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Immunomodulatory effects of royal jelly on aorta CD3, CD68 and eNOS expression in hypercholesterolaemic rats

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KEYWORDS

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Abstract Hypercholesterolaemia (HPC) is a risk factor of cardiovascular disease. Synthetic medicines cause serious side effects that cause an imbalance in the body's functions. Therefore utilization of natural compounds could be an alternative concept in the treatment of diseases, as they have no side effects on human health. The present work is designed to evaluate the immunomodulatory role of royal jelly (RJ) on the aorta in hypercholestrolaemic rats.

Cholesterol (30 mg/kg/day) administration for two months caused a significant increase of total cholesterol, triglycerides, LDL, CD4 and CD8 in serum and diminution in HDL levels. Aortic histopathological lesions are represented by deposition of fats, loss of smooth muscle fibres and an increase in CD3, CD86 and eNOS expression in the tunica intima.

RJ administration (300 mg/kg/day) with CH, produced a counteractive effect, represented in recompense of biochemical alteration, CD4 and CD8 expression. RJ intake also amended the histological picture. The immunohistochemical picture revealed a decrease in CD3, CD86 and eNOS in the aortic tissue. These findings attributed to the significant immunomodulatory effect of RJ remedy suppress deleterious effects of hypercholesterolaemia.

Introduction

Atherosclerosis is a chronic inflammatory disease that results from interaction between oxidized low-density lipoprotein, activated endothelial cells, monocyte-derived macrophages, T cells, and the arterial wall (Libby et al., 2002; Nwichi et al., 2012). The healthy endothelium is maintained in homoeostatic balance by a series of anti-inflammatory, antithrombotic, profibrinolytic, and vasodilatory functions. Endothelium-derived nitric oxide (NO) plays a pivotal role in these functions. In endothelia, NO is synthesized by endothelial NO synthase (eNOS) which can be mediated by many physiopathological factors such as stress and hypercholesterolaemia or metabolites such as glucose and free fatty acids (Stulak et al., 2001; Kim et al., 2005).

$CD4^+$ and $CD8^+$ cells contribute significantly to the progression of atherosclerosis (Hansson, 2005; Bue et al., 2011). Cells of the adaptive immune system include T-cell

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lymphocytes (helper T cells [generally $CD4^+$] and cytotoxic T cells [generally $CD8^+$]), natural killer cells, and B-cell lymphocytes (Baird, 2006). $CD4^+$ T cells are subdivided into: Th1 (which is involved in cell-mediated immunity and secrete pro-inflammatory cytokines such as interferon-gamma [IFN-γ] and interleukin [IL]-2) and Th2 (which promote humoral immunity and secrete anti-inflammatory cytokines such as IL-4 and IL-10) as reported by der Bie (2006).

Honey acts as a natural antioxidant and is becoming increasingly popular because of its potential role in contributing to human health, which has attracted the interest of medical scientists. Honey, propolis, and royal jelly (RJ) are functional foods with phenolic compounds collected by the worker honeybees from the plants where they gather nectar. Beside sugars, honey contains many components with antioxidant activity, among which are amino acids and proteins, carotenes, phenolic compounds and flavonoids, ascorbic acid and organic acids (Erejuwa et al., 2012). It has been proposed that the antioxidant capacity of honey is due mainly to the phenolic compounds and flavonoids they contain, and there is a high correlation between polyphenols and honey antioxidant capacity (Alzahrani et al., 2012). Previous studies have shown that RJ has a number of physiological effects, such as anti-inflammatory, antitumor, antiallergic, and antioxidant activities; that have a protective effect against lipid peroxidation caused by free radicals (Guo et al., 2008; Yamaura et al., 2013).

It has already been stated in the literature that some antioxidant molecules that are influential as scavengers or prevent the formation of reactive oxygen species, eliminate the aortic damage caused by atherosclerosis. Therefore, this study focused on the probable protective and immunomodulatory effects of RJ on the aortic damage caused in cases of hypercholestrolaemia. The hypothesis of the present study was to evaluate the immunological role of RJ on the expression of CD4 and CD8 in serum; CD3, CD68 and eNOS in the aortic tissue of the hypercholesterolaemic rats.

Materials and methods

Animals and experimental protocol

Twenty eight pathogen-free male albino rats (*Rattus rattus*) weighting about 200 ± 10 g were obtained from the Vaccine and Serum Organization at Helwan, Egypt. They were allowed one week for acclimatization, fed standard rat food and tap water *ad libitum*. They were housed in a controlled environmental room, with a light-controlled (12L:12D) cycle. Rats divided into four groups, seven rats each. Rats belonging to the 1st group were administered saline and are considered as the control group (C), while rats in the 2nd group received RJ at a dose level of 300 mg/kg/day (Karadeniz et al., 2011) (Royal Jelly from Queen Bee Jelly, Biomedicals Comp., Santa Ana, CA, USA) through an oro-gastric tube for 8 weeks. The 3rd group was the hypercholesterolaemic group, which was induced by the oral administration of cholesterol (CH) at a dose level of 30 mg/kg cholesterol for 8 weeks (Nwichi et al., 2012). The animals belonging to the 4th group were given both CH + RJ for 8 weeks.

