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Clinicopathological studies on the remodeling effect of Platelet-Rich plasma on lung fibrosis induced by amiodarone in Albino rats

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Abstract

Objectives: This study aimed to evaluate the role of platelet rich plasma (PRP) in the treatment of lung fibrosis induced by amiodarone drug. **Materials and Methods:** Seventy adult male Wistar albino rats were divided into three groups (n=20) plus ten rats were used for PRP collection. The first group used as control group. The rats in second group (gp.2) were injected intraperitoneally (i.p.) daily with amiodarone drug at (80 mg/ kg. bwt) for three weeks, then injected (24 hours after last dose of amiodarone) (i.p.) with phosphate buffer saline (PBS) (0.5 ml/ kg. bwt) two times weekly for three weeks. The rats in third group (gp.3) were injected (i.p.) daily with amiodarone drug at (80 mg/ kg. bwt) for three weeks, plus (i.p.) injection with platelet rich plasma (PRP) at dose (0.5 ml/kg. bwt) (24 hours after last dose of amiodarone injection) two times weekly for three weeks. The animals were examined during the experiment and sacrificed at the end of the experiment. **Results:** Rats in the PRP treatment (gp. 3) showed an increase in the level of WBCs and RBCs counts in comparison with group 2. Significant increase in glutathione reductase and a significant decrease in malondialdehyde levels were detected in group 3 when compared with group 2. The histopathological findings showed an improvement in the fibrosed lungs compared to gp (2). **Conclusions:** This study concluded the remodeling effect of PRP, which was observed clinically and pathologically against the harmful effects of amiodarone in albino rats.

Keywords: *Amiodarone; Platelet rich plasma; Lung injury; Rats.*

1. Introduction

Pulmonary fibrosis is a progressive and fatal lung disease characterized by fibroblasts proliferation and deposition of extracellular matrix in lung tissues. It is considered the end stage of a wide range of lung inflammatory conditions. It is initiated after collagen diseases, scleroderma (Rafii *et al*; 2013), viral infection, rheumatoid arthritis, radiotherapy, inorganic substances as (silica, asbestos) and developed as an adverse effect of some drugs as bleomycin and amiodarone (Gross and Hunninghake, 2001).

Pulmonary fibrosis is the most common interstitial lung disease affecting over five million individuals worldwide with limited therapeutic options and mean survival time about three years (Cottin, 2012). It is induced through different factors contributing the development and persistence of the disease including genetic factors, chronic lung injury, aging, oxidative stress, and impaired healing process (Chanda *et al*; 2019). There is no effective treatment reported for pulmonary fibrosis except lung transplantation (O'Brien *et al*; 2011).

Amiodarone is one of the most common iodine-containing compounds used for treatment of a wide variety of diseases as cardiac arrhythmias (Lapenna *et al*; 2001), ventricular tachycardia, ventricular fibrillation (Dorian *et al*; 2002) left ventricular dysfunction and heart failure (Chevalier *et al*; 2003). It also can cause many adverse effects in lungs as pulmonary toxicity, chronic interstitial pneumonia, diffuse alveolar damage (Chung et al; 2001) and life-threating pulmonary fibrosis (Wolkove and Baltzan, 2009).

The use of biologic therapy in the treatment of various diseases has increased significantly over the last ten years specifically, platelet-rich plasma (PRP). PRP is a modern treatment strategy with worldwide recognition. It was introduced in the 1950s and is currently used in many branches of medicine (Lana *et al*; 2014). PRP is an autologous blood derivative with high platelets concentration in a small volume of plasma and considered an alternative treatment for several diseases as, it is low-cost human by product. It decreases the chances of adverse effects and rejection (Marx, 2004). PRP was used in cardiac surgery, pediatric surgery, gynecology, urology, plastic surgery, and ophthalmology (Andia *et al*; 2015). In addition to, oral, maxillofacial surgery and dermatology (Del Fabbro *et al*; 2015). It also used in wound healing process as it accelerate repairing of the damaged tissues (Villela and Santos 2010).

