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Impact of physical activity in osteoporosis and alveolar bone protection

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Abstract: Physical activity protects body bones against osteoporosis in mysterious way and the reason for such improvement was unknown and unclear. We were inspired to search the possible role of allantoin in such improvement being increased in response to physical activity as a metabolic byproduct. Osteoporosis was simulated by gamma irradiation and allantoin was injected in rats as a radiation treatment or prevention. Allantoin improved different bony concerns related to osteoporosis such as alkaline phosphatase, acid phosphatase, uric acid and histochemical profile by using digital image analysis. This study concluded the possible role of allantoin as a key factor in bone improvement in physically active persons; also allantoin can be used as a treatment for osteoporosis and gout with extra advantage of being antioxidant radiation protector.
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1. Introduction

Increased free radical production associates oxidative rust of the different body compartments showing some similar profiles in different organs because of the organs origin unity. Free radical mainly produced as metabolic by-products for energy metabolism. So any increase in metabolic rate will enhance free radical production. Consequently excessive physical activity induces metabolic rate which is associated with free radicals production ***Warburton et al 2006***. Consequently it is expected that physical activity will enhance excessive body rust and related biological concerns but in real life physical activity was considered as the royal door for health improvement and permanent youth in spite of increased free radical production with respect to histochemical improvement of these concerns such as osteoporosis, edentulous, cancer, cardiovascular and cerebrovascular diseases ***Daniell1983***. Many researchers supposed that causes of such mystery are unknown or unclear reasons while some others supposed wonderful reasons such as better gastric emptying or tissue adaptation ***Warburton et al 2006*** and ***Wactawski-Wende 2001***.

De Pablo et al 2008 elucidated that physical activity improves osteoporosis, periodontitis and edentulous dysfunction with evidence of strong association between these concerns ***Pablo et al 2008***. ***Palmer and Wolfe*** found that fluoride protects both bones and teeth in quite similar profile against osteolytic and biochemical changes in bony organs ***Palmer and Wolfe 2005***. Free radical inducers such as smoking induce quite similar osteoporotic and edentulous concerns profile. Both periodontal diseases and osteoporosis are associated similarly and strongly with alveolar bones and skeletal integrity ***De Stefano et al 1993***. ***Tada 2003***associated between denture integrity and physical activity with other different health concerns such as osteoporosis, edentulism and common health of geriatrics Tada 2003.

One of the forgotten by products in biochemistry world is the allantoin which increased by many folds in response to excessive metabolic rate induced oxidative stress conditions such as physical exercise and amniotic fluids during pregnancy ***Shestopalov et al 2006***. ***Gus'kov et al 2001*** elucidated some redox improved biochemical parameters in response to allantoin administration. Most of the researchers used allantoin as biomarker for oxidative stress not as a signal for healing although its ultimate healing effects by many skin external preparations such ointments and creams. Little or no studies were focused on endogenous healing effect of allantoin ***Jan Gruber et al 2009***.

Physical activity protects and improves human body against chronic and acute diseases for unclear and unknown reasons. In other words physically active persons showed decreased death rates and relative risk

improvement for different organs and tissues. One of the most exercise affected organs is bony tissues. Physical activity improves bony concerns such osteoporosis and denture protection. The improvement of such health concerns is directly proportional to quantity of physical activity. Physical activity improves osteogenic adaptation Warburton et al 2006. osteogenic adaptation occurs in childhood may be persistent to late stages of life, osteogenic adaptation can be affected in many factors such as bone mineral density and PBM. PBM achieved in parallel quality for muscle mass and achieved early in childhood. ***Soliman et al 2016.***

Allantoin is a metabolic by-product increased in response to many conditions such as pregnancy, muscular oxidative stress and muscular exercise. The main organ producing allantoin is the muscle and accordingly allantoin production depends on muscle mass and muscle activity. Allantoin was envisioned as metabolic biomarker for physical activity rather than biologically active molecule induces the protective effect of physical activity.

Aim of the study:

This study was designed to explore some allantoin biological aspects using irradiation as simulator experiments for free radical induced physical activity and osteolytic changes in osteoporosis.

2. Material and methods

Chemicals:

Allantoin was purchased from Fluka as free powder. Allantoin was freshly dissolved in a hotsaline (1%) and set aside to cool prior to I.P. injection (100 mg/kg).

Animals

Animals were purchased from Theodor Bilharz Research institute, Cairo, Egypt. Rats were about 2 month at the time of experiment and were housed in poly carbonate cage with wire lids in standard condition under light standard conditions (light: dark, 13h–11h) and libitum. Animal experiments were conducted with respect of criteria of the investigation and ethics committee of the community guidelines dealing with experimental animals.

Rats were anesthetized with thiopental 50mg/ kg10 minutes prior to scarification by I.P. injection. Animals were sacrificed by blood dragging from retro-orbital venous plexus and blood was oriented for spectro-photometric studies by separation of plasma by centrifugation (EDTA coated tubes) after keeping samples for 1 hour at room temperature. Centrifugation was performed at 4000RPMfor 10min and plasma was kept at -4 for further analysis.

