Antioxidant and anti-inflammatory effects of flaxseed oil and fish oil in fipronil induced oxidative stress in rats

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A B S T R A C T

The present study was designed to evaluate the antioxidant and inflammatory effect of flaxseed and fish oils in rats exposed to fipronil and bacterial wall containing lipopolysaccharides (LPSs). Sixty rats were divided into four groups each of 15 one. Group 1 served as control. Group 2, administered fipronil at dose of 15 mg/kg b.wt. orally for 15 days followed by single injection of LPSs (2×10^6 CFU/ rat) intraperitonially at day 16^{th}. Group 3, treated as group 2 plus administration of fish oil at dose of 270 mg/kg b.wt. orally daily all over the experimental period (30 days). Group 4, treated as group 2 plus administration of flaxseed oil at dose of 270 mg/kg b.wt. orally daily all over the experimental period. Serum was separated and used directly for determination of urea, creatinine, albumin, sodium, potassium, Tumor necrosis factor –alfa and Interleukin 1β. Kidney samples were taken for histopathological examination, Malondiadehyde (MDA), Glutathione peroxidase (GPx) and Superoxide dismutase (SOD). Results revealed that in group 2 there were significant elevations of urea, creatinine, MDA, TNF-α and IL-1β. Furthermore, there were significant decreases in albumin, sodium, potassium, GPx and SOD compared with control group. Meanwhile, administration of flaxseed and fish oils resulted in significant decrease in urea, creatinine, MDA, TNF-α and IL-1β and significant increase in albumin, sodium, potassium, GPx and SOD compared with group 2. Therefore it's concluded that flaxseed and fish oils have strong antioxidant and anti-inflammatory effects.

Keywords: *Oxidative stress, Inflammation, Fipronil, Lipopolysaccharides, Flaxseed oil, Fish oil.*

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1. INTRODUCTION

Oxidative stress is simply defined as a state in which oxidation exceeds the antioxidant systems in the body as a result of loss of harmony between them (Valko et al., 2006) while inflammation is considered an essential component of the immune response for fighting against pathogens invasion (Andrade et al., 2015 and Seki and Schwabe 2015). Several researches has documented that oxidative stress and inflammation are tightly interrelated in many diseases. They seem to occur simultaneously and also promote each other in injury site. In addition, continued oxidative stress can lead to chronic inflammation (Mittal et al., 2014).

Fipronil is the first member of the phenylpyrazole insecticide class with a broad spectrum action against insects, being used to

control fleas, ticks, ants, cockroaches and other insects (Tingle et al., 2003). It can be responsible for increasing the production of reactive oxygen species (ROS) in cells, which in turn lead to increase lipid peroxidation levels and oxidative stress (Bolton et al., 2000).

On the other hand, LPSs are glycoprotein components of the cell wall of gram negative bacteria (Frudenberg and Galanos, 1990) and considered to be potent inducer of the host immune system resulting in overproduction of numerous pro- and anti-inflammatory cytokines in addition to an increase in oxidative stress (Takamiya et al., 2009).

Antioxidants suppress the process of oxidation by neutralizing free radicals. As a result, the antioxidants themselves become oxidized and this explains why there is a constant need to replenish our antioxidant resources (Goldfarb, 1993).

Plant products also are increasingly recognized to have protective roles owing to their antioxidant and anti-inflammatory properties in addition they do not have any adverse effects too (Lam et al., 2016 and Anilla and Vijayalaskshmi, 2002).

Fish oil has been widely studied as a complementary therapy for treatment of inflammatory diseases and inflammatory pain (Nobre et al., 2013) as well as in prevention of oxidative stress (Muga and Chao, 2014).