2. Materials and Methods

Experimental animals

Seventy male Wistar albino rats (each weighing 180-200 gm and two months old) were purchased from the Animal House Facility of the Egyptian Organization for Biological Products and Vaccines (VACSERA), Helwan, Cairo, Egypt, and maintained in clean environment. All animals were allowed to acclimatize in plastic cages (5 animals/cage) inside a well-ventilated room for one week prior to the experiment. The animals were maintained under standard conditions (temperature of 23± 3ºC, relative humidity of 60–70%, and a 12-hour light/dark cycle), fed a diet of standard commercial pellets, and given water ad libitum. The animals were examined for free of parasite.

Preparation of platelet rich plasma (PRP)

Ten age-matched healthy male Wistar rats were used as PRP donors. Whole blood was drawn through median eye can thus vein puncture and mixed with 3.2% sodium citrate at a blood/citrate ratio of 9/1. The whole blood was centrifuged at 1000 r.p.m for 10 minutes at room temperature and the supernatant was separated and centrifuged again at 800 r.p.m for 10 minutes at room temperature then the above two third was discarded and the remaining third (sediment) was used as PRP. The average platelet in PRP was evaluated using a Sysmex XT-1600i system. The platelet count was 800×10^3 platelets/µL (Pazzini *et al*; 2016).

Experimental design

Sixty adult male Wistar albino rats were divided into 3 groups (n=20).

Group (1) the rats were injected intraperitoneally (i.p.) daily with phosphate buffered saline (PBS) at 0.5 ml/rat for 6 weeks and used as a control group.

Group (2) the rats were injected (i.p.) daily with amiodarone drug at (80 mg/ kg. bwt) (0.5 ml /rat) for three weeks, then injected (24 hours after last dose of amiodarone) (i.p.) with (PBS) (0.5ml/kg. bwt) twice weekly until the end of the experiment (Al-Shammari *et al*; 2016).

Group (3) the rats were injected (i.p.) daily with amiodarone drug at (80 mg/ kg. bwt) (0.5 ml drug/rat) for three weeks, plus (i.p.) injection with platelet rich plasma (PRP) at dose (0.5ml/kg. bwt) (24 hours after last dose of amiodarone injection) twice weekly until the end of the experiment (Hesami *et al*; 2014).

All animals in all groups were examined daily along the experiment and clinical signs and mortalities were recorded. The rats in all groups were sacrificed at 3rd week from the beginning of the experiment. Blood samples (about 1.5 ml) were collected from median eye canthus on EDTA-tubes for hematological analysis from each rat.

Animals were euthanized by xylazine (40mg/kg) and ketamine (400mg/kg) (Leary et al; 2013). Tissue samples were collected from lungs of all dead and sacrificed animals for histopathology examinations. Other lung samples were taken from all lungs in all animals and kept frozen at -80°C for glutathione reductase and malondialdehyde determinations.

Hematological analysis

White blood cells count (WBCs), red blood cells (RBCs) count and platelet count were determined using an automated hematology analyzer (Mek-6410).

Bronchoalveolar lavage (BAL) collection

The BAL fluid was collected from the animals by intratracheal injection of saline (3ml/rat) and then collected and centrifuged. The sediments were used to make a smear on slides and then stained with Giemsa stain (Henderson, 2005).

Determination of antioxidant biomarker glutathione reductase

Glutathione reductase was determined using commercially available kit (Bio diagnostic, Co, Egypt) for detection of oxidative stress in lungs homogenate (Goldberg and Spooner, 1983).

Determination of malondialdehyde (MDA)

Malondialdehyde (MDA) was determined using commercially available kit (Bio diagnostic, Co, Egypt) for detection of lipid peroxidase in lungs homogenate (Ohkawa et al; 1979).

Histopathological studies

Specimens from the lungs of the dead and sacrificed animals were collected, then fixed in 10% neutral buffered formalin. Sections about 5µm thickness were prepared and stained with Harries hematoxylin and eosin for histopathological examination (Drury and Willington, 1980) and Masson's trichrome stain for detection of collagen fiber in lungs tissues (Bancroft and Gamble, 2008).

