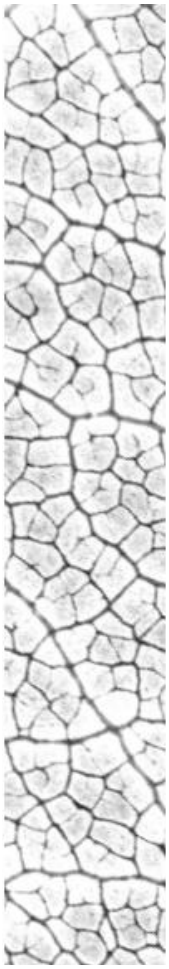

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A weak effects of bee venom on rat superoxide dismutase, catalase and Malondialdehyde activity: Rheumatoid arthritis model

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Abstract
Based on several active molecules and complex mixture component, bee venom used as folk medicine. We measured the biological effects of bee venom on rat three antioxidant staff in the current study, these were the activity of superoxide dismutase, catalase and malondialdehyde, for which forty rats are being used. The experiment groups were randomly sub-grouped into 8 groups (n=5 for each one). Our results showed no significant variation in superoxide dismutase and catalase activity in all groups whereas Malondialdehyde reveled significant decrease in the bee venom 10 µg/kg after 5 days following injection of CFA in comparison with both control and rheumatoid groups. As conclusion a weak evidence of bee venom effects on three antioxidant under consideration.

Keywords: bee venom, superoxide dismutase, catalase a, Malondialdehyde

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INTRODUCTION

Bee venom (BV) is consider a complex combination of toxins, enzymes as well as other trace components with a broad variety of biological activity, including antimicrobials, antirheumotoid disease, anti-cancer and antioxidant activity (Frangieh, et al. 2019). First characterization of the venom from apis mellifera syriaca, a honeybee from the middle east region. *Toxins*, *11*(Baskol, et al. 2005). 191. Entomotherapy, also termed insect use It is well recognized in the treatment of diseases in various areas World pieces include Africa, South America Mexico, China, Ecuador, Brazil, Argentina, Korea, Spain, Nigeria, and India (Oghenesuvwe, & Paul, (2019).

Rheumatoid arthritis (RA) is one of an autoimmune disease complex and Progressive inflammatory Illness involving joints and its advance leads to its demise. The RA prevalence in the general population is 0.5 -1% Globally (Hara, A. V., & Ratnavali, B. www. ijrap. Net).

Studies showed that Bee venom can serve as rheumatoid arthritis modulator that originates from activity of radical oxygen species (ROS) (Mokosuli, Repi, & Worang, 2017).

T-cells and cytokines, together with oxygen radicals, play an important role as productions of hydrogen peroxide by activated macrophages in rheumatoid arthritis. Those reactive oxygen and nitrogen species (ROS and RNS respectively) both have advantageous and toxic effects. Oxidative stress is the condition in which ROS and RNS concentration becomes deleterious and damages the cells and biological macromolecules, and therefore the body. Oxidative stress is caused by a disrupted equilibrium between the body's antioxidant processes and oxidative stress production, and plays an essential role in the advancement of chronic diseases such as autoimmunity (Kumar, Prakash, Gupta, & Khan, 2016; Orhevba, et al, 2016). The idea of crude bee venom used in biomedical field was discussed in many previous data especially those related with inflammatory disease such as rheumatoid arthritis and autoimmune disease that characterized increase ROS and RNS, thus its logically estimated antioxidant staff in case using bee venom as epitheraby to mitigation of disadvantage of ROS and RNS such as cell *damage and deleterious effects.*

MATERIAL AND METHODS

Animals

In the current research male albino rats (*Rattus rattus*) were used as experiment animals. Forty rats (200-250± gm and 8-12 weeks) held at adark-light period (12-12 hours), temperature (25 ° C±1) and humidity (50±). Animals were divided randomly into eight

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groups (n=5 per group), specifically C (negative control was injected with D.W), R (rheumatoid arthritis was only injected with CFA), B1 (BV group (40 µg/kg) +CFA for 4 weeks); B2 (BV treated group , 40 µg/kg after 5 days following injection of CFA for 4 weeks); B3 (BV treated group that injected 10 µg/kg + CFA for 4 weeks); B4(BV treated group at 10 µg/kg after 5 days following injection of CFA for 4 weeks); P1 (BV was treated with 40 µg/kg for 2 weeks before the induction with CFA and then continued the treatment with BV for 4 weeks and P2 (pre-treatment group treated with BV at dose 10 µg/kg for 2 weeks before the induction with CFA and then continued the treatment with BV for 4 weeks at dose 40 µg/kg).

Anesthesia

Rats were anesthetized with ketamine and xylazine hydrochloride, where 100 mg/Kg administered intramuscularly (I.M)(20).

