Influence of Propolis, Nigella sativa on toxicity induced by Chlorpyrifos administration in adult male albino rats.

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ABSTRACT

Background: Chlorpyrifos (CPF) is one of the most widely used Organophosphates (OPs) insecticides in agriculture and public health. Natural products (Propolis and Nigella Sativa) are a promising source for the discovery of new pharmaceuticals. Aim: The present study was an attempt to evaluate the benefits of Propolis and Nigella Sativa alone and in combination against toxicity induced by Chlorpyrifos in lung and heart tissues of adult male albino rats. Materials & Methods: 54 adult male albino rats were equally divided into 9 groups as following: Group I (negative control group); Group II: received the solvent. Group III (Propolis treated group): in which each rat received orally Propolis only rats (400mg/kg body weight) dissolved in corn oil. Group IV (Nigella Sativa oil treated group) each rat received orally Nigella Sativa oil (100 mg/kg) dissolved in corn oil. Group V: (Propolis and Nigella Sativa treated group). Group VI (CPF treated group): rats gavaged orally CPF at a daily dose of 6.75 mg/kg (1/20 of the oral LD50 of CPF (135mg/kg) dissolved in corn. Group 7 (CPF and Propolis treated group): rats gavaged orally Propolis before CPF administration. Group 8 (CPF and Nigella Sativa oil treated group): the rats gavaged orally Nigella Sativa before CPF. Group 9 (CPF, Nigella Sativa oil and Propolis treated group).

Results: Chlorpyrifos induced a highly significant increase in lactate dehydrogenase, creatine kinase activities, elevation of Galctin-3 and troponin levels, and a significant reduction in acetylcholine esterase activity. All these alterations were confirmed histopathologically in lung and heart tissues, where Chlorpyrifos induced myocardial necrosis, muscular hyalinosis, bronchopneumonia and bronchitis. Meanwhile, Propolis and Nigella Sativa showed a protective action against Chlorpyrifos toxic effects especially when used in combination. Conclusion: It can be concluded that Propolis and Nigella Sativa had an ameliorating effect against this toxicity induced in adult albino rats.

INTRODUCTION

Pesticides intended for agricultural and other domestic practices not only produce adverse biological effects against the target species but also have the potential to affect the health of non-target species including human being (¹). Several organophosphorus insecticides are widely used in various purposes. The unavoidable increased use of many new pesticides may cause great hazards to the living organisms, carelessly or wrongly application lead to pollution for the total ecosystem) i.e., air, earth, plant, water, animal and human ecosystem). A considerable numbers of these pesticides were reported to have acute, chronic, histopathologic and teratogenic activities (²). Organophosphates (class of insecticides) have a cumulative toxic effect to wildlife, so multiple exposures to the chemicals amplify the toxicity
Aim: Chlorpyrifos, [O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl)-phosphorothioate], is a broad-spectrum, chlorinated organophosphate insecticide that was first registered in 1965 to control foliage- and soil-borne insect pests on a variety of food and feed crops (4). The chief mechanism of action of OP pesticides is via the inhibition of neuronal cholinesterase, a key enzyme that is involved in neurotransmission (5). Acetyl-cholinesterase (AChE), hydrolyses acetylcholine (ACh) in cholinergic synapses and at neuromuscular junctions (6), this results in the accumulation of ACh in the synapses which in turn induces hyperactivity in cholinergic pathways. Besides, CPF elicits a number of other effects including hepatic dysfunction, immunological abnormalities, embryotoxicity, genotoxicity, teratogenicity, neurochemical, and neurobehavioral changes (7). The following signs and symptoms (weakness, headache, dizziness, visual disturbances, increased salivation, increased lacrimation, nausea, vomiting, lack of appetite, stomachache, restlessness or increased excitement, myosis, bronchial spasms, diarrhea, sweating, bradycardia, hypotonia, tremors, chest pain, difficult respiration, cyanosis of the mucous membrane, generalized convulsions, psychic disturbances, edema of lung, coma) are observed after CPF exposure (8). Nature produces an array of antioxidants to prevent free radical formation or to limit their damaging effects in the cell. Propolis or bee glue is a resinous product, collected by honey bees from plant exudates and mixed with wax and bee enzymes. It contains more than 160 constituents (9, 10). Propolis has several biological and pharmacological properties, as antimicrobial (11), anti-inflammatory (12), antioxidant (13–16), antiparasitic (17), immune modularity and immune stimulant effects and it increases the percentage of protected animals suggesting its use in vaccines as an adjuvant (18,19). Also, Nigella sativa commonly known as black seed or black cumin, are used in folk (herbal) medicine all over the world for the treatment and prevention of a number of diseases and conditions that include asthma, diarrhoea and dyslipidaemia. The seeds/oil has anti-inflammatory, analgesic, antipyretic, antimicrobial and antineoplastic activity (20). Therefore, the present study was aimed to elucidate the possible ameliorating role of Propolis and Nigella sativa oil in alleviating the toxic effects of Chlorpyrifos when administered to male rats separately and combinations to adult male albino rats.