Statistical analysis

Statistical analysis was performed using a one-way analysis of variance (ANOVA). It was done to compare the control and all other treated groups and was followed by a post-hoc analysis (Dunnett's test) using the Statistical Package for the Social Sciences (SPSS), version 17 according to (Borenstein et al; 1997). The data were presented as the mean ± standard deviation. The difference was considered statistically significant when $p < 0.05$.

3. Results

Hematological findings

Figure (1) showed significant decrease in total leucocytic count (leucocytopenia) in group 2 along the experiment when compared to control while, group 3 showed significant increase at 4th, 5th and 6th weeks when compared to group 2 and reached to control one when P>0.05. Red blood cells count (RBCs) showed significant decrease in rats of group 2 (amiodarone group) at 3rd, 4th, 5th and 6th week of the beginning of experiment when compared to control group, while group 3 (platelet rich plasma treated group) showed significant decrease in RBCs count at 4th week in comparison with control and, significant increase in RBCs recorded at 5th and 6th weeks when compared to group 2 when P>0.05.While platelets count showed significant decrease (thrombocytopenia) in amiodarone treated group (gp.2) along the experiment when compared to control rats. While, PRP treated group (gp.3) showed significant thrombocytopenia at 4th week of the experiment when compared to control rats and significant increase at 5th and 6th weeks when compared to group 2 when P>0.05.