Vijaimohan et al., (2006) and Cohen et al., (2005) pointed to the anti-inflammatory properities of flaxseed oil while Newairy and Abdou (2009) illustrated its antioxidant effect and rendered this to being the richest source of lignans. Therefore, the aim of the present study was to evaluate the antioxidant and inflammatory effects of flaxseed and fish oils in rats exposed to fipronil and bacterial wall containing lipopolysaccharides (LPSs) through evaluation of some biochemical, antioxidant, anti-inflammatory parameters and histopathological changes in kidney tissue of rats.

2. Materials and methods

2.1. Animals:

Sixty white male albino rats weighing 170-200 g. were used in the experimental investigation of this study. They were obtained from Laboratory Animals Research Center, Faculty of Veterinary Medicine, Moshtohor, Benha University. Rats were housed in separate metal cages with fresh and clean drinking water supplied ad libitum. Animals were kept at constant environmental and nutritional conditions throughout the experimental period. They were left 7 days for acclimatization before beginning the experiment.

2.2. Chemicals and agents used in the experimental protocol:

Lipopolysaccharides from killed *E.coli*: *Escherichia coli* (serotype O111) was obtained from Animal Health Research Institute, Benha branch. Lipopolysaccharides were obtained after killing *E.coli* strain.

Fipronil present in the form of liquid containing 20% fipronil under trade name (Fipromex 20% SC) obtained from MAC-GMBH Company for Agricultural Products and Chemicals, Germany.

Fish oil present in the form of soft gelatine capsule obtained from South Egypt Drug Industries Co. (SEDICO), 6 October City-Egypt under trade name (Omega-3 plus)® with concentration of 1000 mg.

Flaxseed oil present in the form of soft gelatine capsule obtained from Medizen Pharmaceutical Industries for NAPHA under trade name (Flax seed oil) ® with concentration of 1000 mg.

2.3. Expermintal design:

Rats were divided into four groups each of 15 one placed in individual cages and

classified as follow: Group 1 (Control), rats received no drugs, served as control for all experimental groups. Group 2, rats administered fipronil at dose of 15 mg/kg b.wt. orally for 15 days followed by single I.P. injection of LPSs (2×10^6 CFU/ rat) at day 16th. Group 3, rats administered fipronil at dose of 15 mg/kg b.wt. orally for 15 days followed by single I.P. injection of LPSs (2×10^6 CFU/ rat) at day 16th in addition to administration of fish oil at dose of 270 mg/kg b.wt. orally daily *(Hussein et al. 2013)* all over the period of the experiment (30 days). Group 4, rats administered fipronil at dose of 15 mg/kg b.wt. orally for 15 days followed by single I.P. injection of LPSs (2×10^6 CFU/ rat) at day 16th in addition to administration of flaxseed oil at dose of 270 mg/kg b.wt. orally daily *(Hussein et al. 2014)* all over the period of the experiment (30 days).

2.4. Sampling:

Blood samples for serum separation were collected from all animal groups at day 16th, 17th and 30th from retro-orbital venous plexus for separation of serum. Blood was collected in plain, clean well-dried centrifuge tubes for separation of serum to be used in quantitative determination of urea, creatinine, albumin, sodium, potassium, TNF-α, IL-1β. The collected blood samples were allowed to clot and serum samples were obtained by centrifugation at 3000 r.p.m. for 15 minutes. Sera were obtained by plastic aspiration pipette and transferred into clean, dry and labeled eppendorf tubes and kept in deep freeze till examination.

Kidney tissue specimens were collected at day 16th and 30th from all rats that were sacrificed by cervical decapitation. Kidney specimens were quickly removed, part of it preserved in clean, dry, labeled Eppendorf tubes and kept at - 20°C for MDA, SOD, and GPx determination. The other part was preserved in neutral buffered formalin solution (10%) for histopathological examination. The specimens were dehydrated in different grades of ethyl alcohol, cleared in xylene, embedded in paraffin then sectioned to 6 microns and stained by hematoxylin and eosin stain.

