Impact of Nigella Sativa Supplementation on the Outcome of Systemic Inflammatory Response /Multiple Organ Dysfunction Syndromes in Aged Rats

Nehal M. Bahgat[*1], Gehane M. Hamed[1], Rania A. Salah El Din[2], Ghada Z.A.Soliman[3]

[1]Physiology Department , [2]Anatomy Department Faculty of Medicine, Ain Shams University, [3]National nutrition institute (NNI), Egypt.
[*]nehalgamil@yahoo.com

Abstract: Systemic inflammatory response syndrome (SIRS) is a poorly understood condition that may proceed to multiple organ dysfunction syndrome (MODS), multiple organ failure (MOF) and death. Aged people are more susceptible to SIRS/MODS than adults with more morbidity, mortality and increased cost burden on health care systems. The present study was planned to investigate the effects of pretreatment with *Nigella Sativa* seeds on the prognosis of systemic inflammatory response/multiple organ dysfunction syndromes in aged rats. The present study was carried out on 42 aged male Wistar albino rats (23-25 months). Rats were allocated into three groups; Sham-operated group (C, n==10); Colonic ligation and puncture group (CLP, n=17) comprised of rats given distilled water (10 ml/Kg b.w./day) by gavage for 4 weeks and *Nigella Sativa*/Colonic ligation and puncture group (NS/CLP, n=15) comprised of rats given ground seeds of *Nigella Sativa* (1 g/Kg b.w./day) by gavage. for 4 weeks. Thereafter, CLP and NS/CLP rats underwent colonic ligation and puncture operation (CLP) and after 2 days, rats were examined for body weight, rectal temperature and ECG recording. Blood samples were withdrawn to estimate arterial blood gases, complete blood picture and serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine and adiponectin. Livers, kidneys and lungs were excised for histopathological study. Obtained results revealed that two days after colonic ligation and puncture operation, CLP and NS/CLP groups showed high death rate (35% and 20%, respectively), significant decrease in body weight, hyperthermia, hypoxemia, significant increase in serum levels of ALT and creatinine as well as significant decrease in serum adiponectin compared to C group. CLP group exhibited significantly higher *in vivo* heart rate, deeper Q wave, lymphopenia and elevated serum levels of AST compared to C group, while NS/CLP group exhibited significant decrease in hemoglobin content, packed cell volume and red blood cell count compared to C group. However, when compared to CLP group, NS/CLP rats showed significant decrease of Q wave voltage as well as leukocytosis. Histopathological examination of livers, kidneys and lungs from CLP group revealed leukocytic infiltration, cytoplasmic vacuolation and vascular congestion compared to C group. These changes were less extensive in NS/CLP group. In conclusion pretreatment with ground seeds of *Nigella sativa* in diet improved survival rate as well as some features of SIRS/MODS in aged rats.

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Key words: Multiple organ dysfunction syndrome (MODS), Systemic inflammatory response syndrome (SIRS), Colonic ligation and puncture (CLP), *Nigella Sativa* (NS). Intensive care unit (ICU).

1. Introduction

The concept of multiple organ failure and related abnormalities was first developed in the 1970s, when several reports appeared describing remote organ failure as a complication of severe sepsis **(Baue, 1997).** In the UK and USA, mortality rates due to multiple organ dysfunction syndrome (MODS) are currently comparable with and expected to exceed those from single-organ failure or unexpected cardiac arrest as a cause of death in the ICU **(Brown *et al.*, 2006 and Mayr *et al.*, 2007)**.

Systemic inflammatory response syndrome (SIRS) and eventually MODS are characterized by increased oxygen consumption, hyperglycemia, accelerated protein catabolism and unrecognized perfusion deficits **(Beal and Cerra, 1994)**. It has been reported that five of six patients who develop SIRS for more than 30 days proceed to severe MODS, and three of them die and it has been suggested that early recovery from SIRS might arrest the progression of organ dysfunction **(Yoshio *et al.*, 1997).**

Health benefits of *Nigella Sativa* seeds (and some of its active constituents, e.g. volatile oil and thymoquinone) have been known for centuries in folk medicine for treatment of hypertension, bronchial

asthma and asthmatic bronchitis **(Randhawa and Ghamdi, 2002)**. Scientific research reported anti-inflammatory, analgesic, antipyretic **(Abd–El–Fattah *et al.*, 2000)**, antineoplastic effect of these seeds **(Badary and Gamal El–Din , 2001)**, antimicrobial **(Morsi, 2000)** as well as hypolipidemic and hypoglycemic effects **(Benhaddou–Andaloussi *et al.*, 2010).** Also the seed has cytoprotective and antioxidant actions **(Kruk *et al.*, 2000),** in addition to their effect on some mediators of inflammation **(Alkharfy *et al.*, 2011)**

The present study was designed to investigate the effect of pretreatment of aged rats with *Nigella Sativa* ground seeds on the development of SIRS and progression to MODS.

2. Materials and Methods

Experimental animals

This study was carried out on 42 aged male Wistar albino rats (23- 25 months) weighing 370-410 g at the start of the experiment. Rats were purchased from Ophthalmic Diseases Research Institute (Giza) and housed 3/cage in plastic cages with food and water available *ad libitum* and were maintained in Physiology Department Animal House, Faculty of Medicine, Ain Shams University under standard conditions of boarding at room temperature.

Rats were randomly allocated into the following three groups:-

Sham operated group C (n=10); Comprised of rats fed ordinary rat chow and after 4 weeks were subjected to all the steps of colonic ligation and puncture operation but the cecum was neither ligated nor punctured.

Colonic ligation and puncture group CLP (n=17); Comprised of rats that were given distilled water (10ml/kg b.w./day) by gavage for 4 weeks. Then subjected to colonic ligation and puncture operation.