Arthritis induction

The subchronic disease (rheumatoid arthritis) were triggered into the right hind paw of the plantar surface by administering 100µl of single dose CFA (9). CFA was purchases commercial company (Santa Gruz Biotechnology). Every ml of CFA contains heat killed Mycobacterium tuberculosis (1 mg), paraffin oil(0.85 ml), and mannidemonooleate (0.15) ml).

Bee venom

Stock BVs solution prepared by dissolved powder BV in DW and then stored in dark and cool place. Target dose (40 µg / kg and 10 µg / kg) done according to (2).

Collection of Samples

Blood samples were obtained directly from the heart after anesthetizing rats, then the blood was kept in gel tubes. Serum was obtained by centrifugation at 4000 rpm for 5 minutes, and stored immediately at -20 °C.

Catalase activity

Catalase activity was evaluated by adequate incubation the enzymes in 1.0 ml substrate (totally, 65 mmol / ml hydrogen peroxide in 60 mmol / l sodium – potassium phosphate buffer, pH7.4) for three minutes at 37 ° C. Their operation with ammonium molybdate was halted. Yellow molbdate and H2O2 absorbance complex is calculated against the blank(8) at374 nm.

Estimation of Serum Malondialdehyde (MDA)

Malondialdehyhe were estimated on a spectrophotometer using the thiobarbituric acid (TBA) assay method of (White, Krause, Aust, & Eyster, 1985). 0.6 ml of TCA-TBA-HCl reagents is applied to 0.4 ml of serum. It was well mixed, and kept for 10 minutes in boiling water bath. Added 1N NaOH solution after cooling 1.0 ml freshly prepared, to remove centrifugation. This pink-colored absorbance was measured against blank at 535 nm which contained distilled water instead of serum. Mixed and boiled in blank 0.4 ml of DW and 0.6 ml of TCA-TBA-HCl reagent. Blank was always recorded.

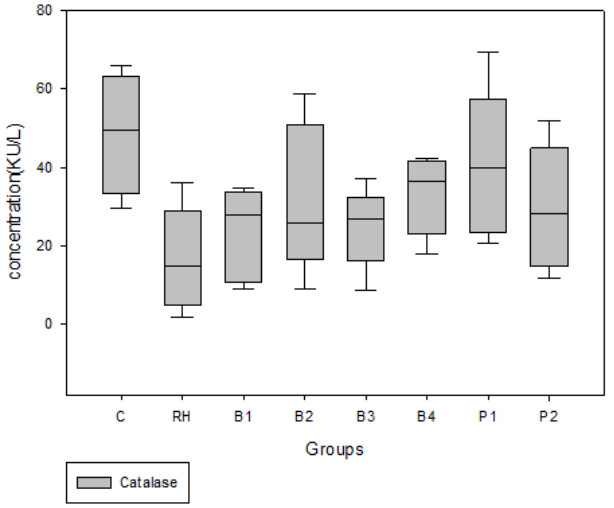

Fig. 1. Mean ± SD of serum Catalase activity (KU/L) in bee venom treated rats

SOD activity determination

According to (Marklund, & Marklund, 1974). the reactant mixture consists of 50 µl crude enzyme and 2 ml tris buffer and 0.5 ml pyragallol that absorbs light at 420 nm. Except for the enzyme extract which was substituted with dH2O, the control tube contains the same. The dH2O was used as a blank. Single enzyme unit is known as the amount of enzyme capable of inhibiting 50 per cent of oxidation of pyragallol.

RESULTS

Catalase

Our results of catalase in all bee venom groups showed non-significant differences as compared with both control negative and rheumatoid groups as shown in **Fig. 1**.

SOD activity

The results of SOD activity revealed no significant differences as compared with rheumatoid group.