2.5. Biochemical analysis:

Serum urea, creatinine and albumin concentration were determined according to the methods described by *Kaplan (1984)*, *Jaffe (1986)* and *Young (1995)* respectively. Also serum sodium and potassium concentrations were determined according to the method described by *Henry (1974).*

MDA, GPx, SOD, TNF-α and IL-1β were determined by Enzyme Linked Immuno-Sorbent Assay (ELISA).

2.6. Statistical analysis:

Data obtained were statistically evaluated for the mean and standard error (S.E). Statistical analysis was performed with statistical package for the social science (SPSS) software. $p<0.05$ was considered statistically significant. Over all differences between groups were determined by one – way ANOVA.

3. RESULTS

The results presented in table (1) revealed that rats treated with fipronil + lipopolysaccharides showed significant increases in serum urea and creatinine concentrations at day 16th, 17th and 30th when compared with control rats. Meanwhile treatment with fish and flaxseed oils caused significant decrease in all measured parameters levels. A significant decrease in serum albumin, sodium and potassium concentrations were observed at day 16th, 17th and 30th when compared with control rats while treatment with fish and flaxseed oils caused a significant increase in the value of parameters determined.

The obtained results demonstrated in table (2) revealed that, rats treated with fipronil + lipopolysaccharides showed significant increases in L-MDA level with marked decrease in SOD and GPx activities in kidney tissue at day 16th and 30th when compared with control rats. In comparison with rats of group (2), rats treated with fish oil (group 3) and flaxseed oil (group 4) showed significant decrease in L-MDA level and marked increase in SOD and GPx activities in kidney tissue.

The current results illustrated in table (3) revealed that rats treated with fipronil + lipopolysaccharides caused significant increases in serum TNF-α and IL-1β concentrations at day 17th when compared with control rats. However, treatment with fish and flaxseed oils showed significant decrease in the values of these parameters.

The histopathological findings in group (2) showed congestion in the cortical inter tubular blood vessels and tufts of the glomeruli associated with focal inflammatory cells infiltration in between the degenerated tubules (fig. 2). Meanwhile treatment with fish oil decreased the toxic effects and showed congestion in the renal blood vessels and intertubular blood capillaries (fig. 3), while treatment with flaxseed oil showed mild degenerative changes which manifested by vesiculation of glomerular tuft and mild degeneration of the epithelial cells lining the renal tubules (fig. 4).

Table 1: Changes in serum biochemical parameters in control, Fipronil + lipopolysaccharides treated group, fish oil and flaxseed oil treated groups at day 16th, 17th and 30th.

Parameters	Periods	G1 (Control)	G2 (Fipronil + lipopolysaccharides)	G3 (Fish oil treated)	G4 (Flaxseed oil treated)
Urea (mg/dl)	Day 16th	19.38±1.15^{c}	44.80±2.00^{a}	24.62±1.13^{b}	21.03±1.19^{c}
	Day 17th	20.68±1.61^{c}	49.59±0.92^{a}	29.06±1.33^{b}	28.04±1.55^{b}
	Day 30th	22.24±2.06^{c}	40.67±0.53^{a}	25.36±1.40^{b}	24.09±1.59^{bc}
Creatinine (mg/dl)	Day 16th	0.70±0.01^{b}	1.68±0.06^{a}	0.67±0.03^{b}	0.70±0.06^{b}
	Day 17th	0.70±0.01^{b}	1.68±0.06^{a}	0.67±0.03^{b}	0.70±0.06^{b}
	Day 30th	0.74±0.03^{c}	1.60±0.12^{a}	0.98±0.08^{b}	0.56±0.15^{d}
Albumin (g/dl)	Day 16th	4.01±0.06^{a}	3.04±0.19^{b}	3.79±0.18^{a}	3.85±0.15^{a}
	Day 17th	4.07±0.09^{b}	2.83±0.16^{c}	4.93±0.08^{a}	4.29±0.20^{b}
	Day 30th	4.15±0.21^{b}	3.19±0.12^{c}	5.00±0.15^{a}	4.02±0.07^{b}
Sodium (mEq/L)	Day 16th	140±1.64^{b}	131±1.05^{c}	148±1.14^{a}	146±1.41^{a}
	Day 17th	141±1.52^{c}	130±0.84^{d}	151±1.41^{a}	146±2.47^{b}
	Day 30th	145±1.73^{b}	131±0.71^{c}	149±1.82^{a}	143±2.76^{b}
Potassium (mEq/L)	Day 16th	4.28±0.17^{b}	3.02±0.06^{c}	5.50±0.16^{a}	4.48±0.32^{b}
	Day 17th	4.34±0.18^{b}	2.86±0.09^{c}	5.30±0.15^{a}	4.38±0.26^{b}
	Day 30th	4.72±0.17^{b}	2.84±0.09^{c}	5.46±0.05^{a}	4.58±0.17^{b}

