International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
DOI: 10.22192/ijarbs Coden: IJARQG(USA) Volume 4, Issue 12 - 2017

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2017.04.12.024

COMPARATIVE EXPERIMENTAL STUDIES ON THE SPLEEN OF YOUNG AND AGED BALB/C AND CD-1 MICE

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Abstract

Immunosenescence is generally related to weaker immune responses since, elderly individuals do not respond to immune challenge as strongly as the youngs. Spleen is the largest secondary lymphoid organ present almost in all vertebrates. In addition of its skill as a main part of immune system, it serves also as a graveyard for old damaged red blood cells, storage of blood as well as production of antibodies and keeping bodily fluids in balance. So, the present study aims to elucidate its complex histological organization in two mice strains outbred (CD-1) and inbred (Balb/C) throughout their different age groups. Spleen in both mice strains is consisted of two histologically distinct components, red pulp and white pulp vastly different in their architecture vascular organization and cellular composition. Spleen weight consequently splenic index significantly amplified, and diagnosed as splenomegaly, with progression of mice age from 2 to 12 months especially in individuals given Poly I:C acid (400µg/100g b.w.). The elderly as well as treated mice showed irregular splenic architecture with more fibrous trabecula, and extra-large sinusoid spaces throughout the splenic parenchyma to the degree that, limits between white and red pulp disappeared. Furthermore, much decrease of lymphocytes population due to cell progressive nuclear pyknosis and subsequently necrosis and cell degeneration at the olders of 12 month age of both strains, especially those received Poly I:C acid.

Keywords: Aging; Spleen; Balb/C, CD-1, Poly I:C

Introduction

Aging can be defined as an increasing proportion in the probability of death. Animals die not from healthy aging but from age-related diseases as cell become aged too, all age-dependent diseases are connected to each other (**Curtis et al., 2005**). **Blagosklonny (2009**) declared that, in addition to aging, many factors contribute to the incidence of a specific disease. So, aging is the biggest and major risk factor for all age-related diseases and he assumed that, age-related diseases are the best biomarker of aging.

Elders are much more susceptible to infections of the soft tissues, skin and both the urinary and respiratory tracts (**Pinner et al., 1996**). The increased prevalence of these conditions and the higher morbidity and mortality from infections strongly suggest functional defects and deterioration of the immune system with advancing age (**Effros, 2001**).With the progressing of age, an increased mortality rates as well as prevalence of specific cancers and certain autoimmune diseases have been also observed by **Castle (2000)** and **Burns & Leventhal (2000**). So, alterations in immune function with age in animals are important to the health of aging individuals.

Aging is a continuous and slow process comprising the morpho-functional features of different animal's organs and systems (**Rose et al., 2012**). It is associated with a weakening of the normal functioning of the immune system that is designated by the roof term, immunosenescence (**Dixit, 2012**). Immunosenescence is generally and popularly related with weaker immune responses which produce a progressive deterioration in the ability to respond new stimulants as a result of retard alteration in both adaptive and innate immune functions mainly observed in olders (**Yan and Wei, 2011**).

Montecino-Rodriguez et al.(**2013**) stated that, the most recognized consequences of aging is a decline in immune function. Since, elderly individuals do not respond to immune challenge as strongly as the young. Consequently, all these age-dependent diseases are connected to each other in a synchronized mode and specific sequence (**Yang et al., 2015**).

Histopathology is essential for identification and description of health effects following exposure to xenobiotics, mainly in lymphoid organs and tissues of rodents **(Kuper et al., 2000)**.Immune response are rising by lymphoid tissue which includes distinct organs such as the thymus, spleen and lymph nodes, as well as more diffuse aggregations of lymphocytes. **Montecino-Rodriguez et al. (2013**) stated that, the effects of aging on the immune system are manifest at multiple levels including reduced production of immune lymphocytes in thymus and diminished function of mature lymphocytes in secondary lymphoid tissues, spleen.

Spleen is a secondary, encapsulated lymphoid organ present virtually in all vertebrates, its most primitive version is found in cyclostomes and acts primarily as a blood filter (**Alves et al, 1996**). It holds a reserve of blood, which can be valuable in case of hemorrhagic shock and metabolizes haemoglobin removed from senescent erythrocytes, so it controls the amount of red blood cells (**Mebius and Kraal, 2005)**.The spleen is important for proper immune function, clears bacteria especially in fighting bacteria. It can be considered analogous to a large lymph node, as its absence causes a susceptibility to certain infections (**Brender et al., 2005**). The same authors also stated that, it serves also as a graveyard for old or damaged red blood cells in addition as a storage site for blood and platelets. Since it is performing as a reservoir for iron, erythrocytes, and thrombocytes and it will save any useful components from the old blood broken cells, including iron to be reused in new cells (**Carey, 2006**).Since, it is the largest lymphatic organ having complex organization and performs number of different functions like as immunological monitoring of blood borne antigens by producing antibodies (**Al-Dahmesh et al., 2011; Babaei et al., 2014**). It is also play a significant role for keeping bodily fluids balanced, but it is possible that, the life keep going without it. This is because other organs, such as the liver and lymph nodes, can take over the duties of the spleen (**Szalay, 2015**). However, removing of the spleen can have serious magnitudes, since animals will be more at risk to develop infections. Spleen has also significant hematopoietic tasks, it is the primary site of extramedullary hematopoiesis (**Suttie, 2006**). This responsibility is employed up until month of gestation and after birth hematopoietic functions cease **(Udo-Affah et al., 2015)**. But as a major lymphoid organ, it preserves the ability to produce lymphocytes, as such remains a hematopoietic organ (**Dennis et al., 2016).**
Spleen is consists of two functionally and morphologically distinct components, the red pulp and the white pulp. Spleen's white pulp plays an important role in the immune system, since it synthesizes antibodies and removes antibody-coated bacteria and antibody-coated blood cells by way of blood and lymph circulation (**Udo-Affah et al., 2015;Abd-Ella, 2016)**. However, the red pulp is a blood filter, removes foreign material and damaged erythrocytes. So, the two compartments are vastly different in their architecture, vascular organization and cellular composition (**Dkhil, 2009**). Later on, **Dennis et al. (2016)** also declared that, spleen works as a very large lymph node. Its immunologic function also includes phagocytosis of foreign materials like as lifeless and feeble red blood cells passing through splenic sinuses. Consequently, it is of variable size, shape, weight and microanatomy which is varied among different vertebrate species (**Mahadevan, 2016**). Because of its filtering function, it is uncommon for the spleen to be a site of infection and so presents immunologic destruction (**Dennis et al., 2016**).