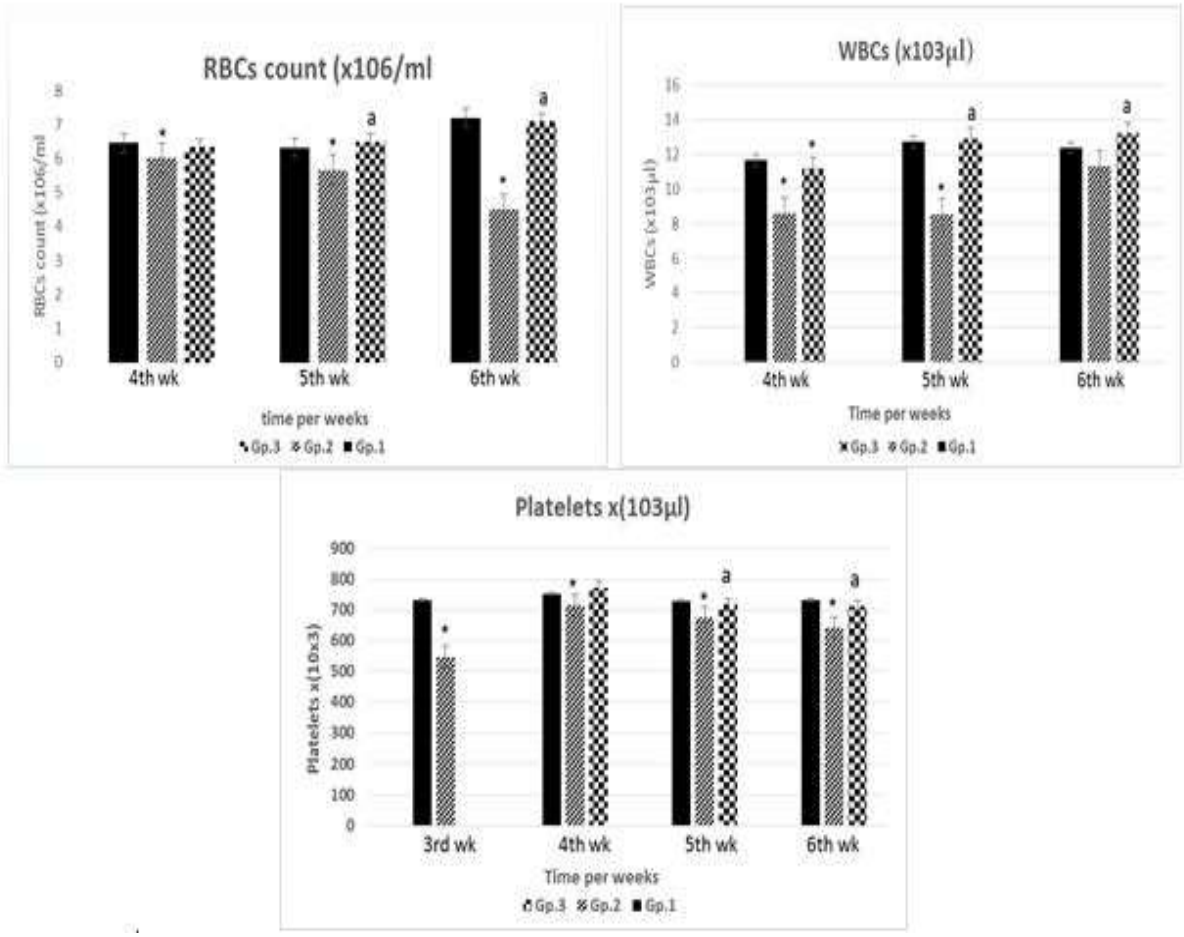

Figure (1): The effect of amiodarone on RBCs count, WBCs count and platelet in rat. Amiodarone was given (80mg/kg/day i.p.) for three weeks and PRP treated group for three weeks. Each group was compared with its respective group. The data represent the mean ± SE. (∗) significant difference from control group when ($P <$ 0.05).