Materials and Methods:

Materials:

Chemicals: Chlorpyrifos powder (CPF) obtained from Sigma, Aldrich, Germany imported by Cairo Chemical Company. Propolis obtained from Martinez Nieto, S.A., Cartagena, Spain as capsules, each capsule contains 400mg of purified Propolis. Nigella Sativa oil be obtained from PHARCO pharmaceutics as capsules, each capsule contains 400mg.

Animals: 54 adult male Swiss albino rats, each weighed 150 to 200 g were housed at the experimental animal house of the Faculty of Medicine, Zagazig University. The animals were maintained in controlled environment of temperature, humidity and light. They were fed on a commercial standard diet and tap water ad libitum.

Experimental design: 54 adult male albino rats were equally divided into 9 groups (6 rats / each group) as following: Group I (negative control group); these rats received only regular diet and tap water for 12 weeks to measure the basic parameters. Group II (solvent control group): each rat gavaged orally with 1 ml corn oil once daily for 12 weeks. Group III (Propolis treated group): in which rats gavaged orally Propolis (400mg/kg body weight) dissolved in corn oil, once daily for 12 weeks (21). Group IV (Nigella Sativa oil treated group): rats gavaged orally with Nigella Sativa oil (100 mg/kg) dissolved in corn oil, once daily for 12 weeks (22). Group V (Propolis and Nigella Sativa treated group): each rat was given both chemicals by the same manner mentioned above. Group VI (CPF treated group) rats were gavaged orally with CPF at a daily dose of 6.75 mg/kg (1/20 of the oral LD50 of CPF (135mg/kg)) (23) dissolved in corn oil for 12 weeks. Group VII (CPF and Propolis treated group): rats received orally Propolis (400 mg/kg body weight) before CPF (6.75 mg/kg body weight) once daily for 12 weeks. Group VIII (CPF and Nigella Sativa oil treated group: in which, rats gavaged orally with Nigella Sativa oil (100mg/kg body weight) before CPF (6.75 mg/kg
body weight) once daily for 12 weeks. And, Group XI (CPF, Nigella Sativa oil and Propolis treated group): rats gavaged orally with Nigella Sativa oil (100mg/kg body weight) and Propolis (400 mg/kg body weight) before CPF (6.75 mg/kg body weight) once daily for 12 weeks. At the end of the experiment, blood samples and tissues were collected from rats for biochemical and histopathological studies.

Methods:

(A) Biochemical study:

1- Assessment of acetylcholine esterase (AchE): Acetylcholinesterase Assay was determined by biodiagnostic kit (Biodiagnostic Company, Dokki, Giza, Egypt), according to the method of Ellman et al. (24).