However, none of these studies describe histological and immunological values of spleen in different strains of mice treated with of viral mimic agent, Polyinosinic:polycytidylic acid (Poly I:C).The present work investigates the general properties and histological architecture of spleen as secondary lymphoid organ in different ages of two mice strains CD-1(Outbred) and Balb/C (Inbred) and its response to low dose of Poly I:C.
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Materials and Methods

Animals and chemicals

Definite pathogen-free male CD-1(Outbred) and Balb/C (Inbred) mice of 2 months, 6 months and 12 months old, weighing 30 ±10g rendering to the mice old, were purchased from the animal house of Vaccsera, Giza, Egypt. First of all mice with evidence of disease (e.g., enlarged spleen, gross tumors) were excluded from these studies. Animals were acclimatized in controlled environmental laboratory conditions at room temperature (25 ± 5 °C), relative humidity (50 ± 10%) and 12-hour light/dark cycle in the animal house of the department of Zoology, Faculty of Sciences. All mice had ad libitum access to standard rodent chow and filtered water throughout the study period.

The Polyinosinic:polycytidylic acid (Poly I:C) 1 vial (10 mg) was purchased from invivogen (San Diego, California, United States). It was dissolved in (1:3) Phosphate-buffered saline (PBS) which is an isotonic water-based salt solution.

Animals were randomly divided into 12 groups (5 mice each), six groups for each mice strain. Individuals of the first group of each mice strain were injected intraperitoneally with a dose of 400µg/100g b.w. Poly I:C dissolved in (1:3) Phosphate-buffered saline (PBS) divided in two sub-doses with 7 days interval. However, individuals of remains six groups of the two strains were administered as positive controls, where they were received only the isotonic solvent, Phosphate-buffered saline (PBS), by the same rout of administration.

All measurements were carried out 24 h post the second sub dose, since delaying of polyinosinic:polycytidylic acid (Poly I:C) treatment, by as little as 48 hr, produces opposite effect **(Peres *et al.*, 1989)**. All mice were examined clinically twice daily before and after inoculation (injection). Present investigations were conducted in compliance with rules and international recommendations for the Protection of Vertebrate Animals used for experimental studies approved by the Animal Ethics Committee at Zagazig and Tanta Universities according to the Laboratory Animal Welfare guidelines.

Body and spleen weight

Afore sacrificing, animal's body weights were detected and recorded as initial and final weights of normal/control and Poly (I:C) treated groups. At the second day of sub-dose treatment, all mice were anesthetized by i.p. injection of 4 mg/100 g b.w. of Sodium thiopental and sacrificed by cervical dislocation and dissected. The spleen of normal/control and treated mice were immediately surgically removed at autopsy within 10 min. The weight of the freshly detached spleens were obtained by using an electronic balance (Sartorius, TE214 S). Percentage of spleen to body weight (splenic index) will be determined for mice of each group in order to estimate the expected hypertrophy following the aging as well as Poly (I:C) treatment.

Histopathology

For histological analysis, the obtained spleen of each animal were divided and fixed in buffered neutral formalin 10% solution for 24 hrs then dehydrated through ascending grades of ethyl alcohols. The fixed specimens were cleaned in xylene, impregnated and embedded in paraffin wax. Sections of five microns thickness were obtained by a rotatory microtome and floated out on clean microscope slides in order to be stained with Ehrlich's haematoxylin and counterstained with eosin stains as recommended by **Drury and Walington (1980).**

Data analysis

Data were analyzed by the one-way analysis of variance (ANOVA). Differences between dose groups were further tested by the Duncan's test. Statistical calculations were performed using a SPSS program software (Statistical Package for the Social Sciences), and differences were considered as significant when $P < 0.05$

Results

Animal's body and spleen weight:

The animal's body weight (initial and final), body weight gain and spleen weight as well as spleen relative weight (splenic index) of different mice groups are presented in table (1). It was found that, the final body weight of normal/control outbred mice (CD-1) in the three investigated ages groups (2, 6 and 12 month) were significantly increased. This was reflected on the mice gain weight as it is realized 0.22±0.107, 0.54±0.121 and 0.30±0.179 grams in the three age groups, correspondingly. Likewise, the gain weight values of the treated mice with Poly I:C acid, increased but insignificantly to give 0.40±0.167, 0.52±0.124 and 0.49±0.172 grams (Tab.1 and Fig.1).

Table 1: Mean of initial and final body weight, body weight gain, spleen weight and relative spleen weight (Splenic index) of the two studied strains CD-1 (Outbred) and Balb/C (Inbred), normal/control and treated mice with Poly I:C.

Mouse groups			Initial body weight in grams	Final body weight in grams	Body weight gain, in grams	Spleen weight, in grams	Relative spleen wt. (Splenic index)
Strain	**Age**	**Exp. status**					
CD-1 Outbred Mice	**2 Month**	**Control**	22.30±0.835c	22.44±0.793c	0.22±0.107a	0.198±0.005d	0.900±0.042d
		Treated	22.80±0.200c	23.12±0.315c	0.40±0.167a	0.201±0.003d	0.888±0.012d
	6 Month	**Control**	25.82±0.630b	25.92±0.594b	0.54±0.121a	0.306±0.005c	1.200±0.028bc
		Treated	27.02±0.583ab	27.30±0.60ab	0.52±0.124a	0.295±0.004c	1.102±0.024c
	12 Month	**Control**	28.38±0.475a	28.64±0.477a	0.30±0.179a	0.376±0.004a	1.338±0.021a
		Treated	28.30±0.680a	28.78±0.622a	0.49±0.172a	0.319±0.009b	1.124±0.031b
Age			0.000 **	0.000 **	0.340	0.000 **	0.000**
Age X Treated			0.282	0.138	0.343	0.000 **	0.000 **
Balb/C Inbred Mice	**2 Month**	**Control**	19.74±0.476d	19.84±0.414d	0.14±0.068b	0.195±0.004c	1.006±0.039c
		Treated	20.14±0.615d	20.20±0.623d	0.10±0.032b	0.196±0.005c	0.986±0.027c
	6 Month	**Control**	22.18±0.483c	22.50±0.449c	0.32±0.058ab	0.252±0.016bb	1.150±0.072b
		Treated	23.04±0.244bc	23.30±0.321b	0.34±0.087ab	0.279±0.007b	1.230±0.033b
	12 Month	**Control**	24.18±0.440ab	24.42±0.491a	0.30±0.110ab	0. 320±0.009a	1.342±0.024a
		Treated	25.26±0.448ab	25.64±0.518a	0.42±0.128a	0.317±0.011a	1.264±0.054a
Age			0.000 **	0.000 **	0.021*	0.000 **	0.000**
Age X Treated			0.051	0.054	0.642	0.319	0.871

*Significance P<0.050; **More Significance P<0.010.