a, b & c: There is no significant difference (*P*>0.05) between any two means for the same attribute, within the same row have the same superscript letter. Data are presented as Mean ± SE. SE+ standard error.

Table 2: Changes of kidney L-MDA, SOD and GPx in control, Fipronil + lipopolysaccharides, fish oil and flaxseed oil treated groups at day 16th and 30th.

Parameters	Periods	G1 (Control)	G2 (Fipronil + lipopolysaccharides)	G3 (Fish oil treated)	G4 (Flaxseed oil treated)
MDA	Day 16th	0.12±0.02^{c}	0.37±0.03^{a}	0.23±0.03^{b}	0.18±0.02^{bc}
(nmol/ g tissue)	Day 30th	0.13±0.05^{c}	0.31±0.01^{a}	0.20±0.00^{b}	0.16±0.02^{bc}
SOD	Day 16th	0.34±0.02^{a}	0.21±0.01^{b}	0.22±0.03^{b}	0.23±0.01^{b}
(u/ g. tissue)	Day 30th	0.38±0.04^{a}	0.16±0.02^{c}	0.21±0.00^{c}	0.27±0.02^{b}
GPx (ng/g. tissue)	Day 16th	0.39±0.04^{a}	0.25±0.03^{b}	0.25±0.01^{b}	0.29±0.02^{b}
	Day 30th	0.35±0.04^{a}	0.19±0.01^{c}	0.23±0.01^{bc}	0.27±0.01^{b}

a, b & c: There is no significant difference (*P*>0.05) between any two means for the same attribute, within the same row have the same superscript letter. Data are presented as Mean ± SE. SE+ standard error.

Table 3: Changes in serum TNF-α and IL-1β concentrations at day 17th in different experimental animal groups.

Parameters	Periods	G1 (Control)	G2 (Fipronil + lipopolysaccharides)	G3 (Fish oil treated)	G4 (Flaxseed oil treated)
TNF-α (pg/ ml)	Day 17th	0.09±0.01^{c}	0.37±0.03^{a}	0.21±0.01^{b}	0.16±0.02^{b}
IL-1β (pg/ ml)	Day 17th	0.12±0.03^{c}	0.33±0.01^{a}	0.21±0.00^{b}	0.17±0.02^{bc}

a, b & c: There is no significant difference (*P*>0.05) between any two means for the same attribute, within the same row have the same superscript letter. Data are presented as Mean ± SE. SE+ standard error.

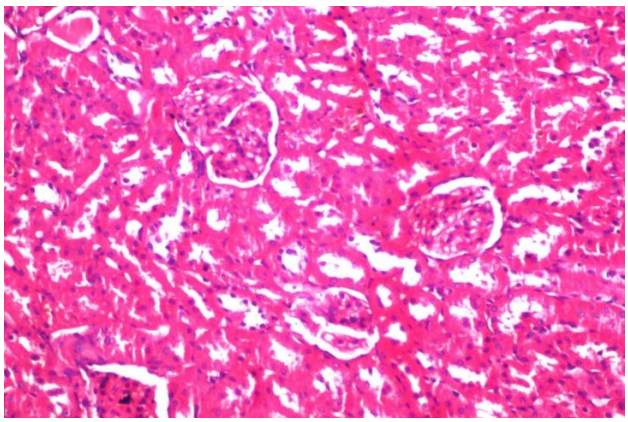

Fig.1. Photomicrograph of Control group at day 30th, showing normal histological structure of the kidney. H & E stain x 100.