Biochemical and cytokine analysis

Total plasma cholesterol (TC) and triglyceride (TG) levels were determined with an automated enzymatic colorimetric technique (Wiesbaden, Germany). High density lipoprotein (HDL) and low density lipoprotein (LDL) cholesterol levels were determined by a HDL and LDL Quantitation Kit (Sigma Chemical Co) via colorimetric tests, according to the manufactured instructions.

Flow cytometric analysis of $CD4^+$ and $CD8^+$

Expression of ($CD4^+$ and $CD8^+$) was determined by flow cytometric analysis on blood lymphocytes. 0.5 ml blood samples were collected in EDTA tubes (Becton, Dickinson & Company, Arizona, USA). Then, the erythrocytes were lysed with ammonium chloride solution, and washed twice in phosphate-buffered saline containing 0.1% sodium azide and 0.1% bovine serum albumin (BSA). The final resulting cell pellet was resuspended in 3 ml of the same buffer. For flow cytometry, the cells were incubated with fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-conjugated monoclonal antibodies (antimouse CD4 [FITC], or antimouse CD8 [PE]) (Bioscience, San Diego, CA, USA) for 20 min. and then washed twice with 1.5% goat serum/PBS. Then cells were fixed with 400 μL of 1% paraformaldehyde immediately before determination. At least 10,000 cells in fluorescent channel 1 for CD4, and fluorescent channel 2 for CD8 were detected using Beckman Coulter EPICS XL, Inc., CA, USA at Faculty of Medicine, Ain Shams University. Results were expressed as the percentage of $CD4^+$ and $CD8^+$ positive cells were taken as the indexes.

Histolpathological investigations

Tissue samples of the aorta were fixed in 10% buffered neutral formalin and embedded in paraffin. The paraffin blocks were cut at 4 μm thicknesses and stained with Haematoxylin and eosin for general histopathology, and Masson trichrome stain for the demonstration of the fine collagen fibres according to Bancroft and Stevens (1982).

Immunohistochemical studies

Immunohistochemistry is the process of localizing proteins in tissues by exploiting the principle of antibodies binding specifically to antigens. The visualization of the antibody is commonly accomplished by conjugating an enzyme to the antibody. This can produce a color changing reaction. The advantage of this method is the ability to show exactly where a given protein is located. The expression of CD3 used for determination of T-lymphocytes infiltration (ab5690, Abcam, Cambridge Science Park in Cambridge, England), CD68 used for analysis of macrophage (ab125212, Abcam) and eNOS for staining of endothelial cells (ab5589, Abcam) in aorta sections was determined immunohistochemically in formalin-fixed, paraffin-embedded tissue. Blocks were cut into 4 mm thick sections mounted on glass slides, and incubated at 4°C overnight. Sections were deparaffinized in xylene and rehydrated. Endogenous peroxidase activity was blocked with 1%

hydrogen peroxide for 20 min. To improve the quality of staining, microwave oven-based antigen retrieval was performed. Slides were probed with either anti-CD3 (1:100, mouse mAb), anti-CD68α (1:100, mouse mAb) or anti-eNOS (1:100). Sections were washed three times with PBS for 10 min each and incubated with biotin-labeled antimouse IgG for 1 h at room temperature. After washing, sections were stained with a streptavidin-peroxidase detection system according to the manufactured instructions.

Statistical analysis

Data are presented as mean ± SE. Statistical significance of differences was calculated using an ANOVA with post hoc Duncan's test, using SPSS (Statistical Package for Social Sciences) (16.0) software. Significance was accepted at the level of $P < 0.05$.

Results

Biochemical studies

The results obtained for the biochemical analysis showed an the increase in TC (185.86 ± 2.84), TG (146.14 ± 1.59), LDL (125.43 ± 1.41) and a decrease in HDL (20.57 ± 1.08) as a result of the HPC induction when compared to normal control (C) as recorded in Table 1. On the other hand, the hypercholesterolaemic rats treated with RJ showed a decrease in TC, TG and LDL levels while increase in values of HDL reached 93.14 ± 1.54, 90.85 ± 2.60, 39.85 ± 2.44 and 36.14 ± 1.24 respectively as compared to the hypercholesterolaemic group.

Flow cytometric analysis

The percentages of $CD4^+$ and $CD8^+$ T lymphocytes conveniently estimate the immune state. In this study, the expression of the differentiation antigens of $CD4^+$ and $CD8^+$ on peripheral blood lymphocytes of CH rats were measured by flow cytometry to further determine the effects of RJ on the cellular immune function (Table 2). The data show that the RJ can efficaciously normalize the immune imbalance significantly raised ($P < 0.05$).