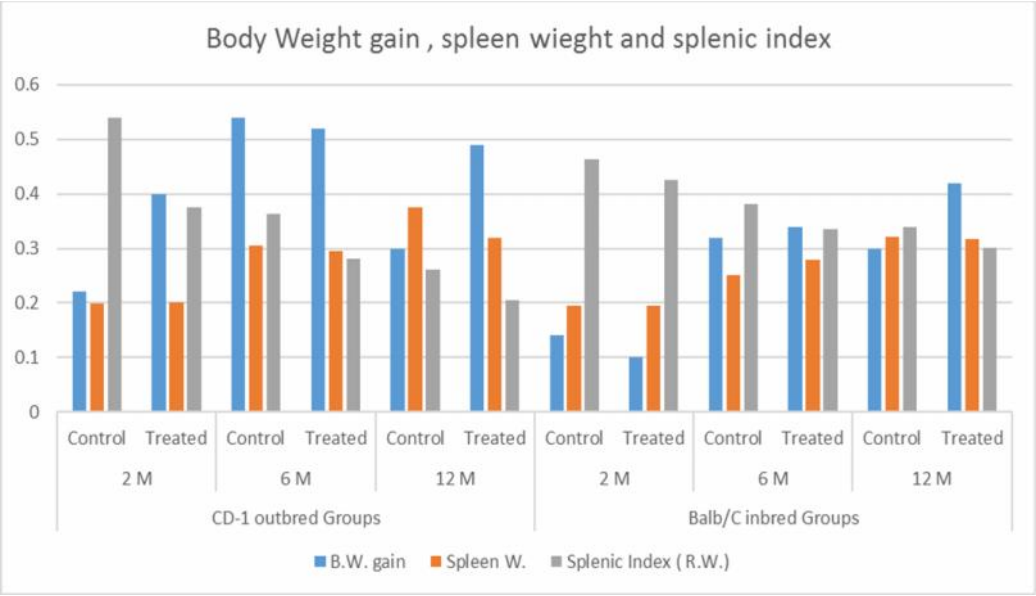

Figure (1): Animal gain weight, spleen weights as well as spleen relative weight (Splenic index) of CD-1 (Outbred) and Balb/C (Inbred) mice strains, controls and treated mice with Poly I:C acid.

In the same way, the normal/control individuals of inbred mice (Balb/C) showed significant rise in both absolute and body gain weights as it getting old. The body gain weights attained 0.14±0.068, 0.32±0.058 and 0.30±0.110 grams in the three studied age groups, respectively. Even this was happened too in the individuals of Balb/C mice exposed to Poly I:C acid, where the body gain weight showed 0.10±0.032, 0.34±0.087 and 0.42±0.128 grams, respectively in the three age groups of 2, 6 and 12 months old, individually (Tab.1 and Fig.1).

In mice of the present studies, spleen is an elongated dark red encapsulated roughly triangular organ, in cross section. It is seated in the peritoneal cavity under the rib cage on the left corner of the abdominal cavity behind the stomach, adjacent to its greater curvature. Its gross appearance, size and weight are variable, depending on the age, strain and animal's health. Healthy spleen of normal/control individuals is of a soft, friable, smooth and dark-red coloured surface with small white spots in a dark-red background, representing the white pulps.

Otherwise, it is known that, the absolute spleen weights as well as splenic index are very important for evaluation of animal's health status. In the present investigation, the absolute spleen weight of normal/control CD-1 mice strain was significantly increased from 0.198±0.005 gram in mice of 2 month age to 0.306±0.005 and 0.376±0.004 gram in those of 6 and 12 months age, respectively. Similarly the splenic index also was raised too, getting 0.900±0.042, 1.200±0.028 and 1.338±0.021 in the same mice individuals of the three age groups, respectively. The treated CD-1 mice with Poly I:C also showed similar elevation of the absolute spleen weight and splenic index from 0.201±0.003 gram in the mice of 2 month old to 0.295±0.004 and 0.319±0.009 grams in mice of 6 and 12 month, realizing splenic index of 0.888±0.012, 1.102±0.024 and 1.124±0.031 in the same three age groups, respectively (Tab.1 and Fig.1). By the same token, spleen weight and splenic index of the normal/control Balb/C mice significantly increased throughout the three age groups of 2, 6 and 12 month old. Since, absolute spleen weight was 0.195±0.004 gram in the first age group raised to 0.252±0.016 and 0. 320±0.009 gram in the second age and third age groups, respectively. Consequently, the splenic index also raised to achieve 1.006±0.039, 1.150±0.072 and 1.342±0.024 in the same specimens of normal/control mice, respectively. Likewise, in the treated Balb/C mice with Poly I:C acid, the absolute spleen weight attained 0.196±0.005, 0.279±0.007 and 0.317±0.011 gram in mice of 2,6 and 12 month old which accomplished splenic index of 0.986±0.027, 1.230±0.033 and 1.264±0.054, correspondingly (Tab.1 and Fig.1).

From the same table (1) it is noted also that, the spleen weight as well as the values of splenic index significantly differed in between the various age groups 2, 6 and 12 months of the normal/control and treated mice of the two studied strains with P values less than 0.050 all. So, in the present studies, the spleen of the studied mice strains (CD-1, Outbred and Balb/C, Inbred) given Poly I:C acid with a dose of (400µg/100g b.w.) slightly affected, considering spleen weight and splenic index of the three age groups (2, 6 and 12 months).