2- Assessment of lactate dehydrogenase activity (LDH): LDH activity was measured through the decrease in NADPH concentration according to Bergmeyer, (25) method.

3- Assessment of creatine kinase-MB (CK-MB): CK activity was measured in the presence of an antibody to CK-M monomer according to Wu and Bowers, (26) method.

4- Assessment of Galctin-3 (Gal-3) levels in serum: Gal- 3 level was determined by using a sandwich Enzyme –linked Immunosorbant Assay Kit method, according to the method of Christenson et al., (27).

5- Assessment of Serum Troponin T (TnT): Troponin T (TnT) level was determined by using a sandwich Enzyme –linked Immunosorbant Assay Kit method (Cloud-Clone Crop), according to the method of Zhang et al., (28).

(B) Histopathological study: for histopathological examination, the lung and heart tissues were dissected, and the tissue samples were fixed in formalin solution for 24 h, processed using a graded ethanol series, and embedded in paraffin. The paraffin sections were cut into 5 mm thick slices and stained with hematoxylin and eosin and then examined microscopically according to Lillie, (29) method.

Statistical Analysis: Statistical analysis was performed using SPSS software II version 14 (30). The effect of each parameter was assessed using the one way analysis of variance. Individual differences between groups were examined using Dunnett’s test and those at p <0.05 were considered statistically significant.

Results

The biochemical and histological results of the first five groups [Group I, II, III, IV, and V] showed no statistically significant differences between them, so we used the results of the control group to compare it with the results of the remaining groups [VI, VII, VIII, and IX].

(A) Biochemical results:

Effect on Acetycholine esterase (AchE) activity: The AchE activity was found to be 362.1± 40.5 (U/L) in negative control group. This activity was significantly reduced in Group VI (CPF treated group) " to 154.2±5.0 by 57.4%, (p<0.001). Meanwhile, AchE activity was elevated to 225.0±12.2, 199.0±6.6, and 278.1±19.3 by 45.9%, 29.1%, and 80.4%; in Group VII (CPF and Propolis treated group), Group VIII (CPF and Nigella Sativa oil treated group "), and Group IX (CPF, Nigella Sativa oil and Propolis treated group); respectively, (p<0.01), compared to group VI, table (1).

Effect on Lactate dehydrogenase activity (LDH) activity: LDH activity was extremely significant increased from 234.2± 19.1 in group I to 3498.7±239.9 (U/L) in group VI by 1393.8%; (p<0.001). This activity was significantly decreased in group VII, group VIII, and group IX, to 1785.7±171.4, 2738.5±255.1, 493.6±56.0 by 48.9%, 21.7%, and 85.8%; respectively, (p<0.01) compared to group VI, table (1).

Effect on Creatine kinase-MB (CK-MB) activity: CK-MB activity was found to be 59.7±2.3 (UL) control group I. This activity was significantly increased in group VI to 226.1±12.3 (UL) by 278.7%, (p<0.001). This activity was reduced significantly to 117.4±7.5, 158.1±16.4, and 82.6±4.7 by 48.1%, 30.1%, and 80.463.5%; in groups VII, VIII, and IX; respectively, (p<0.01), compared to group VI, table (1).

Effect on Galctin-3 (Gal-3) levels in serum: The mean values of Gal-3 levels were highly increased from 3.7±0.08 (ng/ml) in control group to 18.3±1.55 in group VI “CPF treated”, by 394.6%, (p<0.001). These levels were significantly decreased to 10.24±0.96, 11.6±1.2, and 4.2±0.15 by 44.0%, 36.6%, and 77.0%, respectively in group VII, VIII, and IX, (p<0.01) compared to group VI, table (1).

Effect on Serum Troponin T (TnT): Troponin concentrations were significantly elevated to
from 64.9±5.2 in control group I to 276.7±11.55 (pg/ml) in group VI by 326.3%. But in groups VII, VIII, and IX troponin values were decreased to be 199.3±6.2, 232.1±7.88, and 94.6±4.7 by 27.9%, 16.1%, and 65.8%; respectively, (p<0.01), compared to group VI.