***Nigella sativa*/ colonic ligation and puncture group NS/CLP** (n=15); Comprised of rats given *Nigella sativa* ground seeds in oral suspension in a dose of (10 ml / Kg b.w./day) for 4 weeks by gavage ,then subjected to colonic ligation and puncture operation.

Nigella sativa oral suspension was prepared by grinding 10 gm of *Nigella Sativa* seeds and freshly adding 100 ml distilled water to get a final concentration of 100 mg of *Nigella Sativa* ground seeds / ml distilled water. The suspension was mixed thoroughly and given by gavage (10 ml/Kg b.w) to achieve daily intake of *Nigella sativa* (1 g/Kg b.w.) modified from **Al-Hariri et al. (2009).**

Experimental procedure

After 4 weeks, CLP and NS/CLP rats were subjected to induction of sepsis by cecal ligation and puncture operation. C rats were subjected to all the steps of colonic ligation and puncture operation except for ligation and puncture of the colon.

Colonic ligation and puncture operation (CLP):

Rats were weighed to estimate their preoperative body weight then anesthetized using diethyl ether (ADWIC). The rat was fixed on the dissecting table. A midline abdominal incision was made; the cecum was exteriorized and ligated by 3.0 silk ligature at its base without obstructing intestinal continuity. The cecum was punctured twice with an 18 gauge needle, squeezed gently to extrude a small amount of feces from the perforate site. The cecum was returned to the peritoneal cavity and the abdominal incision was closed with 3.0 running silk suture **(Zingarelli *et al.*, 2003)**. Fluid resuscitation of the animal was performed by flushing 0.5 ml sterile saline solution into the peritoneal cavity before closure of the abdominal wall **(Zantl *et al.*, 1998).**

After 2 days, all rats were fasted overnight; weighed to estimate postoperative body weight and rectal temperature was measured using medical thermometer. Rats were anesthetized by thiopental sodium (40mg/kg .i.p). ECG was recorded, then a midline abdominal incision was made to expose the abdominal aorta and blood samples were collected as follows

1. 0.5 ml blood in heparinized plastic syringe for determination of blood gases
2. 2 ml blood in plastic whole blood with spray- coated K_2 EDTA tube for complete blood picture (CBC).
3. 1 ml blood in clean plastic tubes, centrifuged at 4000 r.p.m. for 15 minutes to separate serum then stored at – 80^{o} C for later biochemical study.

1-ECG recording:

Needle electrodes were placed beneath the skin of the 4 limbs of the animal near the paws, and connected through an ECG coupler to a 2 channel oscillograph (Cardimax FX 121, Fukuda Denshi Co, LTD). The electrocardiographic tracing was recorded from lead II at paper speed of 25 mm/sec, heart rate, P-R interval, QRS duration, QT interval, Q wave voltage, R wave voltage and ST segment deviation were measured. The heart rate was calculated using the following equation:

$$HR = \frac{7500}{\text{Distance in mm between 6 successive peaks of R waves}}$$

2-Biochemical measurements:

Biochemical measurement of blood gases, CBC and serum levels of ALT, AST and creatinine were done in Alfa Laboratories, Cairo, Egypt. Blood gases were analyzed by ABL 5 blood gas analyzer (Diamond Diagnostics). CBC was analysed by Cell Dyn 1700

hematology analyzer (Abott Diagnostics). Serum creatinine was measured using Creatinine reagent OSR6178 kinetic color test (Jaffé method) for the quantitative determination of creatinine on Olympus analyzers according to **Mazzachi *et al.* (2000).** Serum alanine aminotransferase (ALT) was measured using OSR6007 kinetic UV test for the quantitative determination of alanine aminotransferase on Olympus analyzers according to **Thomas (1998).** Serum aspartate aminotransferase (AST) was measured using OSR6009 kinetic UV test for the quantitative determination of alanine aminotransferase on Olympus analyzers according to **Thomas (1998).** Serum adiponectin was estimated using Alpco ELISA kit for rat adiponectin (ALPCO Diagnostics) according to the method described by **Shimada *et al*.(2004).**

Lungs, kidneys and livers were excised and kept in 10% formaline for histopathological examinations, dehydrated, cleared in zylol and embedded in parablast. Paraffin sections were cut serially at 6 μm thickness and stained by Hematoxylin and Eosin (Hx & E) as described by **Drury and Wallington, (1980).**

Statistical Analysis:

All statistical data and significance tests were performed by using SPSS (Statistical Program for Social Science) statistical package (SPSS Inc) version 8.0.1 according to **Armitage and Berry (1987).** Statistical significance was determined by one-way ANOVA for differences between means of different groups; further analysis was made by LSD (least significance difference) to find intergroupal differences. A probability of $P< 0.05$ was considered significant. Correlations and Lines of Regression were calculated by linear regression analysis (ranking data directly or indirectly) using the Least Square Method. A probability of $P< 0.05$ (2-tailed). All data were expressed as mean ±SEM.

3. Results

Postoperatively, CLP and NS/CLP groups exhibited death rates 35% and 20% respectively.

Preoperative body weights were comparable between the three studied groups. However postoperative body weights were significantly *(P<0.05)* decreased in CLP and NS/CLP groups compared to C group. Body temperature was significantly *(P<0.05)* increased in CLP and NS/CLP groups compared to C group (Table 1).

ECG recording revealed significant*(P<0.05)* increase of *in vivo* heart rate in CLP group compared to C group and. Q wave was significantly *(P<0.05)* deeper in CLP group compared to C group but showed significantly *(P<0.05)* decreased depth in NS/CLP group compared to CLP group (Table 2; Fig. 1).