Histopathology

In the current studies, in all individuals of the three age groups, 2, 6 and 12 months of the two investigated strains (CD-1 and Balb/C) spleen showed basic structure. In all, the spleen is covered externally by a splenic capsule almost enveloped in the visceral peritoneum. At the medial surface of the spleen there is a cleft, splenic hilum, through which blood vessels enter and leave the spleen.

Histologically, it was found that the surrounded externally splenic capsule composed of dense fibro-elastic connective tissue with mesothelial outermost layer. Irregularly widespread trabecula of smooth muscle and fibro-elastic tissue ramifying and proceeding from the splenic capsule into the splenic parenchyma. These splenic trabecula provide splenic parenchyma with an internal support and it comprise blood and lymph vessels as illustrated in figures (2, 4, 6) for normal/control CD-1 mice strain and figures (8, 10, 12) for normal/control Balb/C mice strain.

The splenic parenchyma of the studied mice is recognized into two large distinct partitions, the red pulp and the white pulp, distinguished histologically by their colouration even in fresh organ sections. The red pulp is composed of splenic sinusoid spaces, blood vessel network and the splenic cords of loose connective tissue embracing blood cells like as lymphocytes and macrophages in addition to some reticular cells in between reticular fibers. Nonetheless, white pulps are built-up mainly of dense and highly organized peri-arteriolar lymphoid cell population comprising lymphocytes and macrophages as well as trabecula framework similar to that found in the red

pulp. Pigment cells may also spread in between cells population of the white and red pulps particularly in the splenic trabecula in mice of both strains as shown in figures (2, 4, 6) for normal/control CD-1 mice strain and figures (8, 10, 12) for normal/control Balb/C mice strain.

However, from the present investigation it was found that, there are some variations between the different studied age groups. As an example, the fibrous splenic capsule and trabecula both became thicker in older mice as of 6 months old individuals (Figs.4 & 10) than the younger ones of 2 months old (Figs.2 & 8). Similarly, sinusoid spaces which became more evident in older mice as a result of pyknosis and consequently necrosis of different splenic cell populations in CD-1 and Balb/C mice (Figs. 6& 12, respectively).

On the other hand, examinations of the spleen sections of mice treated with polyinosinic:polycytidylic acid (Poly I:C), showed irregular splenic architecture with more fibrous trabecula and sinusoid spaces throughout the splenic parenchyma to the degree that, limits between white and red pulp started to disappear in young mice of 2 months age of both strain (Fig. 3 & 9). These distractive changes were multiplied in the older individuals but with variable degree. Thus in 6 months age mice of CD-1 and Balb/C, the white pulp was decolourized and detached from splenic parenchyma so demarcations between the two splenic compartments were lacked in addition to appearing of congested dilated blood vessels as illustrated in figures (5&11) of CD-1 and Balb/C mice, one-to-one. Further more extensive fibrous trabecula and extra-large sinusoid spaces were verified as well much decrease of lymphocytes population due to cell progressive nuclear pyknosis and subsequently necrosis and cell degeneration at the old age (12 months), of both strains (Figs. 7&13, respectively).

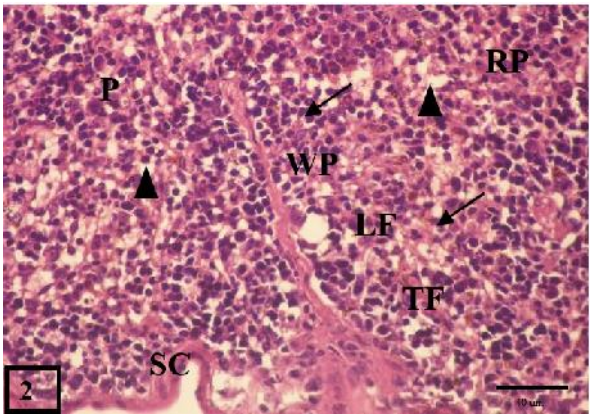

Figure (2): Photomicrograph of spleen section of 2 months age CD-1 mice (normal/control), stained with H&E, showing normal prominent splenic architecture.
Abbreviations: (Arrow) Lymphocytes**; (Double head arrow)** Pyknotic nuclei**; (Head arrow)** Sinusoid spaces**; (P)** Splenic parenchyma**; (Rp)** Red pulp**; (Sc)** Splenic capsule**; (Tf)** Fibro-elastic trabecula**; (Wp)** White pulp.

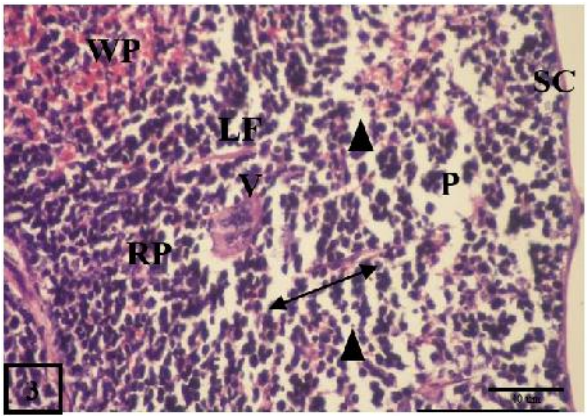

Figure (3): Photomicrograph of spleen section of 2 months age CD-1 mice treated with Poly I: C acid and stained with H&E, showing irregular reticular splenic architecture.
Abbreviations: (Arrow) Lymphocytes**; (Double head arrow)** Pyknotic nuclei**; (Head arrow)** Sinusoid spaces**; (P)** Splenic parenchyma**; (Rp)** Red pulp**; (Sc)** Splenic capsule**; (Tf)** Fibro-elastic trabecula**; (V)** Blood vessels**; (Wp)** White pulp.