(B) Histopathological results: Fig. (I) [A, B, C, D, E] illustrated the photomicrographs of lung tissues in all studied groups. Normal Control group "I" showed normal lung tissue with the appearance of fine lace because most of the lung is composed of thin-walled alveoli (A). Chlorpyrifos treated group "VI" showed bronchopneumonia represented by severe bronchial and interstitial inflammatory reaction, and interstitial perivascular mononuclear cells infiltrations; as the appearance of sever interstitial inflammation and fibrosis leading to alveolar collapse with emphysema of other alveoli (B). In CPF + Propolis treated group "VII", lung tissues showed an interstitial perivascular mononuclear cells infiltrations, as illustrated in (C). Also, CPF + Nigella Sativa oil treated group "VIII" showed bronchopneumonia with bronchial obliteration, (D). While, CPF + Nigella Sativa oil + Propolis treated group "IX" Lung appeared with mild interstitial pneumonia, as showed in (E). Fig. (II) [A, B, C, D, E] showed the alterations in heart tissues in all studied groups. Control group showed normal myocardial muscles with normal arrangement of cardiac muscles (A). There were definite cardiotoxicity changes as a results of Chlorpyrifos treated group "VI", that showed sever myocardial necrosis, with leucocytic cells infiltration. Also, heart muscles appeared muscular hyalinosis, edema, and hemorrhage (B). In group "VII" CPF+ Propolis treated group, Heart showing congested blood vessel (C). CPF + Nigella Sativa treated group "VIII" heart showed minute areas of leucocytic cells infiltration (D). While, CPF + Propolis + Nigella Sativa treated group "IX" heart appeared normal arrangement of cardiac muscles with few hemorrhage (E).

Discussion
Organophosphates (OPs) due to their effectiveness, low cost and easy availability are the most widely used pesticides in agricultural practices and thus pose the greatest risk among all the pesticides to mammals, as they account for more than half of all the insecticides used in the world (31). As, Chlorpyrifos "CPF"-induced toxicity in rats, the present study was carried out to investigate the biochemical and histopathological alterations in lung and heart tissues of adult male albino rats and to evaluate the protective effect of Propolis and Nigella sativa against the possible toxicity caused by CPF-exposure. It was apparent from our results that oral administration of Chlorpyrifos "CPF" induced a marked increase in LDH, CK-MB activities, Gal-3, and troponin leaves compared to normal control group. While, Propolis and Nigella Sativa oil treatments (group VII, VIII, and IX) reduced significantly theses enzymes activities when they compared to group VI (CPF group). Also, CPF resulted in a marked decrease in AChE activity. Meanwhile, AChE activity was elevated in Group VII (CPF and Propolis treated group), Group VIII (CPF and Nigella Sativa oil treated group )", and Group IX (CPF, Nigella Sativa oil and Propolis treated group), (p<0.01), compared to group VI. These changes may be due to CPF induces toxicity through multiple mechanisms (32). Primarily, CPF and other organophosphate pesticides interfere with signaling from the neurotransmitter acetylcholine (33). CPF -induced toxicity results almost entirely from inhibition of neural acetyl cholinesterase by CPF and its bio-activation product, chlorpyrifos oxon (34). CPF is bio-activated in the liver to chlorpyrifos oxon (O, O-diethyl-O-[3, 5, 6, trichloro-2-pyridinyl] phosphate) via cytochrome P-450-dependent desulfuration (35). The oxon is rapidly hydrolyzed to TCP (3, 5, 6,-trichloro-2-pyridinol), probably by A-esterase (8). The oxon is 300-400 times more potent at inhibiting rat brain acetylcholinesterase than the parent compound (36). One metabolite of chlorpyrifos, chlorpyrifos-oxon, binds permanently to the enzyme acetylcholinesterase, preventing this enzyme from deactivating acetylcholine in the synapse (37).