Blood picture revealed that CLP group had significant *(P<0.05)* lymphopenia compared to C group. NS/CLP group showed significant *(P<0.05)* decrease in blood hemoglobin content, packed cell volume and red blood cell count compared to C group and showed significant leukocytosis compared to CLP group(Table 3).

Arterial blood gas analysis revealed significant *(P<0.05)* hypoxemia in both CLP and NS/CLP groups compared to C group. Serum levels of ALT and AST increased significantly *(P<0.05)* in CLP group compared to C group, while in NS/CLP, only ALT increased significantly *(P<0.05)* compared to C group. Serum creatinine increased significantly *(P<0.05)* in CLP and NS/CLP compared to C group. Serum adiponectin showed significant *(P<0.05)* decrease in CLP and NS/CLP groups compared to C group (Table 4).

Correlation study of serum adiponectin versus other parameters in CLP and NS/CLP groups showed that in CLP group, serum adiponectin correlated negatively and significantly with body temperature, *in vivo* heart rate, Q wave voltage and serum levels of ALT and creatinine and correlated positively and significantly with arterial Po_2 and blood hemoglobin content. In NS/CLP group, the significant negative correlations between serum adiponectin and body temperature and serum levels of ALT and creatinine persisted while its correlations with *in vivo* heart rate, Q wave voltage and arterial Po_2 became insignificant (table 5).

Histopathological examination:

Microscopic examination of the livers of sham operated rats showed normal architecture of hepatic lobules with each lobule formed of radially arranged cords of hepatic cells extending from the central vein towards the periphery of the lobule separated by the blood sinusoids. The central vein was lined by flat endothelial cells, blood sinusoids were lined by flat endothelial and Von Kupffer cells. The hepatocytes were polygonal in shape with acidophilic cytoplasm and rounded vesicular nuclei with prominent nucleoli. Portal tracts were seen around the periphery of the lobule, each tract contained branches of hepatic artery, bile duct and portal vein and surrounded by connective tissue. On the other hand, microscopic examination of livers of CLP group revealed loss of hepatic architecture in the form of marked disruption of the cords of hepatocytes and poorly defined portal tracts. Hepatocytes especially at the periphery of the hepatic lobules exhibited signs of degeneration in the form of marked cytoplasmic vacuolation which was extensive in some areas giving a ballooning appearance of the hepatocytes. Prominent cellular infiltration was also observed between degenerated hepatocytes as well as marked congestion of the portal vein branches. Fibrous deposition increased around central veins and

portal tracts. Livers of NS/CLP rats retained almost normal hepatic architecture with less vacuolation of the cytoplasm of the hepatocytes and scanty fibrous deposition around central veins. The central vein and the branches of portal vein appeared normal. No signs of hepatocyte degeneration were observed (Figs.2-a,b,c).

Microscopic examination of the respiratory portion of the lungs of sham operated group showed thin alveolar septa lined with continuous simple squamous epithelium with dark flat nuclei and thin cytoplasm. The bronchioles were lined with normal simple ciliated columnar epithelium and its lumen appeared clear from any cellular debris. On the other hand, lungs from CLP group showed increased thickness of the alveolar septa with marked cellular infiltration, narrowing of alveolar spaces, desquamated cells with vacuolated cytoplasm in the lumen of some bronchioles, congested blood capillaries as well as aggregates of cellular infiltration and pink hyaline material in some areas. Lungs from NS/CLP group exhibited almost normal microscopic picture of the lung with thin alveolar septa and less cellular infiltration, wide alveolar spaces, less congested blood vessels and less desquamated cells in the bronchiolar lumen (Figs.3-a,b,c).

Microscopic examination of the kidneys from sham operated group showed normal appearance of renal corpuscles, proximal and distal convoluted tubules surrounded by sparse interstitial tissue. Each renal corpuscle was formed of a central tuft of anastomosing glomerular capillaries surrounded by Bowman's capsule with no cellular debris in Bowman's space. The proximal convoluted tubules were packed with large cuboidal cells with large spherical basal nuclei and acidophilic cytoplasm with no obvious demarcation between the cells. On the other hand, kidneys from CLP group showed cellular infiltration in some renal corpuscles, narrowed Bowman's spaces and dilated renal tubules as well as tubular cell degeneration in the form of vacuolated cytoplasm. In the NS/CLP group, renal corpuscles appeared almost normal with distinct glomerular capillaries and Bowman's capsule with no degenerative changes in tubular cells (Figs.4-a,b,c).

Table (1): Changes in preoperative body weight (BW_1, g), postoperative body weight (BW_2, g) and rectal temperature (Temp., ^{o} C) in Sham operated control (C), Colonic ligation and puncture (CLP) and *Nigella Sativa*/ colonic ligation and puncture (NS/CLP) groups.

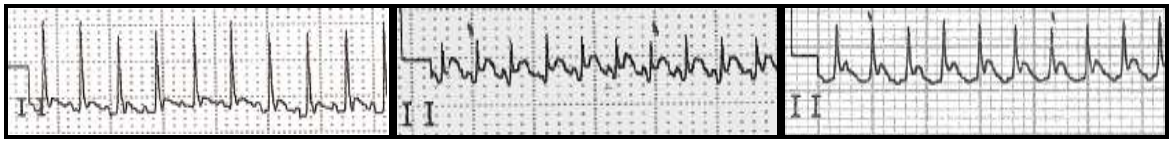
Groups	**BW_1 (g)**	**BW_2 (g)**	**Temp.(^{o} C)**
C(n=10)	390±3.9	390±3.9	37.6±0.6
CLP(n=10)	389±4.9	372±5[a]	38.3±0.1[a]
NS/CLP(n=10)	381±3.8	361±4.7[a]	38.5±0.2[a]
P	*NS*	*<0.001*	*<0.001*

a:significance by LSD at significance level P< 0.05 from C group.
b:significance by LSD at significance level P< 0.05 from CLP group.
P: significance by one way ANOVA among the three studied groups.
NS not significant
In parenthesis is the number of rats.