CH administration caused increase in $CD4^+$ and $CD8^+$ expression reaching 47.28% and 23.57% respectively. This increase promising approach for vascular disease as delineated by the histopathological investigation latter. This values were attenuated by RJ administration in addition to CH reaching 28.00% for $CD4^+$ and 17.57% for $CD8^+$.

Table 2 Percentage of $CD4^+$ and $CD8^+$ in the control and different experimental groups (%).

Groups	Parameters as mean ± SE	
	$CD4^+$	$CD8^+$
C	24.28 ± 0.86	13.42 ± 0.75
RJ	23.71 ± 1.08	13.85 ± 0.91
CH	47.28 ± 1.32[a,b]	23.57 ± 1.08[a,b]
CH/RJ	28.00 ± 0.81[a,b,c]	17.57 ± 0.64[a,b,c]

Values are mean ± SE. Superscript letters denote the significant difference at ($P < 0.05$).
[a] Values are significantly different from the control group.
[b] Values are significantly different from the RJ group.
[c] Values are significantly different from the CH group.

Histopathological studies

Investigating aorta sections from control rats showed a normal appearance of 3 different layers intima, media and adventitia. The intima is composed of a single layer of endothelial cells with their oval nuclei. Elastic lamella was seen in both layers intima and media. The media contains multiple layers of vascular smooth muscle cells. The adventitia is the outermost

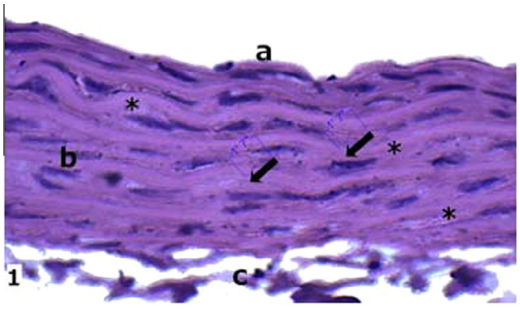

Fig. 1 Photomicrograph of aorta cross sections of the control rat showing normal appearance of different layers.: intima (a), media (b) and adventitia (c), with normal endothelial cells (head arrows) with their oval nuclei in the intima, elastic lamellae (*) in both layers intima and media. The media contain multiple layers of smooth muscle fibres (arrows) (H-E, 400×).

Table 1 Averages of serum lipid profile in the control and different experimental groups (mg/dL).

Groups	Parameters as mean ± SE			
	TC	TG	LDL	HDL
C	86.14 ± 1.29	76.28 ± 1.08	35.28 ± 1.32	44.14 ± 1.50
RJ	84.14 ± 1.01	74.28 ± 1.86	33.00 ± 1.41	45.57 ± 1.33
CH	185.86 ± 2.84[a,b]	146.14 ± 1.59[a,b]	125.43 ± 1.61[a,b]	20.57 ± 1.08[a,b]
CH/RJ	93.14 ± 1.54[a,b,c]	90.85 ± 2.60[a,b,c]	39.85 ± 2.44[a,b,c]	36.14 ± 1.24[a,b,c]

Values are mean ± SE. Superscript letters denote the significant difference at ($P < 0.05$).
[a] Values are significantly different from the control group.
[b] Values are significantly different from the RJ group.
[c] Values are significantly different from the CH group.

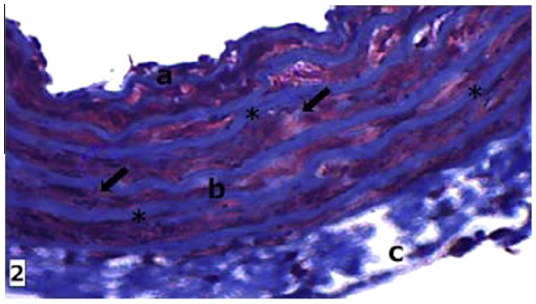

Fig. 2 Photomicrograph from cross sections of the aorta from RJ treated rat showing the normal architecture of the intima (a), media (b) and adventitia (c). Elastic (*) and smooth muscle fibres (arrows) appeared with their normal distribution (Masson-trichrome, 400×).

layer of the blood vessel (Fig. 1). No histological differences were found in the RJ treated group (Fig. 2). The aorta sections showed the normal architecture of intima, media and adventitia. Elastic and smooth muscle fibres appeared with their normal distribution.

Concerning to the influence of CH on rats, examined sections of the aorta manifested augmented accumulations of fat cells in the tunica intima and media. These epitomized in the form of fat deposition under the intima and the presence of foam cells and fat vacuoles that were aligned side by side as appearing in Fig. 3. Detached elastic fibres were taken from the tunica intima, distribution of fat vacuoles at the expense of smooth muscle cells seen in the tunica media and the presence of lymphocytes seen in the adventitia. Also, the elastic fibres appeared straight (Fig. 4), which led the aorta losing its ability to contract and pump blood.