By irreversibly inhibiting acetylcholinesterase, chlorpyrifos leads to a build-up of acetylcholine between neurons and a stronger, longer-lasting signal to the next neuron. The inhibition of the activity of cholinesterase enzymes causes an increase in the level of endogenous acetylcholine
in the organism and results in its binding to muscarinic and nicotinic receptors in both the peripheral and central nervous systems (CNS). Moreover, CPF has been reported that it was able to generate reactive oxygen species (ROS) in different tissue organs leading to the elevation in lipid peroxidation. The electrophilic metabolites or radicals can readily interact with essential biomolecules, including DNA, proteins and lipids, leading to oxidative modification, hence, structural and functional alterations. CPF has been shown to impair antioxidant enzyme activities either directly or through the induction of free radicals resulting in oxidative stress. A decrease of antioxidant enzyme activities was observed after CPF-exposure in rats. CPF-induced oxidative stress, that defined as the disequilibrium between pro-oxidants and antioxidants in the biological systems. Once this imbalance appears, cellular macromolecules may be damaged by the predominant free radicals. Although certain compound has been tested for the detoxification of CPF, there are no previous studies that carried out to evaluate the curative effect of Propolis against CPF-toxicity that cause dysfunction of the lung and heart. On a hand, Propolis is considered as one of the most promising natural products presenting not only therapeutic action, but also a preventive one. Propolis alone tended to prevent the damage and suppressed the leakage of enzymes through cellular membranes. The primary mechanism of Propolis effect may involve the scavenging of free radicals that cause lipid peroxidation and tissue damage. The other mechanism may comprise the inhibiting effect of Propolis on the activity of xanthine oxidase, which is known to cause free radicals to be generated. The biological effects exhibited by Propolis could be related to an overall effect of the phenolic compounds present in Propolis. It contains more than 300 compounds from different groups. It contains mostly a mixture of polyphenols, flavonoids (major ingredients), phenolic acids and their esters, caffeic acid and their esters, phenolic aldehydes and ketones; moreover, proteins, amino acids, vitamins (A, B1, B2, B3 and biotin), minerals (calcium, phosphorous, magnesium, manganese, iron, zinc, silicon, potassium, cobalt and copper). Flavonoids and esters of phenolic acids in Propolis have been recognized as antiseptic, cytostatic, antimicrobial, antibiotic, antiviral, antifungal, antibacterial and heptato-protective. On the other hand, the seeds of *Nigella sativa* contain both fixed and essential oils, proteins, alkaloids and saponin. Much of the biological activity of the seeds has been shown to be due to thymoquinone, the major component of the essential oil, but which is also present in the feed oil. The pharmacological actions of the crude extracts of the seeds (and some of its active constituents, e.g. volatile oil and thymoquinone) that have been reported include protection against nephrotoxicity and hepatotoxicity induced by either disease or chemicals. *Nigella sativa* has a protective effect against oxidative damage in isolated rat CPF- induced. It was found that the fixed oil of *Nigella sativa* has both antioxidant and anti-eicosanoid effects greater than thymoquinone which is its active constituent. *Nigella sativa* decrease lipid peroxidation and liver enzymes, and increase antioxidant defense system. Our results are reinforced by Abbassy *et al.*, who showed that, plasma cholinesterase activity of rats fed single doses of Chlorpyrifos (32 mg/kg body) was significantly reduced after 24 hours post feeding.

Also, our data were consistent with Abd El-Aziz *et al.*, who reported that, Propolis and ginseng have an effective antioxidant activity against Chlorpyrifos toxicity. Chronic CPF exposure caused significant hippocampal neuron damage (neurotoxicity), especially in CA3 region and significant decrease in brain AChE activity.