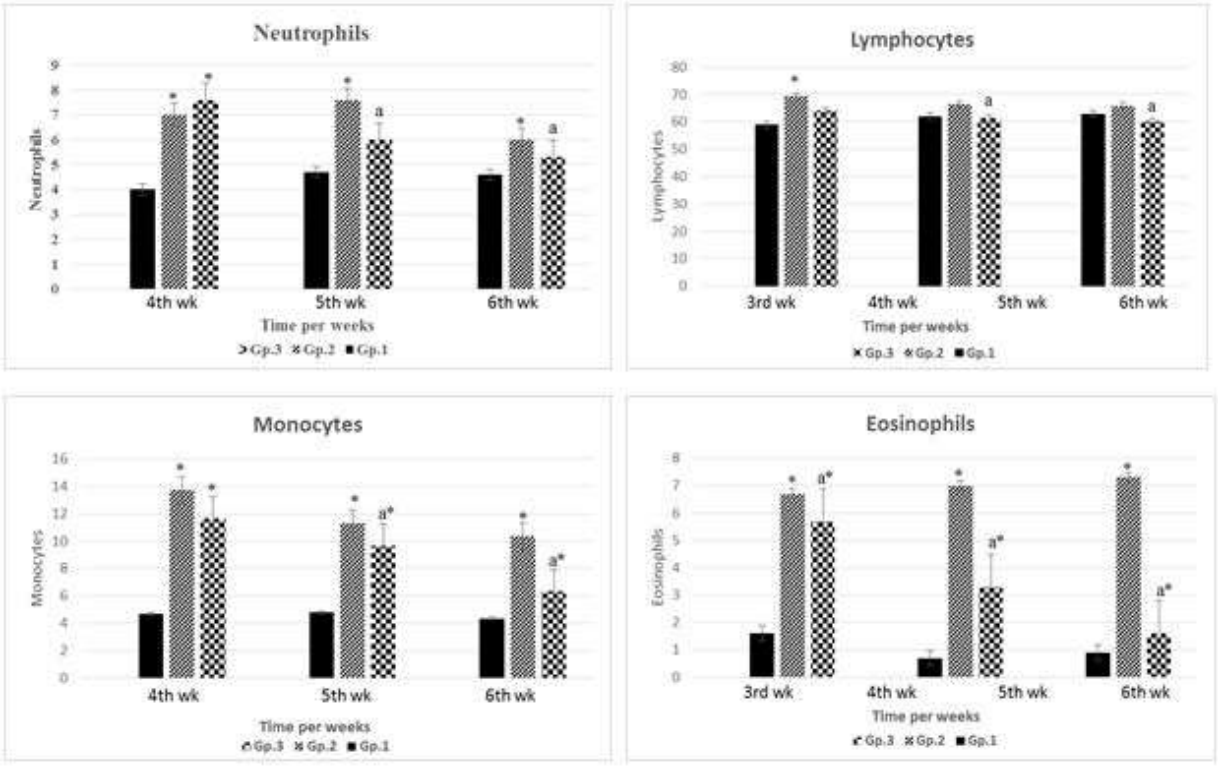

Figure (2): The effect of amiodarone on differential leukocytic count in BAL in rats. Amiodarone was given (80mg/kg/day i.p.) for three weeks and PRP treated group for three weeks. Each group was compared with its respective group. The data represent the mean ± SE. (∗) significant difference from control group when ($P < 0.05$).

Glutathione reductase

Figure (3) showed significant decrease in glutathione reductase level in rats of group 2 along the time of experiment when compared to control rats. While, group 3 which treated with platelet rich plasma showed significant decrease in glutathione reductase level at 4th week in comparison with control rats but, significant increase recorded at 4th, 5th and 6th week of the experiment when compared to group 2 when P>0.05.

Malondialdehyde (MDA)

Figure (3) showed significant increase in lungs tissue MDA level in group 2 in comparison to control group till the end of experiment. Meanwhile, group 3 showed significant decrease in MDA level at 5th and 6th weeks of the experiment when compared to group 2 and significantly increased at 4th week of the experiment when compared to control rats when P>0.05.

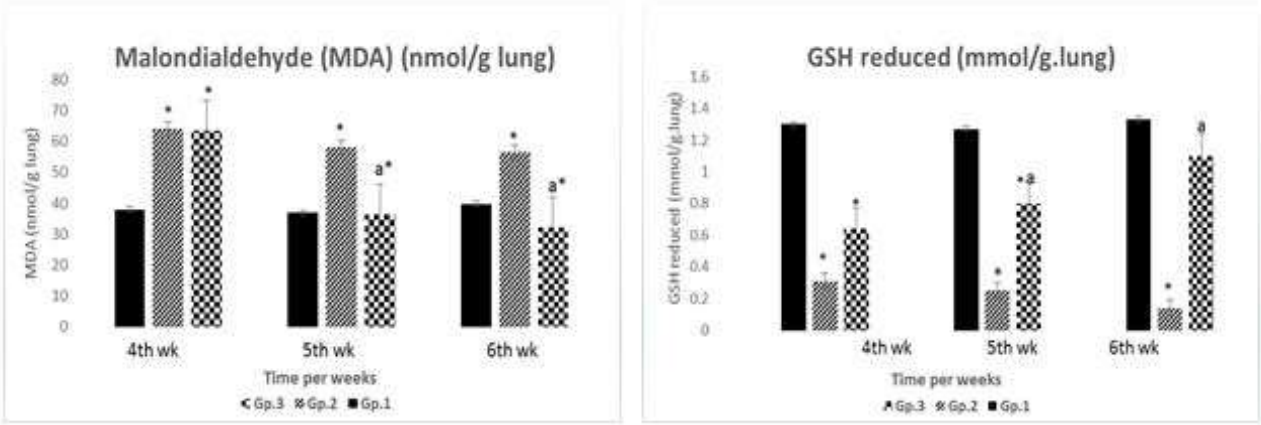

Figure (3): The effect of amiodarone on glutathione reductase and malondialdehyde activity in rat lungs homogenate. Amiodarone was given (80mg/kg/day i.p.) for three week and PRP treated group for three week. Each group was compared with its respective group. The data represent the mean ± SE. (∗) significant difference from control group (a) significant difference from gp.2 when ($P < 0.05$)

Pathological examination

The rats of group 2 lungs displayed interstial pneumonia with emphysematous areas. Bronchioles exhibited bronchiolitis characterized by lining epithelial cells desquamation and neutrophilic debris obstruct the lumen. While, in gp. 3 lungs appeared apparently normal with peribronchiolar mononuclear inflammatory cells infiltration.