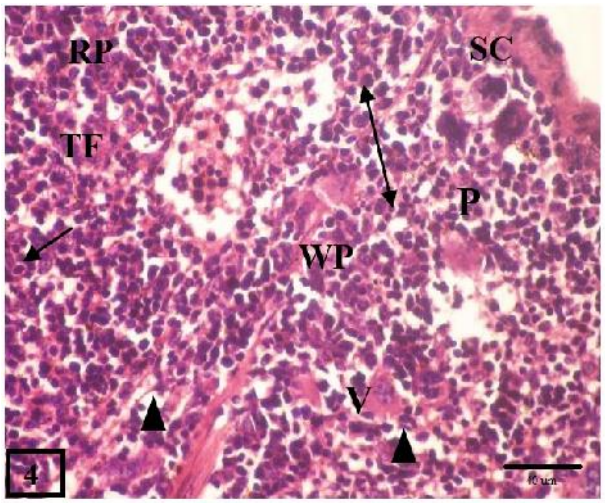

Figure (4): Photomicrograph of spleen section of 6 months age CD-1 mice (normal/control), stained with H&E, showing prominent splenic architecture.
Abbreviations: (Arrow) Lymphocytes**; (Double head arrow)** Pyknotic nuclei**; (Head arrow)** Sinusoid spaces**; (P)** Splenic parenchyma**; (Rp)** Red pulp**; (Sc)** Splenic capsule**; (Tf)** Fibro-elastic trabecula**; (V)** Blood vessels**; (Wp)** White pulp.

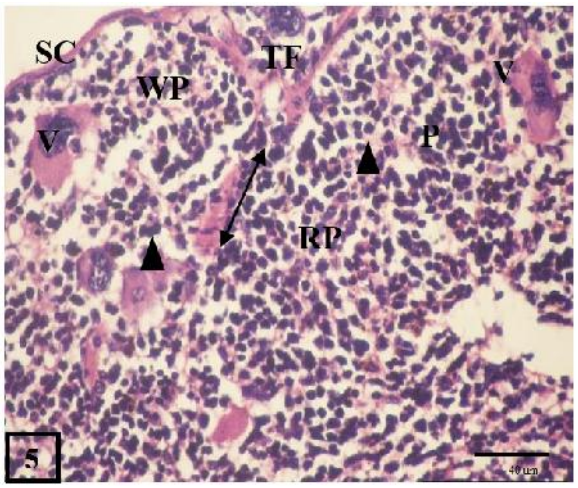

Figure (5): Photomicrograph of spleen section of 6 months age CD-1 mice treated with Poly I:C acid and stained with H&E, showing irregular reticular splenic architecture.
Abbreviations: (Arrow) Lymphocytes**; (Double head arrow)** Pyknotic nuclei**; (Head arrow)** Sinusoid spaces**; (P)** Splenic parenchyma**; (Rp)** Red pulp**; (Sc)** Splenic capsule**; (Tf)** Fibro-elastic trabecula**; (V)** Blood vessels**; (Wp)** White pulp.

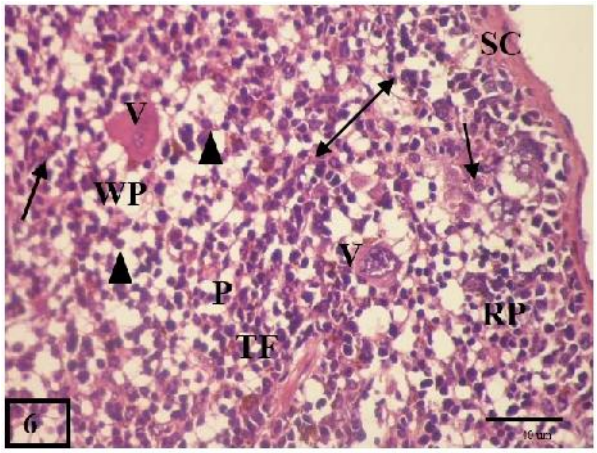

Figure (6): Photomicrograph of spleen section of 12 months age normal/control CD-1 mice (normal/control), stained with H&E, showing irregular splenic architecture.
Abbreviations: (Arrow) Lymphocytes**; (Double head arrow)** Pyknotic nuclei**; (Head arrow)** Sinusoid spaces**; (P)** Splenic parenchyma**; (Rp)** Red pulp**; (Sc)** Splenic capsule**; (Tf)** Fibro-elastic trabecula**; (V)** Blood vessels**; (Wp)** White pulp.

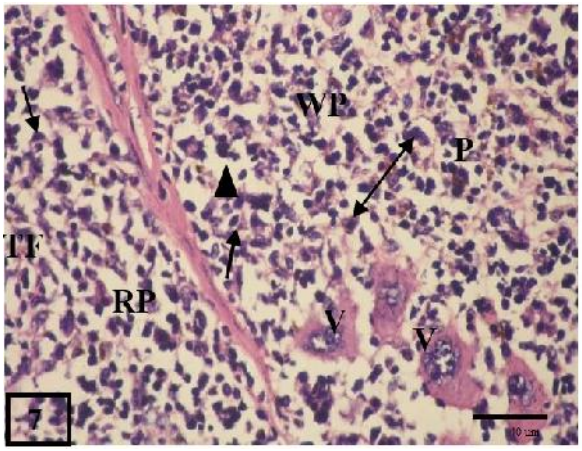

Figure (7): Photomicrograph of spleen section of 12 months age CD-1 mice treated with Poly I:C acid and stained with H&E, showing irregular reticular splenic architecture.
Abbreviations: (Arrow) Lymphocytes**; (Double head arrow)** Pyknotic nuclei**; (Head arrow)** Sinusoid spaces**; (P)** Splenic parenchyma**; (Rp)** Red pulp**; (Sc)** Splenic capsule**; (Tf)** Fibro-elastic trabecula**; (V)** Blood vessels**; (Wp)** White pulp.

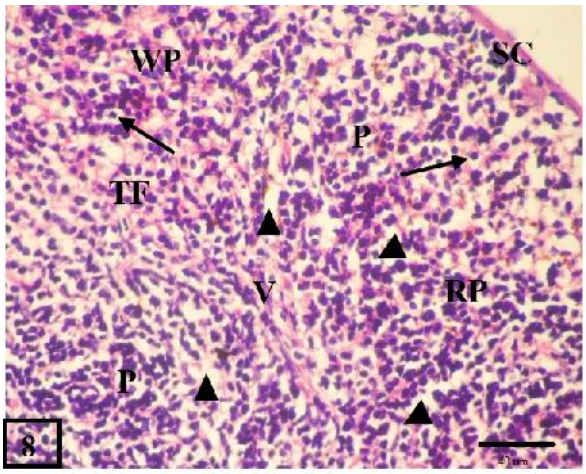

Figure (8): Photomicrograph of spleen section of 2 months age Balb/C mice (normal/control), stained with H&E, showing normal prominent splenic architecture.
Abbreviations: (Arrow) Lymphocytes**; (Double head arrow)** Pyknotic nuclei**; (Head arrow)** Sinusoid spaces**; (P)** Splenic parenchyma**; (Rp)** Red pulp**; (Sc)** Splenic capsule**; (Tf)** Fibro-elastic trabecula**; (V)** Blood vessels**; (Wp)** White pulp.