As RJ was introduced to the hypercholesterolaemic rats, aorta sections revealed an improvement in its histological picture resembling that in the well-defined intima, media and adventitia. Tunic intima appeared with normal endothelial layer, normal arrangement of the elastic fibres with its zigzag appearance and smooth muscle cells in the tunica media (Figs. 5 and 6).

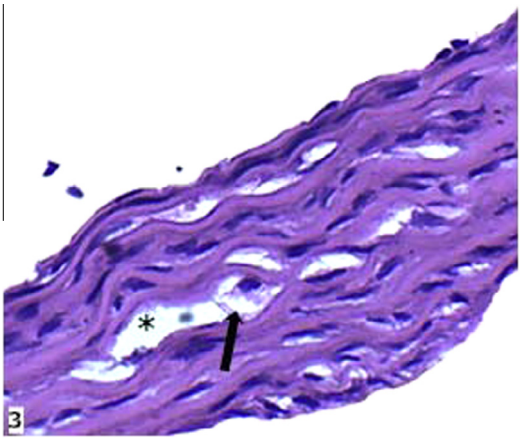

Fig. 3 Photomicrograph from cross sections of the aorta from CH treated rat showing fat deposition under the intima and the presence of foam cells (arrow) in the tunica media. Some areas lost their smooth muscle cells (*) (H-E, 400×).

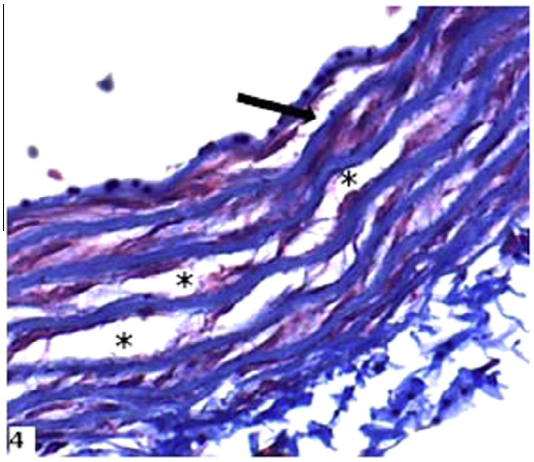

Fig. 4 Photomicrograph from cross sections of the aorta from CH treated rat showing detached elastic fibres from the tunica intima (arrow), distribution of fat vacuoles at the expense of the smooth muscle cells (*) in the tunica media and the presence of macrophages in the adventitia. Note: The elastic fibres appeared straight and lymphocytes appeared in the tunica adventitia (Masson-trichrome, 400×).

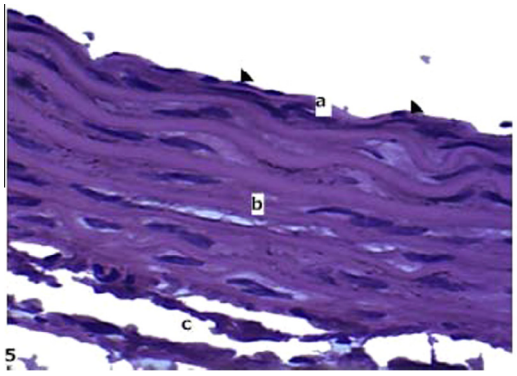

Fig. 5 Photomicrograph from cross sections of the aorta from CH + RJ treated rat showing that aortic tissue regained its architecture with the intima (a), media (b) and adventitia (c). Normal endothelial cell (head arrows) structure, elastic fibres arrangement and smooth muscle fibres (H-E, 400×).

Immunohistochemical investigations

CD3 expression

Cross sections of the aorta from the control and RJ treated rats stained immunohistochemically for CD3 delineated no CD3 positive cells distributed in the endothelium (Figs. 7 and 8). These circulating leukocytes adhere poorly to the normal endothelium under normal conditions. On the other hand, aorta sections from rats that received CH, revealed CD3 distribution in the aortic tissue, referring to the inflammation (Fig. 9). Aorta sections of rats treated with CH + RJ showed decreased numbers of CD3 positive T-lymphocytes (Fig. 10), as compared with the CH group.

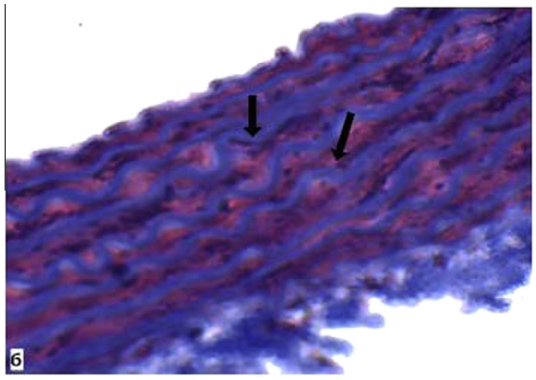

Fig. 6 Photomicrograph from cross sections of the aorta from CH + RJ treated rat showing normal arrangement of elastic fibres (arrows) with its zigzag appearance between smooth muscle fibres (Masson-trichrome, 400×).