Table (2): Changes in Heart rate (HR, beats/min.), PR interval (msec.), QRS duration (msec.), QT interval (msec.), Q wave (μv), R wave (μv), and ST segment elevation (μv) in Sham operated control (C), Colonic ligation and puncture (CLP) and *Nigella Sativa*/ colonic ligation and puncture (NS/CLP) groups.

Groups	**HR** (beats/ min.)	**PR** (msec.)	**QRS** (msec.)	**QT** (msec.)	**Q** (μv)	**R** (μv)	**ST** (μv)
C (n=10)	426±11.4	52±3.2	46±3.7	92±6.1	55±5	625±61.5	130±25.7
CLP(n=10)	490±24 [a]	51±2.7	51±3.1	96±4.9	95±8.9[a]	495±70.4	95±11.6
NS/CLP(n=10)	474±19.1	52±4.4	45±3.7	90±6.8	65±7.6[b]	645±66.8	105±11.0
P	*NS*	*NS*	*NS*	*NS*	*<0.01*	*NS*	*NS*

a:significance by LSD at significance level P< 0.05 from C group.
b:significance by LSD at significance level P< 0.05 from CLP group.
P: significance by one way ANOVA among the three studied groups.
NS not significant
In parenthesis is the number of rats.



A *B* *C*

Figure (1): ECG tracing of Sham operated control group (C), *panel (A)*; Colonic ligation and puncture group (CLP), *panel (B)* and *Nigella Sativa*/ colonic ligation and puncture group (NS/CLP), *panel (C)*.

Table (3): changes in hemoglobin content (Hb, g/dl), Packed cell value (PCV, %), red blood cell count (RBC, 10^6/mm^3), white blood cells (WBC, 10^3 /mm^3), Neutrophil (N,%) and lymphocyte (L., %) in Sham operated control (C), Colonic ligation and puncture (CLP) and *Nigella Sativa*/ colonic ligation and puncture (NS/CLP) groups.

Groups	**Hb** (g/dl)	**PCV** (%)	**RBC** (10^6/mm^3)	**WBC** (10^3 /mm^3)	**N** (%)	**L** (%)
C(n=10)	13.6±0.1	39.9±0.4	7.1±0.1	9.1±1.2	30.1±6.2	62.7±6.7
CLP(n=10)	12.4±0.1	36.7±0.6	6.8±0.1	6.8 ±1.2	42.8±4.9	45.6±4.4[a]
NS/CLP(n=10)	11.8±0.9[a]	34.9±2.6[a]	6.23±0.4[a]	11.1±1.4[b]	43.4±4.5	50±4.6
P	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*

a:significance by LSD at significance level P< 0.05 from C group.
b:significance by LSD at significance level P< 0.05 from CLP group.
P: significance by one way ANOVA among the three studied groups.
NS not significant
In parenthesis is the number of rats.

Table(4):Changes in arterial blodd gases (Po_2, PCo_2, mmHg). Serum levels of alanine aminotransferase (ALT, U/L), aspartate aminotransferase (AST,U/L), creatinine (Cr., mg/dl) and adiponectin (ng/ml) in Sham operated control (C), Colonic ligation and puncture (CLP) and *Nigella Sativa*/ colonic ligation and puncture (NS/CLP) groups.

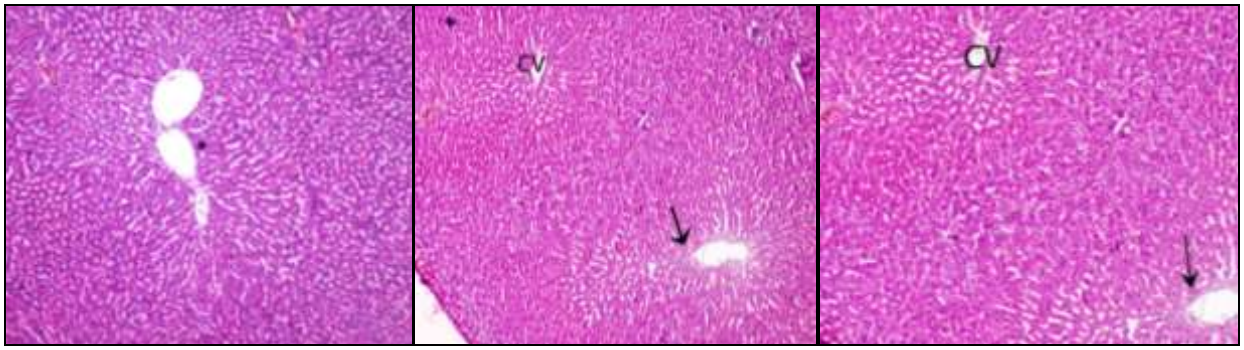
Groups	**Po_2**, (mmHg)	**PCo_2** (mmHg)	**ALT** (U/L)	**AST** (U/L)	**Cr.** (mg/dl)	**Adp.** (ng/ml)
C(n=10)	110±8.3	33.2±4.6	44.3±1.5	223.3±13.9	0.5±0.01	1.06±0.05
CLP(n=10)	82.7±6.6[a]	34.4±1.9	84.5±6.1[a]	291.4±24[a]	0.6±0.0[a]	0.2±0.04[a]
NS/CLP(n=10)	82.7±9.9[a]	33.6±2.1	71.6±5.4[a]	282.4±25	0.6±0.1[a]	0.4±0.02[a]
P	*<0.05*	*NS*	*<0.001*	*NS*	*<0.001*	*<0.001*

a:significance by LSD at significance level P< 0.05 from C group.
b:significance by LSD at significance level P< 0.05 from CLP group.
P: significance by one way ANOVA among the three studied groups.
NS not significant
In parenthesis is the number of rats.