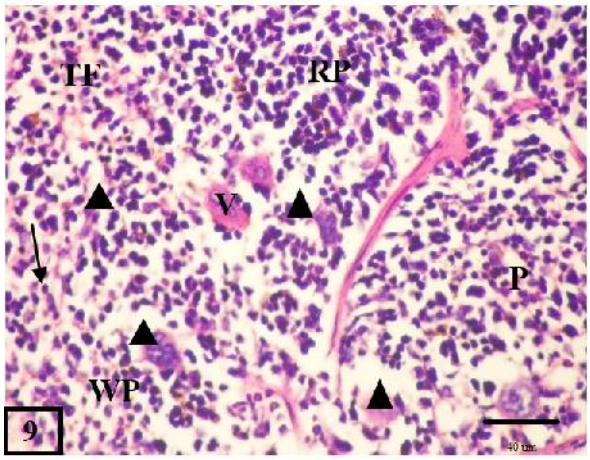

Figure (9): Photomicrograph of spleen section of 2 months age Balb/C mice treated with Poly I:C acid and stained with H&E, showing irregular reticular splenic architecture.
Abbreviations: (Arrow) Lymphocytes**; (Double head arrow)** Pyknotic nuclei**; (Head arrow)** Sinusoid spaces**; (P)** Splenic parenchyma**; (Rp)** Red pulp**; (Sc)** Splenic capsule**; (Tf)** Fibro-elastic trabecula**; (V)** Blood vessels**; (Wp)** White pulp.

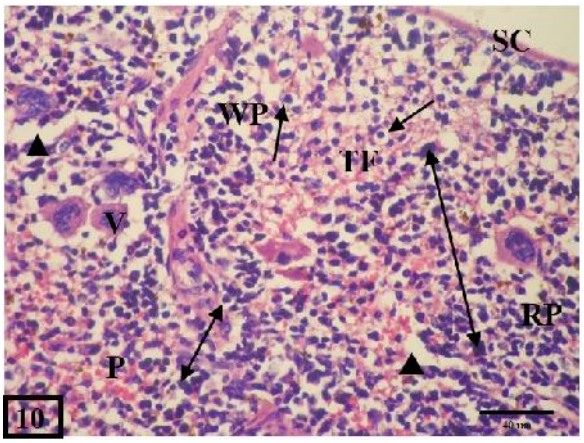

Figure (10): Photomicrograph of spleen section of 6 months age Balb/C mice (normal/control), stained with H&E, showing prominent splenic architecture.
Abbreviations: (Arrow) Lymphocytes**; (Double head arrow)** Pyknotic nuclei**; (Head arrow)** Sinusoid spaces**; (P)** Splenic parenchyma**; (Rp)** Red pulp**; (Sc)** Splenic capsule**; (Tf)** Fibro-elastic trabecula**; (V)** Blood vessels**; (Wp)** White pulp.

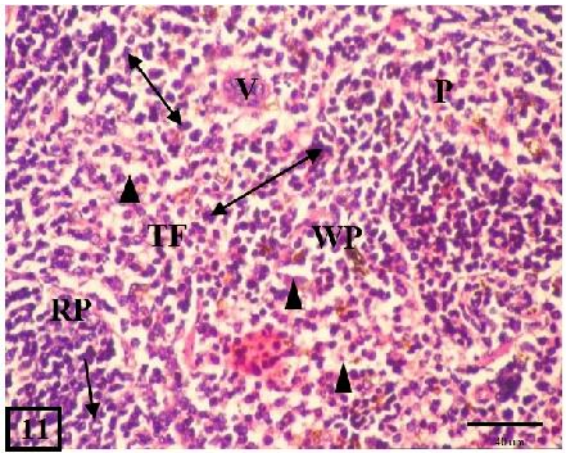

Figure (11): Photomicrograph of spleen section of 6 months age Balb/C mice treated with Poly I:C acid and stained with H&E, showing irregular reticular splenic architecture.
Abbreviations: (Arrow) Lymphocytes**; (Double head arrow)** Pyknotic nuclei**; (Head arrow)** Sinusoid spaces**; (P)** Splenic parenchyma**; (Rp)** Red pulp**; (Sc)** Splenic capsule**; (Tf)** Fibro-elastic trabecula**; (V)** Blood vessels**; (Wp)** White pulp.

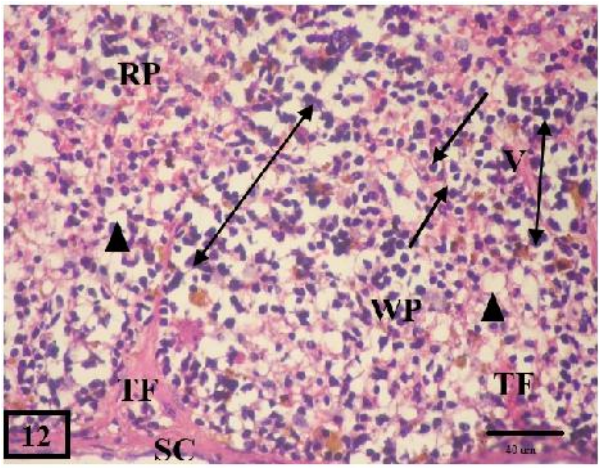

Figure (12): Photomicrograph of spleen section of 12 months age Balb/C mice (normal/control), stained with H&E, showing irregular splenic architecture.
Abbreviations: (Arrow) Lymphocytes**; (Double head arrow)** Pyknotic nuclei**; (Head arrow)** Sinusoid spaces**; (P)** Splenic parenchyma**; (Rp)** Red pulp**; (Sc)** Splenic capsule**; (Tf)** Fibro-elastic trabecula**; (V)** Blood vessels**; (Wp)** White pulp.