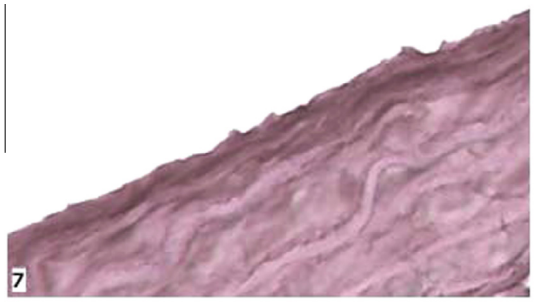

Fig. 7 Photomicrograph of aorta sections from the control rat showing that there is no CD3 positive cells distributed in the tunica intima (Immunohistochemical stain, 400×).

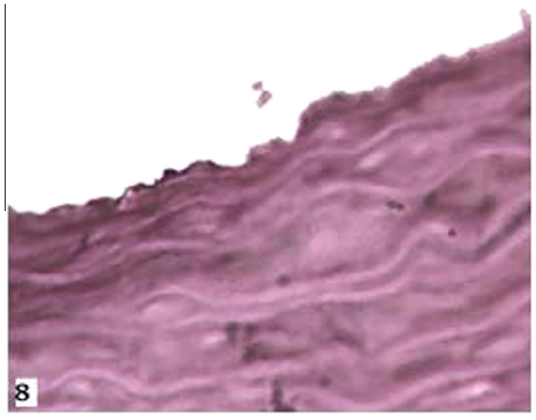

Fig. 8 Photomicrograph of aorta sections from rat treated with RJ showing that there is no CD3 positive cells (Immunohistochemical stain, 400×).

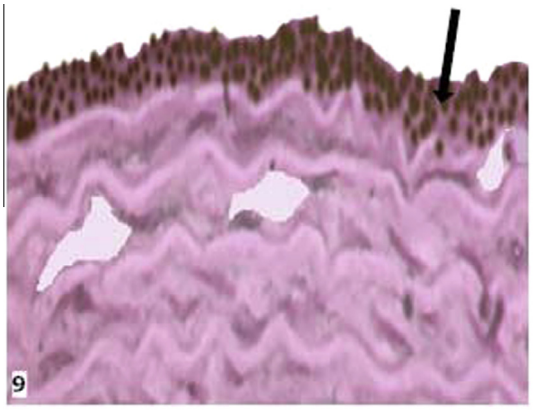

Fig. 9 Photomicrograph of aorta sections from rat treated with CH showing CD3 positive cells stained in brown (arrow) in the tunica intima (Immunohistochemical stain, 400×).

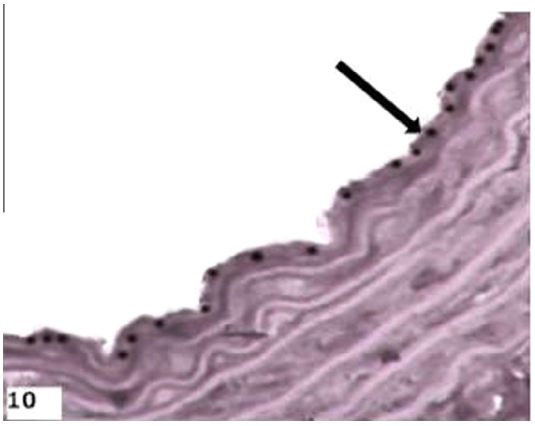

Fig. 10 Photomicrograph of the aorta sections from rat treated with CH + RJ showing a decrease in CD3 positive cells (arrow) distribution as compared with the CH treated rat (Immunohistochemical stain, 400×).

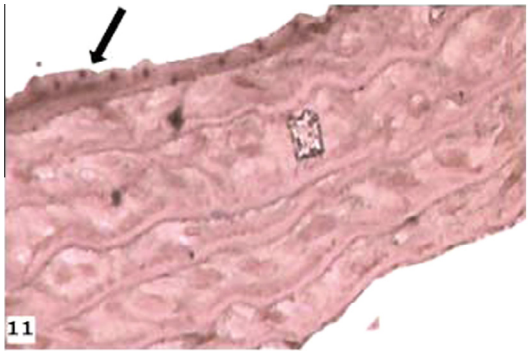

Fig. 11 Photomicrograph of the aorta sections from the control rat showing the normal expression of CD68 (arrow) in the tunica intima (Immunohistochemical stain, 400×).