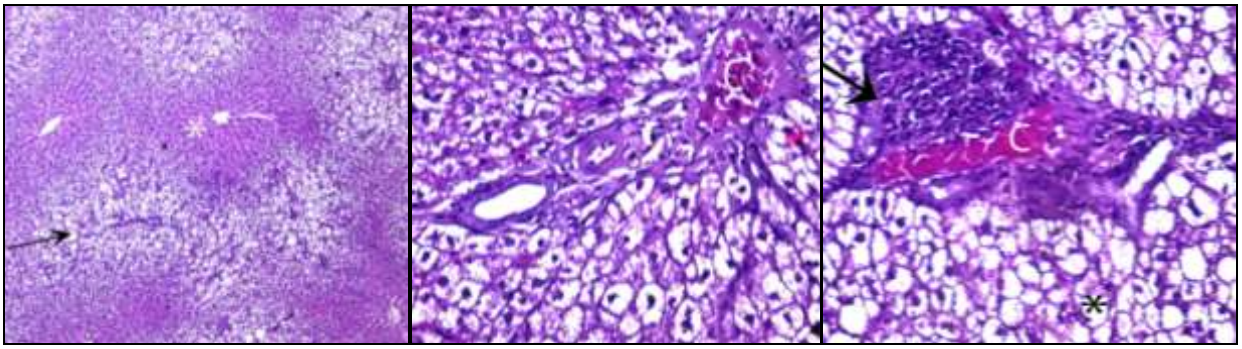
Table(5):Correlations of serum adiponectin with rectal temperature, heart rate, Q wave voltage, Po_2, Hemoglobin content (Hb), white blood cells (WBC), alanine aminotransferase (ALT) and creatinine (Cr.) in colonic ligation and puncture (CLP) and *Nigella Sativa*/ colonic ligation and puncture (NS/CLP) groups.

Groups		**Temp.**	**HR**	**Q**	**Po_2,**	**Hb**	**WBC**	**ALT**	**Cr**
CLP	*r*	-0.73	-0.48	-0.69	0.44	0.66	0.19	-0.82	-0.57
	p	*<0.001*	*<0.05*	*<0.01*	*<0.05*	*<0.01*	*NS*	*0.001*	*<0.05*
NS/CLP	*r*	-0.7	-0.28	0.32	0.39	0.447	-0.36	-0.7	-0.58
	p	*<0.01*	*NS*	*NS*	*NS*	*<0.05*	*NS*	*<0.01*	*<0.01*

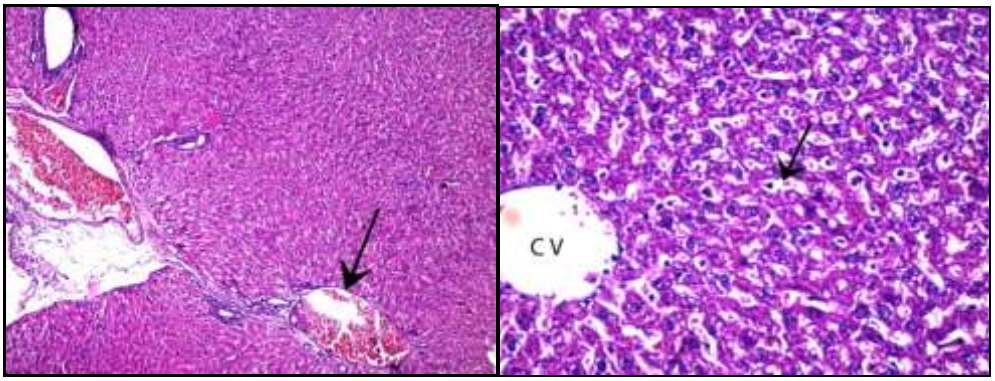
r: correlation coefficient *P: significance at the 0.05 level*



A (Hx &E . x200, x100 , x 200)
CV: central vein, Arrow: portal tract.

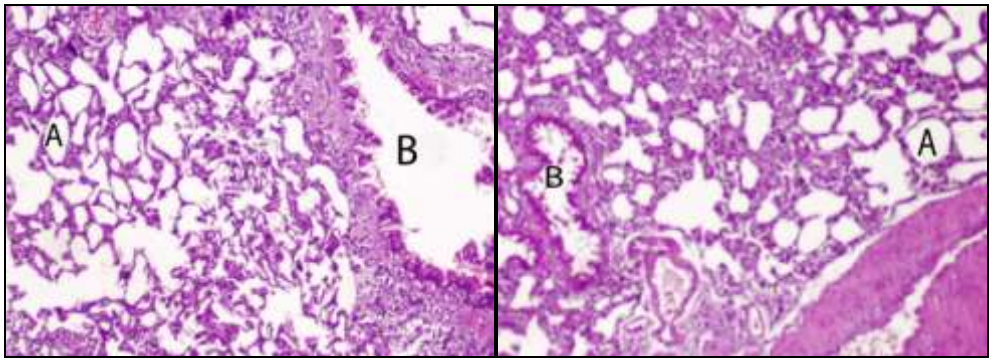


B (Hx &E . x100, x400 ,x 400)
C: congested portal vein, *: areas of vacuolations., Arrow: mononuclear infiltration.

