied withamelioration excessive ROS production and lipid peroxidation (MDA) product has been documented (Wiegand *et al*., 2009)

Another mechanism for intestinal GSH elevation by QCT could be though down regulation the transcription of AOP3, an H_2O_2 transporting protein present in the cell membrane that facilities uptake of H_2O_2 (Thiagarajah *et al*., 2017), in H_2O_2 exposed cell (*in vitro*) caused depression in intracellular H_2O_2 and elevated GSH (Dong *et al*., 2020).Flavonoids compounds such as Quercetin are characterized by presences of one or more phenol ring and two or more hydroxyl groups linked directly to aromatic ring (Cutillo *et al*., 2006), have been associated with their anti - proliferative, anti-inflammatory and antioxidant properties (Sarkar *et al*., 2016).

Concerning the effect of H_2O_2 on intestinal antioxidant status, the current study showed significant elevation in intestinal MDA and decrease in intestinal GSHin H_2O_2 treated group. At low level, ROS including H_2O_2 were essential for cell differentiation ion ,apoptosis and function as secondmessenger, however, an elevation in H_2O_2 and induction ofoxidative stress, decreased intra cellular GSH and or decrease MDA level has been documented in vitro (Dong *et al*., 2020). Besides, H_2O_2 caused reduction in GSH level in serumand in different organs has been documented in vivo (Khudair, 2010).

Malondialdehyde (MDA), is a cytotoxic products (biomarkers of LPO), has been associated with pathogenesis of IBD and colon cancer (Nairetal, 2007). Its elevation by H_2O_2 and depression in GSH indicateda case of oxidative stress and inflammation (Bhattacharyya *et al*., 2014). Over production of ROS and elevated formation of H_2O_2 results in lipid peroxidation (LPO), protein oxidation, DNA damage &induced intestinal damage (Rezai *et al*., 2015) and disruption of intestinal barrier (Aw *et al*., 2005), where ROS induced intestinal epithelia cell damage has been associated

with pathogenesis of inflammatorybowel disease including cohran disease and ulcerative colitis(Rezai *et al*., 2015)

Anti-inflammatory status

In the current study, significant decrease in serum TNF-α and elevation in serum IL-10 concentration was observed in QCT treated group, indicated its anti- inflammatory effects, which was documented by many investigators in vitro (Nikfarjametal., 2017;) and in vivo (Egert *et al*., 2009). One of the most remarkable properties of QCT is its ability to modulate inflammation. It inhibit cyclooxygenase and lipooxygensis pathway thereby decreasing inflammatory mediators such as prostaglandins and leukotriens (Lee etal., 2010).Quercetin has been used in patients with neutrophil mediated inflammatory disease (Nikforjametal., 2017) and inhibit production of pro inflammatory cytokines such as IL-6 and TNF - α from macrophage in lipopolysaccharide induced inflammation (Huang *et al*., 2015). Besides, Quercetin anti-inflammatory properties in relation to obesity and type-2 diabetes is documented (Chen *et al*., 201 6).

Fiedles and his colleagues (2020) demonstrated that quercetin 3-Rutinord possess anti- inflammatory, cytoprotective and gastro protective activities, which was attributed to suppression of TNF-α, IL-6 and blocking activities of nuclear factor kB (NFkB) transcription andpromote expression of inflammatory cytokines (Lee *et al*., 2018). Quercetin may be the best flavonoids candidates to provide anti- inflammatory reflex in vivo, attributed to their inhibitory effect on TNF–and iNOS synthase expression coupled with enhancement of IL-10 release(Comalada *et al*., 2005).

Besides, the reduction of TNF-alpha by QCT may be though down regulation significantly myeloperoxid as activity, which is indicative of decrease neutrophil infiltration and thereby reduced generation of ROS (Toth *et al*., 2017). On conclusion, the use of QCT in H_2O_2 exposed rats, prevent intestinal damage and enhance intestinal recovery via oxygen radical scavenger activity, nitric oxideand xanthan oxidase inhibition, lipid oxidation inhibition & metal chelating activity (Leyva *et al*., 2016).