Our histopathological findings showed that, CPF induced severe alterations in both lung and heart tissues. Lung showing bronchopneumonia represented by severe bronchial and interstitial inflammatory reaction, interstitial perivascular mononuclear cells infiltrations. Also, Lung showing severe bronchitis, and peribronchitis. And, Heart showing severe myocardial necrosis, with leucocytic cells infiltration, muscular hyalinosis, edema, and hemorrhage. These results were consistent with Razavi *et al.*, who proved that OPs have chronic toxic effects have been accompanied with oxidative stress damages in...
different organs such as cardiovascular system. Acute-duration oral exposure to CPF has been shown to cause respiratory distress resulting from cholinesterase inhibition. Following chlorpyrifos exposure due to the insecticide's ability to cause bronchoconstriction and increase mucous secretions in the airways. Heart disease may also represent a group at particular risk due to both direct cardiac effects and restriction in airway diameter. As undetermined amounts of Chlorpyrifos has been shown to cause tachycardia. The initial response after exposure to an acetyl cholinesterase inhibitor is likely to be bradycardia because of stimulation of muscarinic receptors in the heart. When the cell membrane is damaged, varieties of enzymes normally located in the cytosol are released into the blood stream. Also, the elevation in the activity of LDH and CK-MB suggests an increase in lysosomal mobilization, cell necrosis and heart damage due to pesticide toxicity.

In this study, treatment with Propolis and Nigella sativa separately or in combination, ameliorated the lung and heart damage, especially in a combination treatment. In CPF+ Propolis treated group "VII" lung showed interstitial perivascular mononuclear cells infiltrations, and heart showed congested blood vessel. Moreover, in CPF + Nigella Sativa treated group "VIII", lung showing bronchopneumonia with bronchial obliteration, and heart showed minute areas of leucocytic cells infiltration. While, in CPF + Propolis + Nigella Sativa treated group "IX" lung showing mild interstitial pneumonia, and heart appeared normal arrangement of cardiac muscles with hemorrhage. These findings confirmed the protective effect of Propolis and Nigella Sativa.

General medicinal uses of Propolis have also been described; they include treatment of cardiovascular, blood system and respiratory disorders, and cancer, digestive tract disorders and immune system support and dermatological disorders. Propolis has been shown to stimulate various enzyme systems, cell metabolism, circulation and collagen formation. Caffeic acid phenethyl ester (CAPE) is an active component of Propolis and has been used in traditional medicine to treat a number of diseases, CAPE treatment have been shown to protect tissues from ROS mediated oxidative stress and reduce lipid peroxidation in ischemia and toxic injuries. The antioxidant activity of CAPE is due to the presence of two hydroxyl groups in its structure. Also, Propolis contains acid derivatives such as benzoic-4- hydroxy benzoic which improves the digestive utilization of calcium, phosphorus and magnesium. Therefore, the flavonoids of the Propolis can increase the activities of the antioxidant enzymes and reduce the levels of the ROS. It has also been shown that compounds isolated from of Nigella sativa seeds (including thymoquinone, carvacol, tanethole and 4-terpineol) have appreciable free radical scavenging properties. Therefore, the antioxidant action of Nigella sativa, particularly thymoquinone, may explain its claimed usefulness in folk medicine.

Many authors agreed with our findings, Jasprica et al., showed that Propolis and related flavonoids exercise their activity through the scavenging of hydroxyl, superoxide free radicals and lipid peroxides. The antioxidant activities of Propolis and its polyphenolic/flavonoid components are related to their ability to chelate metal ions and scavenge singlet oxygen, superoxide anions, peroxyl radicals, hydroxyl radicals and peroxynitrite. Also, Yousef et al., showed that the treatment with isoflavonones as antioxidant in combination with cypermethrin minimized its hazardous effects. Kanbura et al. found decreases in the plasma and the tissues malondialdehyde (MDA) levels and normalization in the antioxidant enzyme parameters of animals that were administered Propolis in association with propetamphos, in comparison to the group that was administered propetamphos alone.