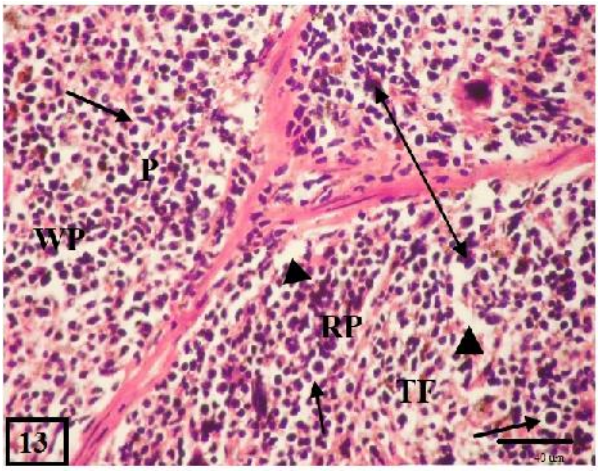

Figure (13): Photomicrograph of spleen section of 12 months age Balb/C mice treated with Poly I:C acid and stained with H&E, showing irregular reticular splenic architecture.
Abbreviations: (Arrow) Lymphocytes**; (Double head arrow)** Pyknotic nuclei**; (Head arrow)** Sinusoid spaces**; (P)** Splenic parenchyma**; (Rp)** Red pulp**; (Sc)** Splenic capsule**; (Tf)** Fibro-elastic trabecula**; (V)** Blood vessels**; (Wp)** White pulp.

Discussion

In the present study, the histology and some histochemical aspects of two lymphoid organs, spleen and thymus gland were investigated in two different mice strains; CD-1 (Outbred) and Balb/C (Inbred). This was done in normal animals of three different ages of 2, 6 and 12 months old as well as those treated with polyinosinic:polycytidylic acid (Poly I:C). This acid is commonly used as an immuno-stimulant in scientific research (**Loewen and Derbyshire, 1986**),it is a potent interferon inducer in rodents (**Hartmann *et al*., 1987**). The latter authors presumed that, Poly I:C showed significant therapeutic activity in a number of preclinical tumor models and significant toxicity in clinical settings. It can also stimulate and activate the major cellular components (lymphocytes, macrophages and dendritic cells) of the lymphoid organs of immune system (**Fortier *et al*., 2004**).

Concerning spleen which is the largest lymphatic organ is commonly found in all vertebrate animals. It is not only arrested reserve of blood and controls the amount of blood cells (**Mebius and Kraal, 2005**) but also it fights bacteria and serves also as the burial ground for damaged blood cells (**Brender *et al*., 2005**) and saves any useful components from blood broken cells, including iron to be reused (**Carey, 2006**). Spleen also watches of blood borne antigens by producing antibodies (**Al-Dahmesh *et al*., 2011**). So, it shows many variances between different animal species even within the strain individuals of the same species around life span.

The present studies showed that, in the both mice strains; CD-1 (Outbred) and Balb/C (Inbred), the size and weight of spleen are changed, depending on the animal's age and health status and either treated with Poly I:C or not. The spleen weight of CD-1 (Outbred) and Balb/C (Inbred) mice strains, in the three age groups, non-significantly increased as animal grow in age, from 2 months through 6 moths to the 12 months age. Similarly, the splenic index (spleen relative weight) of both strains also increased but with much less amplitude in the treated mice than normal/control individuals of the three age groups. Since, the spleen of the both strains (CD-1 and Balb/C) given Poly I:C acid with a dose of (400µg/100g b.w.) slightly affected, considering spleen weight and splenic index even within the same age group. Nonetheless, comparing the various age groups of the same mice strain, the oldest mice (12 months) were the more affected and vice versa the youngest (2 months) ones. The splenic index was fluctuated between 0.888 and 1.338 in CD/1 strain, but in Balb/C strain was between 0.986 and 1.342 for youngest and oldest in both, respectively. However, it is noted that spleen weight as well splenic index are higher in the treated individuals than the normal/controls within the same age group in both mice strains.

Losco early in **(1992)** explained that, in spite of the splenic index remains justly constant regardless of age and it is typically around 0.2% in rodents, the spleen is still a site of hematopoiesis, particularly in newborn individuals of high splenic index. The same author also showed that, spleen as a lymphoid organ contains about one-fourth of the body's lymphocytes, consequently it initiates immune responses to blood-borne antigens (**Balogh *et al*., 2004**). However, **Elmore (2006a**) hypothesized that spleen weight and consequently splenic index are relatively insensitive sign of immunotoxicity. Splenic index also increased in both Albino Oxford and Dark Agouti strain rats after cadmium-treatment (**Demenesku *et al*., 2016)**.

The present histological studies were done, since **Suttie** (**2006**) said that histological evaluation of the spleen, involves routine examination of cross-sections, may be adequate to diagnose pathological changes following senescence and/or treatment with any xenobiotic agent. So, in all mice individuals of the three age groups, 2, 6 and 12 months of the two investigated strains (CD-1 and Balb/C) spleen showed the basic histological architecture. It is found that the mice's spleen is surrounded externally by fibro-elastic splenic capsule as also reported by **Mahadevan (2016)**. He declared that the spleen is encased in a thin capsule almost enveloped completely in visceral peritoneum. In the present mice specimens, this capsule involves the splenic parenchyma which is distinguished into two partitions, the red and white pulps recognized primarily by their colouration. However, both pulp partitions are composed of loose connective tissue embracing sinusoid spaces, blood vessel and blood cells like as lymphocytes and macrophages. Some reticular and pigment cells may be also diffuse in between the irregularly widespread fibro-elastic trabeculae framework radiated from splenic capsule into the splenic parenchyma forming an internal support. However, from the present investigation it was found that, there are many variations between the different studied ages and also following treatment with polyinosinic:polycytidylic acid (Poly I:C).