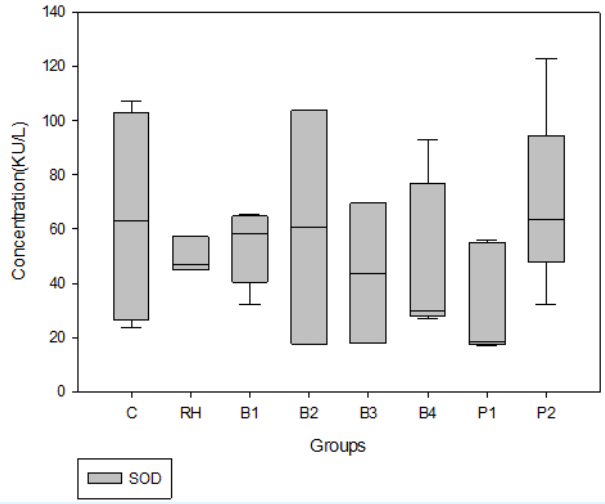

Fig. 2. Mean ± SD of serum SOD activity (KU/L) in bee venom treated rats

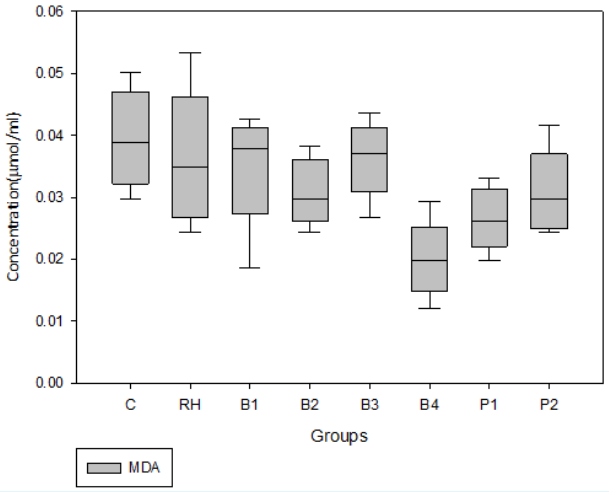

Fig. 3. Mean ± SD of serum MDA activity (KU/L) in bee venom treated rats

MDA

The results of MDA reveled significant decrease in the bee venom 10 µg/kg after 5 days following injection of CFA in comparison with both control and rheumatoid groups, and also with bee venom (10 µg/kg) + CFA.

DISCUSSION

Because a few studies that focus on effects of bee venom on antioxidant staff especially in rheumatoid arthritis model , the study focus on this. Numerous studies show that ROS plays a significant role in RA pathogenesis (Sarban, et al. 2005. Kamanlı, Nazıroğlu, Aydılek, & Hacıevlıyagil, 2004). Macrophage, neutrophils, and lymphocytes occur at high rates in synovial fluid containing ROS (Baskol, et al. 2005). So, it's well known a significantly raise in ROS due to oxidative stress and peroxidation of lipids, thus we used three main antioxidant Catalase, SOD, and MDA that deal with oxidative stress as predictor for this oxidative stress from one hand and the effect of bee venom on decrease or increase of antioxidant staff on other hand(Oghenesuvwe, & Paul, 2019).

Our result showed non-significant differences in catalase, SOD activity in all bee venom groups in comparison with control and rheumatoid groups, only the MDA activity showed significant decrease in the bee venom 10 µg/kg after 5 days following injection of CFA in comparison with both control and rheumatoid groups, and also with bee venom (10 µg/kg) + CFA. A study done by (Souza, Peluffo, & Radi, 2008). demonstrated the reduction in antioxidant enzyme activity may be clarified by saturation of the antioxidant enzyme systems and enzymatic suppression, like hydrogen peroxide inhibition of SOD. In particular, another study showed unchanged CAT activity; this enzyme shows no substantial physiological activity due to its lower affinity to H2O2 than GPX, but has become an important enzyme in a high concentration of H2O2(Fedorova, Kuleva, & Hoffmann, 2009).

We hypothesis two possible causes for ours experimental findings may be that in CFA-rat 's arthritis-affected muscles (i.) the development of ROS is increased but scavenged faster because the time of testing not daily (ii.) the origin of ROS is differ than that of mitochondria and NOX2 and near that particular but unidentified site the scavenging ability of ROS is inadequate that findings in ROS scavenging.

One the same line several studies revealed that an imbalance between ROS synthesis and scavenging can cause oxidative stress (Yamada, et al. 2015). Yamada, et al. 2015). (Yamada, et al. 2017). (Nathan, & Cunningham-Bussel, 2013). RA-associated oxidative stress promotes muscle weakness through the addition of oxPTM on actin, this lead to hypothesize that the balance between ROS sources and ROS scavengers is altered in arthritis-affected muscles. Where different studies was demonstrated frequently which oxidative stress is found in the serum affected with arthritis (Grönwall, et al. 2017). (Nunes-Miranda, et al. 2017). as well as skeletal muscles, also been shown oxidative stress may contribute to muscle weakness(Yamada , et al. 2015).

A research performed by (Quiñonez-Flores, et al. 2016). showed that reducing antioxidant enzyme activity could also be clarified by saturation of antioxidant enzyme systems and enzymatic suppression, such as hydrogen peroxide inhibition of SOD, in particular, unchanged CAT activity; this enzyme displays no noticeable physiological activity because of its lower affinity to H2O2 than GPX, but has become an important enzyme in a situation where H2O2 concentration is high. Alternatively, the use of different parameters to determine the function of enzymes can yield different results.

CONCLUSIONS

Based on our data a weak evidence of bee venom effects on three antioxidant under consideration except minor decreasing effects in MDA activity in two groups.

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