CD68 expression

Sections of the aorta from control rats stained for CD68 revealed a normal distribution of macrophage cells in the aorta (Fig. 11). Normal distribution of CD68 appeared in the aortic tissue from RJ treated rats (Fig. 12). Augmentation of positive staining with the anti-CD68 antibody was seen in the aorta from rats that received CH (Fig. 13). Sections of the aorta from

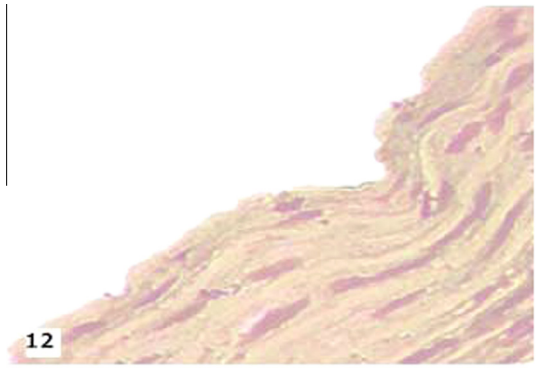

Fig. 12 Photomicrograph of the aorta sections from rat treated with RJ showing no expression of CD68 in the tunica intima of the aortic tissue (Immunohistochemical stain, 400×).

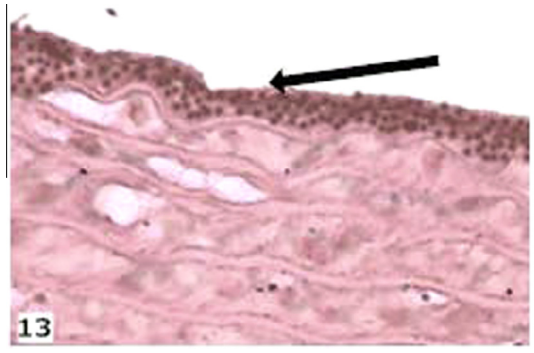

Fig. 13 Photomicrograph of the aorta sections from rat treated with CH for 14 weeks showing an increase in CD68 expression (arrow) (Immunohistochemical stain, 400×).

rats treated with CH + RJ revealed near to normal distribution in CD68 (Fig. 14). The present study shows that RJ prevents macrophage infiltration in the endothelial aortic layer.

eNOS expression

Sections of the aorta from control and RJ treated rats stained for eNOS revealed a normal distribution of endothelial cells of

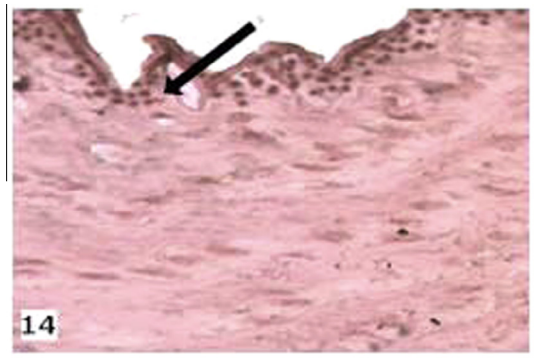

Fig. 14 Photomicrograph from rat aorta sections treated with CH + RJ showing a slight decrease in CD68 expression (arrow) as compared with CH treated rat (Immunohistochemical stain, 400×).

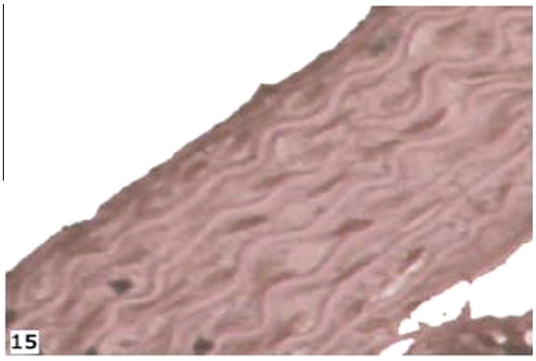

Fig. 15 Photomicrograph of aorta sections from the control rat showing the normal cytoplasmic expression of eNOS in the endothelial cells (Immunohistochemical stain, 400×).

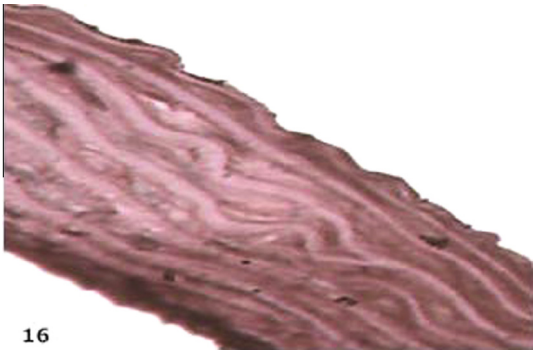

Fig. 16 Photomicrograph of aorta sections from rat treated with RJ showing the normal cytoplasmic expression of eNOS in the tunica intima of the aortic tissue (Immunohistochemical stain, 400×).

the aorta (Figs. 15 and 16). Augmentation of positive staining with the anti-eNOS antibody was seen in the endothelial cells and rarely in the subintimal layer of the aorta from rats that received CH and the strongest reaction was confined to the