The most prominent variations under the effect of aging and/or Poly I:C acid are presented in irregularity of splenic architecture to the degree that, limitations between white and red pulps disappeared and they were detached from the splenic parenchyma. Also more obvious domination of sinusoid spaces and cellular vacuoles in addition to thickening of the fibrous splenic capsule and trabecula in older mice especially in those treated with (Poly I:C). Somewhat early, **Hartmann *et al*. (1987)** showed that, the high dose of Poly (I:C), significantly reduced the mice body weight and gotten some histological abnormalities like as reduction of blood platelets and also brought hepatic necrosis resulted in the production of tumor necrosis. Nonetheless, **Black *et al*. (1992)** declared that Poly (I:C)-LC significantly increases antitumor effector cell functions in many organs including spleen, lungs and peritoneum. Furthermore, **Wu *et al.* (2014)** confirmed that, hydrodynamic injection of Poly (I:C) enhances innate and adaptive immune responses in mice.

Morphological and histological alterations of splenic partitions, white and red pulps, in outbred albino rats and inbred Balb/C mice and Swiss albino mice under the effect of fluoride salts were avowed also by **Bely (2000)**, **Machalinska *et al*. (2002)** and **Podder *et al*. (2010)**, respectively. All of them declared also that, these alteration are potent at the higher dose of fluoride salt especially in spleen contrast to the other organs like as liver, kidneys and bone marrow showed little alteration**.** Since, fluoride salts induces apoptosis in spleen (**Chen *et al*., 2009**).Chili pepper in addition to its induced increase of spleen weight (splenomegaly) it altered splenic histological architecture in the form of enlargement of white and red pulps as well the splenic capsule (**Al-Dahmesh *et al*., 2011**).

Chemical toxicity of splenic cell populations, particularly lymphocyte may resulted in nucleic pyknosis followed by cellular necrosis of these populations typically characterize the cellular apoptosis as disclosed also by **Suttie (2006)**. He also declared that, these degenerative histological lesions as well as loss of absolute body weight and body weight gain, displayed in the aged mice of 12 month of the present study, are similar to that common in elderly rats timely investigated by **Suttie (2006)**. Furthermore, he also presumed that, these degenerative lesions displayed in the aged mice can occur naturally as an age-related change or as atrophy and fibrosis which may occur as direct or indirect related changes.

The splenomegaly is associated with enlargement of both white and red pulp due to proliferation of fibrous support and numbers of macrophages (**Tolosano *et al*., 2002**).In addition to that and because spleen is the body's filter against any foreign materials from the blood stream, large number of neutrophils migrate from the peripheral blood to be participating in the splenic parenchyma (**Fawcett, 1986**).Enlargement of the red pulp may be also attributed to the receiving of arterial blood, since it is the site where blood borne cells. This zone, red pulp, also traps circulating antigens and so it is important in the immunological activities of the spleen (**Cesta, 2006**).

Elderly animals by no mean, are immuno-deficient (**McElhaney and Dutz, 2008**). The main provider to decline immune function in the elders is the lymphocytes changes **(Nikolich-Zugich, 2009)**. **Kumar and Kumari (2013**) also assumed that, the adversely effect in the structure and function of the spleen may particularly due to the apoptosis of splenocytes in the degenerated white and red pulps in the wharf rats under the effect of Sodium fluoride, leading to the reduction of immunogenic response.

Otherwise, the parenchymal and capsular fibrosis demonstrated in elderly mice of both present strains can be occurred as a reparative process following splenic tissue injury as explained by **Goodman *et al*. (1984**). The same authors declared also that, splenic fibrosis can be prompted by the induced chemicals as Poly I:C used in the present studies. Fibrosis may be focal or diffused and it is primarily localized to the red pulp, although extension into the white pulp may be happened **(Suttie**, **2006**). However, capsular fibrosis which is usually focal is the more common and may occur in association with toxic lesions of the spleen. The same author said also that fibrotic regions may contain areas of hemorrhage and pigment deposition as cleared in the present elderly treated mice especially of Balb/C strain. However, **Babaei *et al*. (2014)** found that, no obvious difference in splenocytes morphology between control and treated rats with saffron petal extract. This extract only causes an increase in antibody response without any change in spleen histology. Even so, Ginger, *Zingiber officinale*, extract produces lymphoid hyperplasia in the splenic cells, so it may have harmful effects on the spleen cells at high doses (**Udo-Affah *et al*., 2015**). Otherwise, the deposition and presence of pigments in splenic

parenchyma of elderly treated mice especially of Balb/C, may be happened as result of transformation of hemoglobin of phagocytized erythrocytes to hemosiderin to be stored in the splenic tissue **(Losco, 1992).** Since, **Tolosano *et al*. (2002)** stated that, the iron pigments are the most present ones in the injured splenic parenchyma. **Suttie (2006)** also affirmed the scattering of hemosiderin pigments throughout the splenic parenchyma of aged rodents. Otherwise, **Ward *et al.* (1999)** indicated that melanocytes containing melanin may be also present in the spleen, particularly in the fibrous trabecula of red pulp.

Finally speaking, it can be said that, immunosenescence may change the innate immune system which is the first line of defense and is the early defense against pathogens. With age, cells of the innate system, particularly neutrophils, monocytes, macrophages and dendritic cells, undergo changes leading to disclosing function of the immune response. Since, neutrophils display reduced functions (**Fulop *et al*., 2004**) and vice versa monocytes have been increased in numbers (**Della Bella *et al*., 2006**). The spleen is the location of hematopoiesis, particularly in fetal and neonatal as well it is a center of phagocytes activity in animals including rodents **(Brender *et al*., 2005)**. **Swirski *et al*. (2009)** and **Jia and Pamer (2009)** explained that, in mice the red pulp of the spleen forms a reservoir that contains over half of the body's monocytes. These monocytes, upon moving to injured tissue turn into macrophages while promoting tissue healing **(Premi *et al*., 2010).** With age the continued production of lymphocytes, even though is limited and so, the presence of relatively slight numbers of lymphocytes in spleen elicited why functional immunity only declines in the elderly (**Montecino-Rodriguez *et al*., 2013**).However, the same author showed that, the effects of aging on the immune system will not be uniform between individuals.

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DOI:10.22192/ijarbs.2017.04.12.024	

How to cite this article:
Al-ZahabyA.S.; Salem M.L.; Abdul-Aziz K.K. and Serageldeen H.M. (2017). Comparative experimental studies on the spleen of young and aged Balb/c and CD-1 mice. Int. J. Adv. Res. Biol. Sci. 4(12): 226-241.
DOI: http://dx.doi.org/10.22192/ijarbs.2017.04.12.024