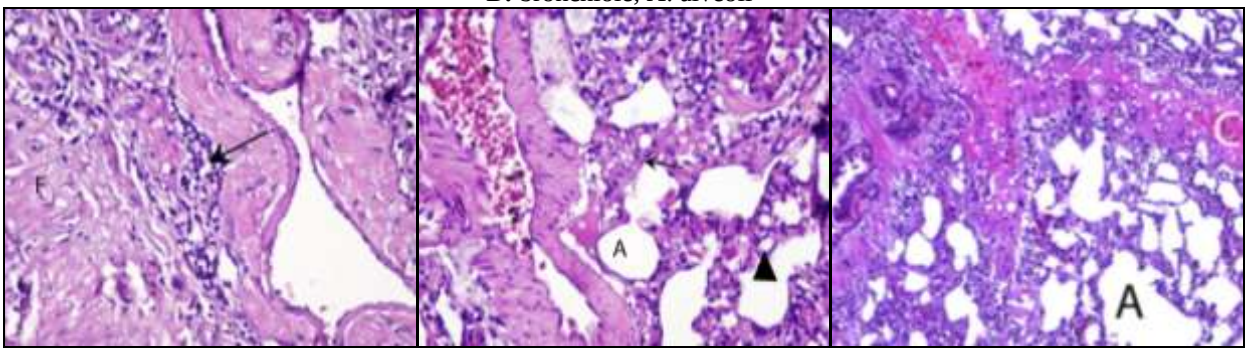


C (Hx &E . x100, x 400)
Arrow: portal tract, CV: central vein.

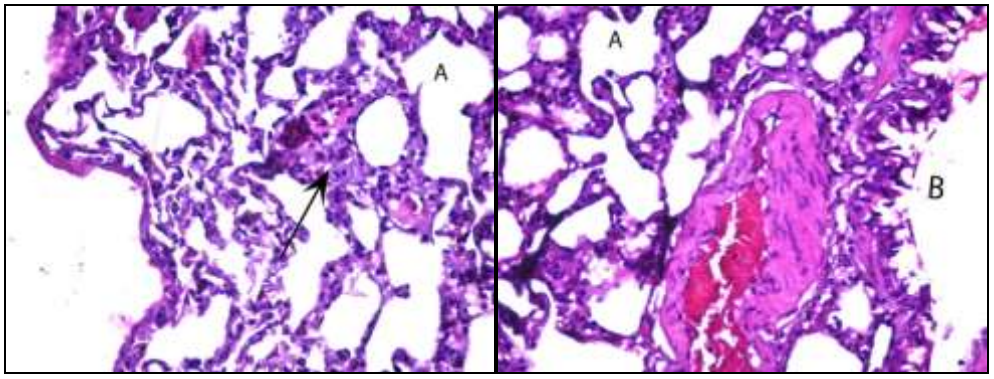
Figure .**(2)** (A) sham operated group(C) showing normal liver architecture; (B) colonic puncture and ligation group (CLP) showing disrupted liver architecture, poorly defined portal tracts, degenerated hepatocytes, cellular infiltration and congested blood vessels; (C) *Nigella Sativa*/colonic ligation and puncture group (NS/CLP) showing almost normal hepatic architecture.



A (Hx &E . x100 x 100)
B**:** bronchiole, A: alveoli

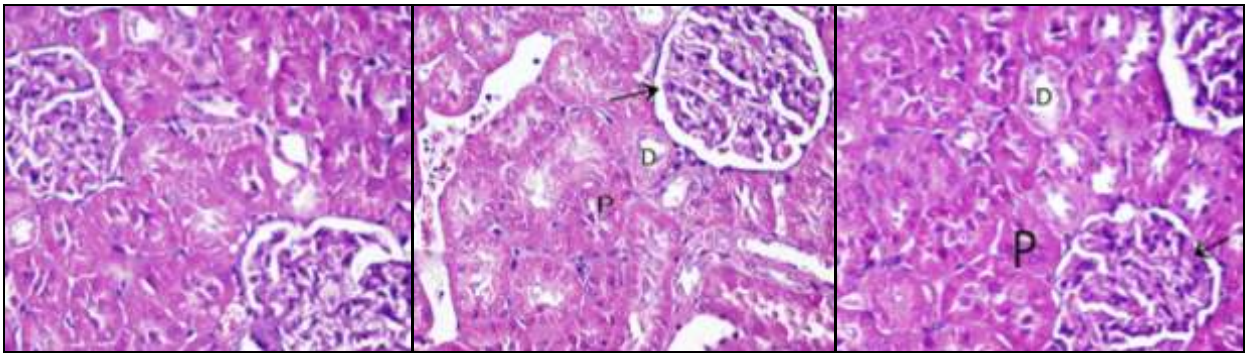


B (Hx &E . x400, x400, x 100)
Arrow: mononuclear infiltration, Arrow Head: vacuolations, A: alveoli.

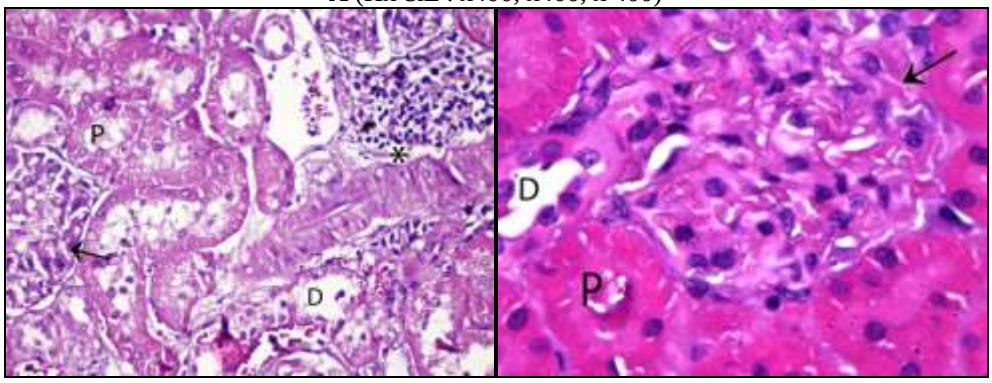


C (Hx &E . x400, x400)
Arrow: mononuclear infiltration, A: alveoli, B**:** bronchiole.