In the current study, significant elevation in serum TNF-and depression in serum IL-10 was observed in H_2O_2 (T2) treated group comparing to other treated groups which is attributed to inflammatory status. The pro-inflammatory effect of H_2O_2 was documented in vitro through increased expression of COX2, inflammatory cytokines, such as TNFalpha, IL-6 (Okoko, 2018) and pro inflammatory transcription factor NF-kB (Gupta *et al*., 2012). Hydrogen peroxide could be produced by lymphocyte, monocyte and neutrophil that coming from leukocyte infiltration which is characteristic features of intestinal inflammation (Moura *et al*., 2015). Besides, ROS (to which H_2O_2 is belong) coordinate the inflammatory response of tissue (Nrethammer *et al*., 2009), where TNF-alpha is acentral mediator of inflammation (Oncel *et al*., 2016).

The redox sensitive -nuclear-factor-erythroid-2 related factor-2 (Nrf2) transcription factor, is the main defense mechanism against various harmless stress, it improves the bod oxidative status andmaintain cellular redox homeostasis (Hafezetal., 2019). Reactive oxygen species (H_2O_2) may cause decrease in expression of this cytoprotective (Nrf2) factor (Mouetal, 2019), leading to oxidative stress (Pickiringetal, 2013) and decrease in anti- inflammatory response that may be accompanied with depression in TNF-alpha and IL-10 concentration.

An association between IL-10, a key anti-inflammatory cytokines, and intestinal mucosal homeostasis is documented, where IL-10 and it's receptorsignaling modulate innate and adaptive immune response in GIT and play role in inhibition of upregulation of inflammation &oxidative stress (Cheng *et al*., 2018) and prevention of IBD (Shouval *et al*., 2014). Depression of this interleukins by H_2O_2 indicating a case of inflammation. Besides, H_2O_2 activates the release of high morbidity group -1 protein from macrophages, resulting in amplification of pro inflammatory stimulation (Sies *et al*., 2017) could be a mechanism.

Morphometric changes

The optimal gut health is characterized by several ways, one ofwhich is villus height, crypt depth ratio, a high ratio indicated matureand well-functioningvilli, with shallowcrypt, that is constantly providing cell renewal (Cuie *et al*., 2020).

The intestinal histomorphological parameters measurement in groups T2 and T3 showed significant elevation in VH and CD, suggested improvement of absorptive and digestive capacity of small intestine (Zhang *et al*., 2020) which could be a mechanism for QCT Besides, an elevation in morphometric and physiological performance of intestinal mucosa such asVH and number of goblet cell by QCT, could be through elevation of mucosal proliferation , differentiationand enzymatic activity (Sun *et al*., 2020)

The improvementofabovementioned criteria by QCT could be through stimulation activity ofprobiotic bacteria associated with elevationin short chain fatty acidmainlybutyric acidthat participatein elevation intestinal absorption and digestion capacity (Yadava and Jha, 2019)

As we, an elevation in length of absorptive surface in determined by villus height and crypt depth, where elevation in villus height score indicated an elevation in absorption capacity and healthyand well developed small intestine (Cui *et al*., 2020). Accordingly, depression in theses morphometric criteria by H_2O_2 indicated decreased absorptive capacity ofintestine, besides, the measurement of villus are correlated very well with total number ofepithelialcell in villus (Krndija *et al*., 2019).

Histomorphometricalterations, such as decreased in villus height, crypt necrosisand inflammatoryinfiltrationare reportedin H_2O_2 treated groups. H_2O_2 treated rats showed sever, atrophy epithelia flatting, extensive crypt loss in vitro (Sukhotnik *et al*., 2018)

As we previously mentioned, An elevationin VH/CD ratio result in slowerturnover of intestinal mucosa, that could result in higher growth efficacyof animal (Parker *et al*., 2019).

Depleted in goblet cell number in H_2O_2 treated rat, indicated a case of inflammation and ulceration colitis associated with low mucin secretion that damage epithelial tight junction leading to inflammation (Lin *et al*., 2016) whichcould bedue to inhibition of probiotic bacteria and decrease in pathogenicbacteria, accompanied with deceased in fermentationand loweringofshortchain fatty acid production (akey anti-inflammatory metabolitesinduced by commensal bacteria) (Chen *et al*., 2019) leading to

pathogenic change in histopathological picture of intestine. On the contrary, another research reported that expose to stress developed an adaptationmechanism characterized by elongationin villus anddeeping of crypt which increase absorption capacity and digestion /unit length (Cormula *et al*., 2019).

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