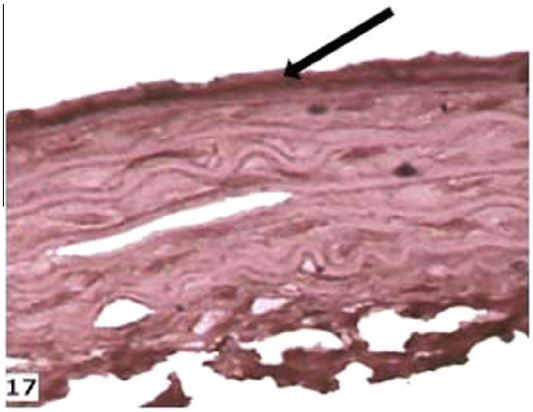

Fig. 17 Photomicrograph of aorta sections from rat treated with CH showing an increase in the expression of eNOS confined to the cytoplasm (arrow) of intimal endothelial cells (Immunohistochemical stain, 400×).

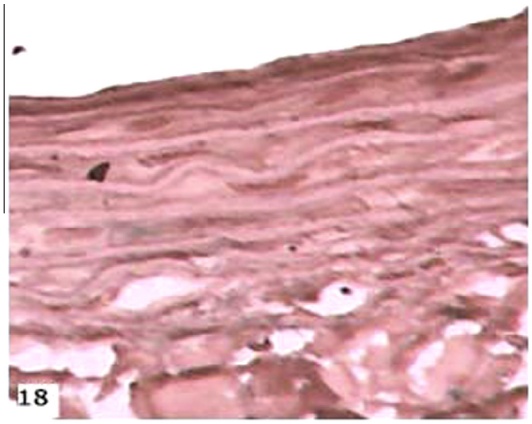

Fig. 18 Photomicrograph from rat aorta sections treated with CH + RJ showing a slight decrease in eNOS expression in the endothelial cells as compared with the CH treated rat (Immunohistochemical stain, 400×).

cytoplasm (Fig. 17). Sections in rat aorta treated with CH + RJ revealed a slight decrease in eNOS expression in the endothelial aortic cells (Fig. 18).

Discussion

To the best of our knowledge, this study is the first to explore the protective effect of RJ against CD3, CD68, eNOS, $CD4^+$ and $CD8^+$ expression in cases of hypercholestrolaemia.

The present findings revealed an increase in TC, TG, and LDL and a decrease in HDL levels as a result of CH administration. This comes in harmony with Nwichi et al. (2012), who reported that the increase of these parameters may be related to the antioxidant status in cases of CH intake. Decreased levels were seen in glutathione and catalase; and increased in malondialdehyde and superoxide dismutase enzymes was reported accompanied with hypercholesterolaemia.

RJ accompanied with intake reported improvement for the previous parameters, proofing its hypolipidemic effect. This may be related to its antioxidant activity, which caused an increase in HDL levels. In 1995, Vittek reported that RJ significantly decreased serum total lipids and cholesterol levels in rats and rabbits and also in human trials. The author suggested that RJ at approximately 50–100 mg per day, decreased serum TC levels by about 14%, and total serum lipids by about 10% in the group of patients studied. The decrease in TC and LDL may be as a result of lowering very low density lipoprotein (VLDL) levels as reported by Guo et al. (2008), due to RJ strongest hydroxyl radical scavenging quality.

Increased values of $CD4^+$ and $CD8^+$ values were reported in the present study as a result of CH intake (Bue et al., 2011). Lymphocytes (e.g., $CD4^+$ Th1 cells) contribute to and may accelerate atherosclerotic plaque formation (Libby et al., 2002). $CD4^+$ secretes high levels of IFN-γ, which are directly cytotoxic. These cells preferentially infiltrate unstable plaque and contribute to increased endothelial cell lysis in patients with acute coronary syndromes (Nakajima et al., 2002). The imbalance occurred between reactive oxygen species (ROS) generation and antioxidants, leading to oxidative damage which spreads over most cell targets such as DNA, lipids and proteins (Ortial et al., 2006). Free radicals can induce oxidative damage to the body and this damage might induce dysfunction of cells, organs and the whole body.

RJ administration returned $CD4^+$ and $CD8^+$ levels near to normal levels which normalize the immune imbalance. Seasonal variation, chemical composition and antioxidant activity of Brazilian propolis samples are studied. The phenolic compounds found in RJ are known to counteract oxidative stress in the human body by helping in maintaining a balance between oxidant and antioxidant substances (Siddhuraju, 2006). Mechanisms of antioxidant action may include suppression of ROS formation, removal or inactivation of oxygen reactive species and up-regulation or protection of antioxidant defenses (Teixeira et al., 2010). Antioxidants intercept the free radical chain oxidation by donating hydrogen from the phenolic hydroxyl groups, thereby forming stable end products, which does not initiate or propagate further oxidation. This phenolic compound undergoes intestinal absorption and prevents oxidative damage in hepatocytes and is assumed to prevent degenerative diseases by acting on cellular DNA (Jayaprakasha et al., 2006). Oral administration of RJ (1 g/kg) significantly improves the Th1/Th2 ratio in favour of Th1 (Oka et al., 2001). This leads to the secretion of anti-inflammatory cytokines such as IL-4 and IL-10, which are Th2 subtypes. RJ has produced the proliferation of healthy lymphocytes and the increased secretion of various cytokines (IFN-γ), while decreasing the production of others. In lymphocytes from patients treated with RJ, the ratio between Th1/Th2 (IFN-γ/IL-4) cytokines has changed in favour of Th1 (Erem et al., 2006).