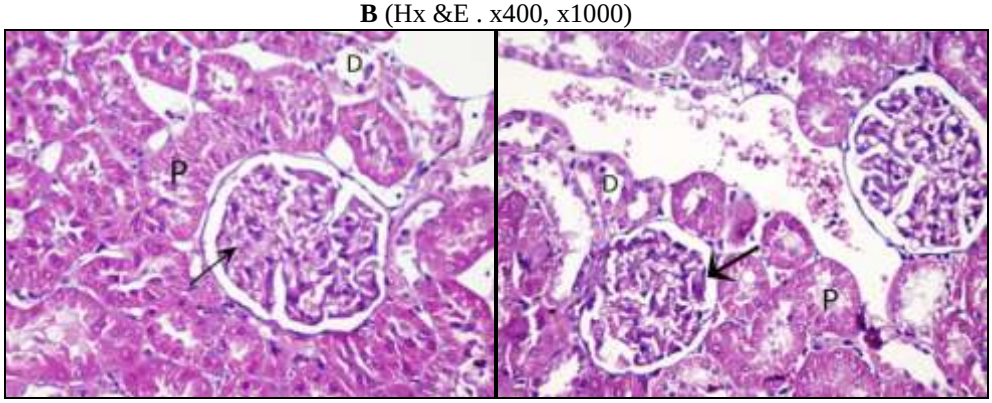
Fig.ure **(3)** (A) sham operated group(C) showing normal appearance of the lung; (B) colonic puncture and ligation group(CLP) showing thick alveolar septa with cellular infiltration, narrowing of alveolar spaces, desquamated cells with vacuolated cytoplasm in the bronchioles and congested capillaries; (C) *Nigella Sativa*/colonic ligation and puncture group (NS/CLP) showing almost normal appearance of lung tissue.



A (Hx &E . x400, x400, x 400)



B (Hx &E . x400, x1000)



C (Hx &E . x400, x400)

Arrow: Glomeruli, P: proximal convoluted tubules, D: distal convoluted tubules, *: mononuclear infiltration.

Figure.**(4)** (A) sham operated group (C) showing normal appearance of renal corpuscles and tubules;(B) colonic ligation and puncture group(CLP) showing cellular infiltration of renal corpuscles, widening of Bowman's spaces, dilated renal tubules with degenerative changes in tubular cells; (C) *Nigella Sativa*/colonic ligation and puncture group (NS/CLP) showing almost normal appearance of renal corpuscles and tubules.

4. Discussion

The concept of systemic inflammatory response syndrome (SIRS) was established in 1992, it describes a hyperinflammatory state represented by elevated levels of proinflammatory mediators with development of multiple organ dysfunction (MOD), multiple organ failure (MOF) and finally death **(Bone *et al.*, 1992).** Various therapeutic approaches were tried in experimental models of SIRS/MODS with variable success **(Zingarelli *et al.* 2003; Barichello *et al.*, 2007 and Park *et al.*, 2011)**. However, when these approaches were tried in human cases of SIRS/MODS, the results were disappointing partly due to the poorly understood pathology of this syndrome and partly due to the mismatching between animal models and human patients. One aspect of this mismatching is that most humans with sepsis and SIRS are above 50 years **(Martin *et al.*, 2003 and Derek *et al.*, 2001),** while animals used in

experimental studies were of young age. It had been reported that SIRS is a preventable condition and that early intervention in septic patients with supportive non specific measures could be beneficial **(Rivers *et al.*, 2001).**

In the present study, we chose CLP model of sepsis in aged rats as a surrogate of human sepsis, SIRS and MODS as previously described by **Overhaus *et al.(*2004) and Rittirsch *et al.*(2007).** Forty eight hours after the CLP operation, all rats showed wound sepsis and adhesions between the perforated colon and intestinal loops. Survival rate was 65 % for CLP group and 80 % for NS/CLP group which was consistent with the findings of **Zingarelli *et al.* (2003).** CLP and NS/CLP rats showed hyperthermia compared to sham operated rats which agree with the findings of **Zingarelli *et al.* (2003) and Rittirsch *et al.* (2007)** as well as leukocytic infiltration of remote organs which denotes a systemic inflammatory reaction rather than discrete organ affection. Manifestations of multiple organ dysfunctions were also observed in the form of tackycardia, low arterial Po_2, elevated serum levels of ALT and creatinine. The significant increase in body core temperature with sepsis can be explained by release of the endogenous pyrogen (IL-1) from blood leukocytes, tissue macrophages and large granular killer lymphocytes **(Guyton and Hall, 2006).** CLP rats showed insignificant change in their total leukocytic count from control values with observable decrease in lymphocytes. However, their vital organs (livers, kidneys and lungs) were heavily infiltrated with leukocytes which indicate exaggerated extravasation of white blood cells from unhealthy blood capillaries possibly due to accumulation of chemotactic mediators in these organs **(Neumann *et al.*, 1999).** On the other hand, the significantly higher leucocytic count in NS/CLP rats compared to CLP rats with less organ infiltration denotes potent immune response with less inflammatory reaction in the vital organs which might reflect restoration of the normal balance between immunity and inflammation, the disruption of which had been claimed to be the underlying pathology of this syndrome **(Butt and Shrestha, 2008)**. The significant decrease of hemoglobin content in NS/CLP group compared to normal control rats could be the result of excessive nutrient utilization by stimulated leukopoietic cells in the bone marrow, thus limiting the rate of erythropoiesis. Although both CLP and NS/CLP rats showed evidence of multiple organ dysfunctions, yet cardiac dysfunction was more evident in CLP rats. This can be deduced from the observation that although CLP and NS/CLP rats exhibited significant comparable body temperature, yet *in vivo* heart rate increased significantly only in CLP group compared to C group which was consistent with the findings of **Zingarelli *et al.* (2003).** Moreover, Q wave was significantly deeper in CLP rats compared to C rats which might indicate myocarditis **(Goldberger, 2006).** The significant drop in arterial Po_2 in both CLP and NS/CLP groups with no significant change in arterial Pco_2 indicates decreased diffusing capacity of alveolocapillary membrane, thus interfering with O_2 diffusion rather than Co_2 **(Barrett *et al.*, 2009).** Lack of significant difference in liver and kidney functions between NS/CLP and CLP rats despite regression of most of the inflammatory signs by microscopic examination of these organs in NS/CLP rats might be due to the short time interval between induction of sepsis and sacrifice of rats.