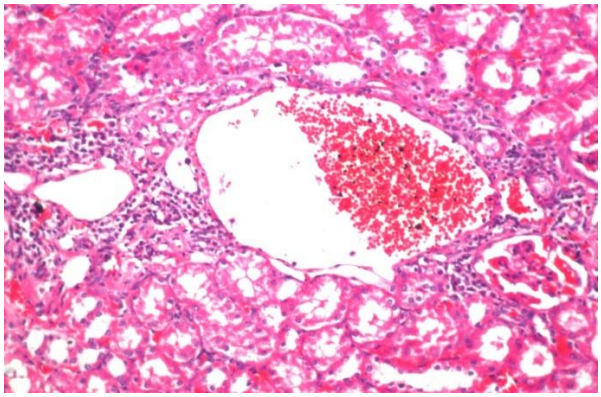

Fig.2. Photomicrograph of Fp + LPSs treated group at day 30th, showing severe congestion in renal blood vessels and glomerular tuft with focal mononuclear eosinophilic cellular infiltration in between the degenerated tubules. H & E stain x 200.

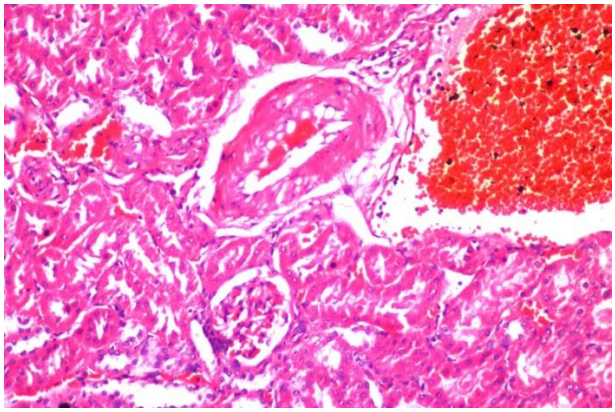

Fig.3. Photomicrograph of Fish oil treated group at day 30th, showing congestion in the renal blood vessels. H & E stain x 200.

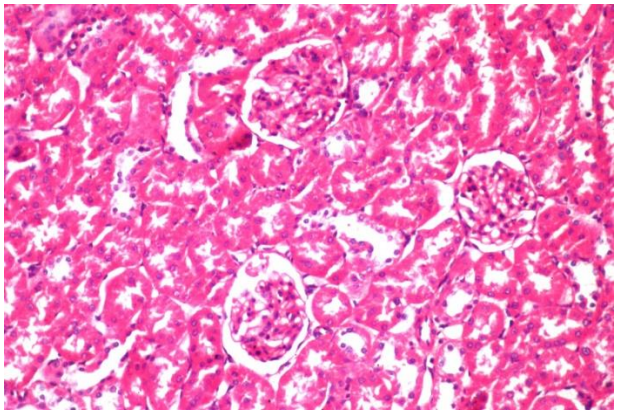

Fig.4. Photomicrograph of Flaxseed oil treated group at day 30th, showing mild degenerative changes in the form of vesiculation in glomerular tuft and mild degeneration of the epithelial cells lining the renal tubules. H & E stain x 200.

4. DISCUSSION

In the present study, the obtained data showed a significant increase in urea and creatinine concentrations in rats administered Fp + LPSs compared to control group which come in agreement with the study of Prarabdh et al. (2014); Er and Dik (2014).