4. Discussion

Amiodarone is an iodinated class III antiarrhythmic drug widely used in treatment of many forms of life-threatening cardiac arrhythmia and sudden cardiac death (Bargout *et al*; 2000 and Saad *et al*; 2004). However, its use related to many side effects involving different organs as lungs causing pulmonary complications (Uhal *et al*; 2003). Lung fibrosis is a lethal pathological process with gradual increasing incidence worldwide and limited therapeutic options. It is characterised by abnormal deposition of collagen following tissues damage (Cooper, 2000). Platelet rich plasma is widely used nowadays in various medicinal fields as bone defects (Mehta, 2010) oral and maxillofacial surgery (Albanese *et al*; 2013) aesthetic plastic surgery (Cervelli *et al*., 2009), spinal surgery and later its applications extended to wound healing and tissue regeneration (Okamoto *et al*; 2012). As it contains growth factors it plays role in angiogenesis and tissue regeneration (Fortier *et al*; 2011).

In our work amiodarone treated group recorded leucocytocytopenia, characterized by significant decrease in total leukocytic count (WBCs) similar to that reported by (Erie *et al*; 2010). (Mohamed *et al*; 2007) referred that signs to bone marrow granuloma formed by amiodarone due to accumulation of iodine in tissues or accumulation of phospholipid-like substance due to inhibition of phospholipase enzymes causing pancytopenia.

In our experiment, blood samples were collected from all groups to detect effect of amiodarone (gp.2), which, recorded anemia characterized by significant decrease in RBCs count at 3rd, 4th, 5th and 6th week when compared to control gp. and gp. 3. This was attributed to amiodarone induced bone marrow granuloma through inhibition of phospholipases, leading to accumulation of phospholipids in bone marrow (Lossos and Matzner 1992). (Mukhopadhyay *et al*; 2014) suggested that granuloma formation occur due to immunological reaction. Other author (Chang and Ng, 2008) suggested that amiodarone initiate (erythropoietin) EPO-resistant anemia after treatment for arrhythmia which consequently cause decrease in RBCs synthesis and anemia. Amiodarone using also caused hemolytic anemia (Arpin *et al*; 1991). PRP treated group showed significant increase in RBCs count at 5th and 6th week when compared to gp.2. As platelets stimulate the mitogenic activity of human bone cells to increase the proliferation of stem cells, thus lead to regeneration of tissues (Marx *et al*; 1999).

The present study showed that amiodarone administration causes significant thrombocytopenia in gp.2 along the experiment when compared to control one. On the other hand, group 3 showed significant increase in platelet count at 5th and 6th week when compared to gp.2 this result caused by drug-dependent antibodies specific for platelet glycoproteins GPIa/IIa and/or GPIIb/IIIa which, produced by amiodarone in patient serum (Sahud *et al*; 2013). Slow return of platelet levels occurs even, after discontinuation of amiodarone related to slow clearance of this lipophilic drug from body tissues (Aster & Bougie, 2007). Aster *et al*; (2009) found that amiodarone bound to plasma protein and localized in glycoproteins of megakaryocytes and platelets producing structural changes which, are immunogenic in some individuals enabling amiodarone-induced antibodies to be detected and destruction of platelets occurred.

In our work, group (2) showed leukocytosis in bronchoalveolar lavage (BAL) fluid along the time of the experiment characterised by lymphocytosis and abundant macrophages with and eosinophilia same result reported by (Ohar *et al*; 1992 and Kaushik *et al*; 2001). On the other hand, rats treated with PRP showed a significant decrease in (BAL) leukocyte count at 5th and 6th week of the experiment. This was attributed to platelet rich plasma has a 5 to 10-fold higher concentration of growth factors than whole blood. These growth factors promote natural healing processes (Lai *et al*; 2015) as the first response of the body to tissue injury is to deliver platelets to injured area and attract stem cells to the site of the injury (Wang *et al*; 2015). PRP suppress cytokine release and limit inflammation (Marx, 2004).

5. Conclusion

In conclusion, amiodarone caused lungs injury in rats following daily i.p. administration resulted in inflammatory reactions, and imbalance between antioxidant oxidative stress ending ultimately in severe lung toxicity. platelet rich plasma can cause moderate regeneration in lung damage.

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