Therefore, it is probable that RJ cures $CD4^+$ and $CD8^+$ activities through its immunomodulatory and antioxidant mechanisms. That is, RJ probably inactivates the hydroxyl radicals, which are very reactive, thus protecting rats from the harmful effects of free radicals.

The above biochemical and flow cytometric analysis results correlated well with the histological and the immuohistochemical investigations from the aortic tissue, which revealed fat deposition, smooth muscle disappearance and inflammatory cell infiltrations.

Atherosclerosis is characterized by arterial lesions containing cholesterol, fibrosis, and inflammatory infiltrates (Ross, 1999). The latter consist mainly of macrophages and T lymphocytes (Jonasson et al., 1986). Degeneration is preceded by focal endothelial damage with subsequent inflammatory cell infiltration, specifically, T-lymphocytes and macrophages indicating chronic inflammation aggregate in aortic tissue, as shown in the immunohistochemical investigation. Macrophage-derived foam cell formation is enhanced upon a relative increase in cholesterol uptake or by a defective cholesterol efflux (Li and Glass, 2002), that may account for this increased LDL uptake by macrophages. Skowasch et al. (2005) showed that the degenerative areas contained both CD3 T-lymphocytes and CD68 macrophages lining the aortic border. It is known that inflammation plays an important role in the development and/or progression of native degeneration (Bellamy et al., 2002; Rajamannan et al., 2005). Remarkably, all participating cells were predominantly localized in the aortic tissue from the circulation due to the endothelial injury.

When the aortic endothelium becomes inflamed, it expresses adhesion molecules that bind relative ligands on leukocytes. Selectins mediate a loose rolling interaction of leukocytes with the inflammatorily activated endothelial cells. Integrins mediate firm attachment. Chemokines expressed within atheroma provide a chemotactic stimulus to the adherent leukocytes, directing their diapedesis and migration into the intima, where they take residence and divide as shown by Libby et al. (2002).

The administration of RJ retarded the formation of atheromas in the aorta of rabbits fed a hyperlipemic diet as explained by Vittek (1995). The phenolic compounds present in honey have promising effect in the treatment of some chronic diseases, by acting directly as radical scavengers leading to decreased intracellular oxidation due to increased levels of endogenous antioxidant proteins (Pérez-Pérez et al., 2013). RJ counteracts the distribution of CD3, CD68 and eNOS expression in the aortic tissue.

The impairment of NO production is considered to be at least one of the causes of endothelial dysfunction. The ability of RJ to inhibit eNOS might contribute to the regulation of vascular permeability. In agreement with this hypothesis, eNOS deficiency in genetically engineered mice is associated with reduced vascular endothelial growth factor-induced permeability (Fukumura et al., 2001). In addition, royal jelly is regarded as an excellent food for maintaining the physiological equilibrium and body chemistry, strengthening the immune system (Saritas et al., 2011), thus maintaining regular functioning of the kidney and liver, maintaining healthy blood LDL and HDL cholesterol levels (Karabag et al., 2010).

Kohno et al. (2004) reported that RJ suspensions were added to a culture of mouse peritoneal macrophages stimulated with lipopolysaccharide and IFN-γ, the production of pro-inflammatory cytokines, such as TNF-α, IL-6 and IL-1 was efficiently inhibited in a dose-dependent manner without having cytotoxic effects on macrophages. This suggests that RJ contains factors responsible for the suppression of pro-inflammatory cytokine secretion.

Administration of RJ to hypercholesterolaemic rats reducing the expression of $CD4^+$ and $CD8^+$, which are related to the progression of atherosclerosis. Improving in the histological picture of the aorta, restoring its ability to contract and pump blood. Inhibiting the expression of CD3, CD68 and eNOS expression in the aortic tissue so, reducing the inflammatory action and regained the endothelium function.

The delineated data in this study, concluded that oral administration of RJ prevents the increase in CD3, CD4, CD8, CD68 and eNOS expression in blood leukocytes and aortic tissue of rats significantly. This result shows that RJ has a potential regulatory effect on these parameters; therefore, RJ can be used as a protective natural product against the aortic damage caused by CH.

RJ could also be used as a prophylactic agent (food protector) to prevent the increase of total cholesterol and its harmful types.

Conflict of interests

The author declares that there is no conflict of interests.

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