Prarabdh et al. (2014) rendered the increase in serum urea and creatinine concentrations to that fipronil compromised the ability of the

kidney to filter waste products from blood. In addition, Er and Dik (2014) and Wen-Tien et al. (2010) illustrated that LPSs can induce hemodynamic changes and acute renal injury resulting in the reduction of glomerular filtration rate.

In fish oil treated group, there was a significant decrease in serum urea and creatinine concentrations compared to Fp + LPSs treated group. Similarily, De Caterina et al., (1993) documented that, administration of omega 3 causes an increase in plasma eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which is able to stimulate renal function. Some experimental studies have shown that omega 3 may increase thromboxane A_3 (TxA_3) formation, with a fall in thromboxane A_2 (TxA_2) and a significant increase in total prostacyclin levels. Thus this potential vasodilatory effect of ω3 may promote improved renal functions (Holm et al., 2001).

Flaxseed oil treated group revealed a significant decrease in urea and creatinine levels compared to Fp + LPSs treated group. This agree with the study of Khan et al. (2012) who suggested that omega-3 polyunsaturated fatty acids-enriched flaxseed oil attenuated the nephrotoxicity and oxidative damage induced by sodium nitroprusside intoxication. This also matches with the study of Omar (2016) who rendered this improvement of renal function to the antioxidant effect of flaxseed oil.

Concerning serum albumin concentration, the current study revealed significant decrease in Fp + LPSs treated group compared to control group. This comes in agreement with Balali-Mood, (2008) who rendered the decrease in albumin to the decreased synthesis by the liver in response to insecticide exposure. Also Mackiewicz et al. (1992) stated that inflammatory hypoalbuminemia occurs because albumin is a negative acute phase protein and this may be related to the production of IL-6 that increases the synthesis of acute-phase reactants by the hepatocytes while decreasing the production of albumin.

Fish oil treated group showed a significant increase in serum albumin level compared to Fp + LPSs treated group which come in agree with Hozayen et al. (2011) who rendered this to that fish oil treatment has the ability to restore the normal functional status of the poisoned liver and also protect against subsequent nephrotoxicity.

Flaxseed oil treatment also showed a significant increase in serum albumin level compared to Fp + LPSs treated group which come in harmony with the study of Farag et al., (2007) who suggested that this improvement could be returned to the relieving effects of flaxseed oil on hepatic architecture which is important for metabolism and excretion of toxic materials.

Referring to electrolytes, our study showed a significant decrease in serum Na^+ and K^+ levels in rats administered Fp + LPSs compared to control group which come in agreement with Prasad et al. (2011) who attributed this to alterations in influx/efflux of electrolytes at the kidney in response to pesticide exposure. On contrast, fish oil treated group showed a significant increase in serum Na^+ and K^+ levels compared to Fp + LPSs treated group which matches the study of Mannaa et al. (2011) who said that this may be due to the fact that dietary fish oil rich in eicosapentanoic and docosahexaenoic fatty acids may prevent the changes in Na^+/K^+-ATPase activity and by this mechanism prevent the membrane alteration.

Flaxseed oil significantly increased Na^+ and K^+ levels which come in agreement with Rizwan et al. (2014) who rendered this to that flaxseed oil improved integrity of renal

proximal convoluted tubules and its brush border membrane which is responsible for reabsorption of important ions and that may be altered due to toxic insult.

Regarding to parameters of oxidative stress, our study showed significant increase in MDA level in Fp treated group compared to control group. This come in agreement with Bolton et al. (2000) and Banerjee et al. (1999) who said that this elevation could be attributed to the fact that Fp can be responsible for increase in the production of reactive oxygen species (ROS) in cells, which in turn result in increased lipid peroxidation levels and oxidative stress (Bolton et al., 2000 and Banerjee et al., 1999).