Conclusion:
It can be concluded that, Chlorpyrifos can produce toxic effects on the lung and heart tissues of the adult male albino rats, and use of the Propolis and Nigella sativa especially in combination form can ameliorate this toxicity.

Recommendations:
1- Exposure to CPF and similar compounds should be reduced.
2- Propolis alone or in combination with Nigella sativa was proved to be beneficial in decreasing the levels of free radicals and increasing the activities of the antioxidant enzymes so the intake
of these natural products as supplement should be encouraged as a beneficial way to overcome the toxicity of CPF-exposure.

3- Periodical examination of occupationally exposed workers should be done.

4- The use of protective clothes is essential to minimize exposure to such chemicals during production, carriage, storage, and use.

5- Further studies on Propolis and Nigella sativa should be encourage.

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vitro antifungal and antioxidant activity of the essential oil and methanolic extract of Teucrium sauvagei Le Houerou,”. Natural Product Research; 20 (12), pp. 1089–1097.


Table (1): Biochemical markers in all studied groups:

<table>
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<tr>
<th>Group</th>
<th>AChE (U/L)</th>
<th>LDH (U/L)</th>
<th>CK-MB (U/L)</th>
<th>Gal-3 (ng/ml)</th>
<th>Troponin (pg/ml)</th>
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<td>362.1±40.5</td>
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<tr>
<td>% change</td>
<td>29.1%</td>
<td>-21.7%</td>
<td>-30.1%</td>
<td>-36.6%</td>
<td>-16.1%</td>
</tr>
<tr>
<td>IX</td>
<td>278.1±19.3</td>
<td>493.6±56.0</td>
<td>82.6±4.7</td>
<td>4.2±0.15</td>
<td>94.6±4.7</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>493.6±56.0</td>
<td>82.6±4.7</td>
<td>4.2±0.15</td>
<td>94.6±4.7</td>
<td></td>
</tr>
<tr>
<td>% change</td>
<td>80.4%</td>
<td>-85.8%</td>
<td>-63.5%</td>
<td>-77.0%</td>
<td>-65.8%</td>
</tr>
</tbody>
</table>

[a]: The mean difference at p<0.05, group compared to control normal group; [b]: The mean difference at p<0.05, groups compared to CPF treated “VI” group. 
Fig. (I): (A): Photomicrograph of Normal control group "I" Lung showing Apparently normal tissue.  
(B): CPF treated group "VI" Lung showing bronchopneumonia represented by severe bronchial and interstitial inflammatory reaction, interstitial perivascular mononuclear cells infiltrations. Also, Lung showing severe bronchitis (blue arrow), and peribronchitis (black arrow).  
(C): CPF+ Propolis treated group "VII" Lung showing interstitial perivascular mononuclear cells infiltrations (arrow).  
(D): CPF + Nigella Sativa treated group "VIII" Lung showing bronchopneumonia with bronchial obliteration (arrow).  
(E): CPF + Propolis + Nigella Sativa treated group "IX" Lung showing mild interstitial pneumonia (arrow).  

(H&E X 400)
Fig. (II): (A): Photomicrograph of Normal control group "I" heart tissues showing Heart showing normal myocardial muscles. (B): CPF treated group "VI" Heart showing sever myocardial necrosis (blue arrow), with leucocytic cells infiltration (black arrow). Also, muscular hyalinosis (blue arrow), edema (black arrow), and hemorrhage (arrow head). (C): CPF+ Propolis treated group "VII" Heart showing congested blood vessel (arrow). (D): CPF + Nigella Sativa treated group "VIII" heart showed minute areas of leucocytic cells infiltration (arrow). (E): CPF + Propolis + Nigella Sativa treated group "IX" heart appeared normal arrangement of cardiac muscles with hemorrhage (black arrow). (H&E X 400).