Up till now, pathogenesis of SIRS/MODS is not well understood but several clinical observations reported that a wide variety of systemic insults including surgery, trauma, burns or severe infection prime the host immune system with subsequent overwhelming reaction of the innate immune system **(Butt and Shrestha, 2008).** Primed polymorphnuclear neutrophils, macrphages and monocytes exhibit generation of reactive oxygen and nitrogen species (ROS and RNS), degranulation of enzymes, expression of cytokine and delayed apoptosis **(Schaeffer *et al.*, 2007).** Also, proinflammatory cascades (.e.g. the complement cascade) become activated with appearance of various mediators like TNF-α, IL-1, IL-6, C5a, **(Cavaillon *et al.*, 2003)**. This inflammatory milieu in different tissues will recruit more leukocytes with release of too much proinflammatory mediators and uncontrolled inflammatory response resulting in intavascular coagulation, tissue hypoxia, cellular damage, consumptive depletion of the clotting system, excessive release of anti-inflammatory mediators **(Bone, 1996)** , immunosuppression, anergy and SIRS/MODS **(Rittirsch *et al.*, 2007).** Nevertheless, a question remains to be answered about the triggering event that shifts the normal immune response into widespread uncontrolled inflammation of vital organs with subsequent failure and death. **Butt and Shrestha, (2008)** proposed the two hit hypothesis to explain the SIRS/ MODS which postulated that a severe insult like trauma or infection prime the host immune system so that a subsequent trivial insult produces a markedly exaggerated immune response of the host leading to MODS and death. Signs of disturbed metabolism in the form of increased levels of gluconeogenic hormones, diversion of skeletal muscle protein to splanchnic area, altered transport of glucose, and insulin resistance are serious sequelae of uncontrolled release of inflammatory mediators which would add more

risk to those patients with sepsis making them more susceptible to mortality **(Michie, 1996).**

Insight into this interwoven pathogenesis of SIRS / MODS requires that the effective therapy should have the potency of interrupting all these vicious circuits to restore the normal balance between inflammation and immunity. *Nigella Sativa* oil was reported to inhibit 5-lipoxygenase products in polymorphonuclear leukocytes of rats **(El-Dakhakhny *et al.*,2002)** as well as endothelin -1 production and oxidative stress induced by sepsis in rats **(Alici *et al.*, 2011)**. Thymoquinone- a constituent of *Nigella Sativa* was also reported to decrease inflammatory markers in septic rats like IL-1, IL-10, TNF and IL-2 **(Alkharfy *et al.*, 2011),** to normalize liver GSH and to decreases liver MDA and caspase-3 activity and serum levels of TNF-alpha and total bilirubin **(Helal, 2010)**. The observation that *Nigella sativa* -pretreated rats could retain almost normal microscopic picture of their lungs, livers and kidneys during sepsis was consistent with the findings of **Helal (2010)** and further confirmed the previous reports of the antinflammatory, antioxidant protective properties of Nigella Sativa seeds. Our study demonstrated that all rats with sepsis (CLP and NS/CLP groups) had significant decrease in serum adiponectin compared to sham operated rats which was consistent with the findings of **Lago *et al.*(2007)** and although serum adiponectin showed substantial increase in NS/CLP group, yet it was not significantly different from CLP group. Hepatic, cardiac and renal protection conferred by adiponectin can be deduced from the significant inverse relationship between it and body temperature, liver enzymes and creatinine in both CLP and NS/CLP groups and came in accordance with the results of **Kondo *et al.* (2010; Wang *et al.* (2010) ; Hamed *et al.* (2011) and Latif *et al.(*2011)**. Whether or not using a higher dose of *Nigella Sativa* for a longer duration would elevate serum adiponectin level towards normal values thus adding more protection during sepsis is a matter of debate that needs further investigation to be clarified.

Results of the present study demonstrated that dietary supplementation of aged rats with ground *Nigella Sativa* seeds improved their survival on exposure to SIRS/MODS that complicated a septic insult. *Nigella sativa* -pretreated rats showed amelioration of inflammatory changes in vital organs (liver, kidney and lungs) although organ dysfunctions were not evidently improved. *Nigella sativa* seeds can be included as dietary supplement for elderly people to improve their prognosis on exposure to sepsis. Clinical studies should be encouraged to extrapolate these findings to human patients.

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Corresponding author
Nehal M. Bahgat
Physiology Departmen**,** Faculty of Medicine, Ain Shams University
nehalgamil@yahoo.com

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