Also, the present study recorded a significant decrease in kidney tissue SOD and GPx activities in rats administered Fp compared to control group which come in harmony with the study of Mossa et al. (2015) who rendered this reduction to several causes as excess production of O_2^- which is rapidly converted to H_2O_2 by SOD and to water by GPx, cellular injury and death of healthy cells that are able to respond to the oxidative insult as well as the insufficient detoxification capacity to Fp and damage caused by reactive oxygen species.

Fish oil treated group revealed a significant decrease in MDA level which matches the study of Gopal et al. (2011) who rendered this reduction to the ability of FO to inhibit lipid oxidation.

Concerning SOD and GPx as antioxidant enzymes, fish oil treatment showed a significant increase in their activities compared to Fp treated group. This comes in agreement with Priyamvada et al. (2014) and also with Mori and Beilin (2004) who illustrated that, omega 3 fatty acids up-regulate gene expression of antioxidant enzymes and down-regulate genes associated with production of ROS. In addition, Ozgomen et al. (2000) suggested that the cause of this improvement is that ω3 PUFAs, which has been supplemented, may be replaced with PUFA components of the membranes that had been attacked by oxygen free radicals such as O_2^-, H_2O_2 and OH^-.

Flaxseed oil treatment induced a significant decrease in MDA level and a significant increase in SOD and GPx activities in kidney tissues which come in harmony with Han et al. (2017) and Naqshbandi et al. (2012). Han et al. (2017) suggested that, the potential effect of flaxseed oil in preventing oxidative stress could be due to the ability to reduce free radical production or through increased free radical scavenging activity. On the other hand Makni et al. (2011) rendered these improvements to the relieving effects of flaxseed oil on hepatic and renal architectures which are important for metabolism and excretion of toxic materials.

Regarding to pro-inflammatory cytokines (TNF-α and IL-1β), the current study revealed a significant increase in these cytokines in Fp + LPSs treated group compared to control group which come in agreement with Korish and Arafa (2011) and Wu et al. (2000). This may be due to bacterial LPSs acts on macrophages to release TNF- α and then the secreted TNF- α induces the cells to produce IL-1 β and IL-6 as stated by Jean-Baptiste (2007) and Xaus et al. (2000). On the other hand, fish oil treated group showed a significant decrease in TNF-α and IL-1β compared to Fp + LPSs treated group which agree with Cao et al. (2011). This can be attributed to inhibition of the activation of nuclear factor κB (NFκB) that down-regulate the inflammatory response (Novak et al. 2003 and Calder, 2010). Meanwhile, flaxseed oil treated group showed a significant decrease in TNF-α and IL-1β concentrations compared to

Fp + LPSs treated group which agree with Archana et al. (2010).

The possible explanation may be attributed to α-linolenic acid (ALA) in the oil which could diminish the production of pro-inflammatory cytokines as stated by James et al. (2000). Also Ren and Chung (2007) rendered the down-regulation of TNF-alpha gene expression to that ALA can block the activation of nuclear factor-kappa β and mitogen-activated protein kinases in lipopoly-saccharide stimulated cells.

Concerning histopathological alterations, the present study revealed congestion in inter tubular blood vessels and tufts of the glomeruli with focal inflammatory cells infiltration in between the degenerated tubules in Fp + LPSs treated group which come in agreement with Mossa et al. (2015); Budin et al. (2013) and Abdel-Mottaleb and Zaki. (2008). Fish oil treated group when compared to Fp + LPSs treated group showed fewer infiltration with inflammatory cells and also lesser congestion of blood vessels which come in harmony with the study of Hussein et al. (2013) and Hozayen et al. (2011). Flaxseed oil treated group in the current study revealed no histopathological alterations which agree with the study of Omar (2016); Abdel Moneima et al. (2011) and Farag et al. (2007).

5. Conclusion

It could be concluded that flaxseed oil and fish oil are effective in improving the oxidative and inflammatory state generated by administration of fipronil and lipopolysaccharides, taking into consideration that flaxseed oil is more effective than fish oil.

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