Animals were dissected and bones were isolated, preserved directly in 10 % formalin solution for histochemical studies performance.

Experimental design:

The study was carried out using 36 male Sprague Dawley rat (weighing approximately about: 100 g±10 g) were grouped into 6 groups. The first group, Gp A (negative control) was injected with saline (0.1 ml / rat) for 7 consecutive days as sham operated group for Gp Ă which is treated with allantoin only. Group B (prevention control) is injected with saline for 7 consecutive days prior to irradiation while Gp B̆injected with allantoin (100 mg/ kg) prior to irradiation. Gp C (treatment control) is injected with saline after irradiation for 7 consecutive while Gp Č is allantoin treated post- irradiation.

All groups were injected I.P. as the following: Group A: sham (saline for 7 days), while Group Ă: allantoin (100 mg/ kg / day for 7 days). Group B: irradiation prevention control (saline 7 days, sacrification after 6 Gy irradiation for1 days), Group B̆: allantoin 100 mg/ kg/ day for 7 days administration prior to irradiation (6 Gy). Group C: irradiation treatment (sacrification after 6 Gy irradiation and saline 7 days) as control for Group Č: allantoin administration 100 mg/ kg/ day for 7 days post irradiation (6 Gy) prior to scarification.

Irradiation protocol:

Animals were irradiated at Egyptian Atomic Energy Authority using previously installed an AECL (137cesium) Gamma Cell-40 as whole animal irradiation device. Animals exposed to 6 Gy as one shot dose rate of 0.012Gy/s.

In-vivo biochemical studies:

All biochemical experiments were carried out in Fungi Center Al-Azhar University. Uric acid was measured calorimetrically in the plasma using diagnostic kit supplied by Biodiagnostic Company, Egypt, and Acidphosphatase was measured calorimetrically (510 nm) due to cleavage of Phenyl phosphate into phenol at acidic pH in the presence of 4-aminophenazone and potassium ferricyanide, while alkaline phosphatase was measured by the same technique but at pH 10 (Biodiagnostic Kit).

In vitro oxidative stress determination: SOD activity of allantoin was determined by adding 0.1 of allantoin to human serum (first set), 0.1 ml of allantoin solution (1% in saline) was added to 0.9 saline (second set) and third set was the negative control (0.1 saline and 0.9 ml of serum) Superoxide dismutase (SOD) activity was assayed by measuring the inhibition of the formation of blue colored formation at 560 nm (Kakkar et al. 1984).

Histochemical studies:

All histochemical experiments were carried out in Mansura University. Slides were photographed

using Olympus® digital camera installed on Olympus® microscope with 1/2 X photo adaptor, using 40 X objective. The result images were analyzed on Intel® Core I3® based computer using VideoTest Morphology® software (Russia) with a specific built-in routine for area, % area measurement and object counting.

Statistical analysis:

Statistical analysis was performed by using SPSS software version II. Data are figured as mean± S.E. and analyzed by student's T-test. Level of significance was set at $p<0.05$.

3. Results

Effect of allantoin and gamma-R on the bone matrix biochemical parameters:

The current study was conducted to screen the protective and healing effects of allantoin on the damaging effect on bone matrix caused by gamma-irradiation as a simulator for excessive free radical production conditions like osteoporosis, physical activity, inflammation etc. allantoin confers protective effect against irradiation by improvement of uric acid, Alkaline phosphatase and acid phosphatase shown in figures 1-3.

In vitro antioxidant activity:

Addition of allantoin to human serum increased the SOD activity as shown in table 1.

Histochemical profile as DIA to elucidate the effect on the bone matrix:

DIA pictures elucidated osteolytic changes in irradiated control groups similar to osteoporosis which showed marked resorption. Such osteolytic changes improved in allantoin groups which showed improvement in bone remodeling.

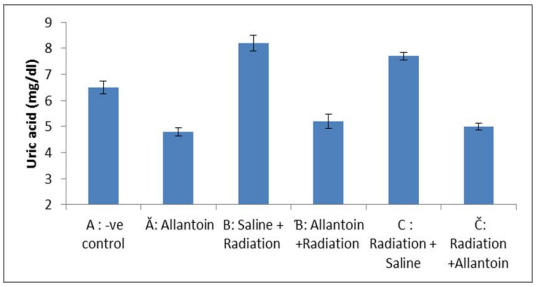


Fig 1: Plasma uric acid concentration (Mean ±SE) in the different studied groups.

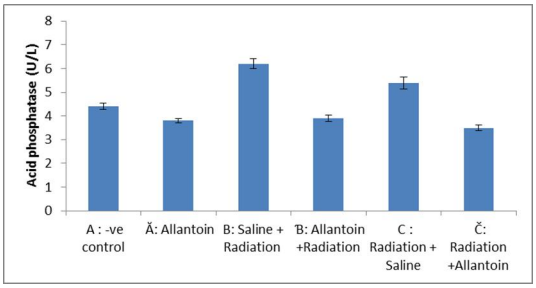


Fig 2: Plasma Acid phosphatase (Mean ±SE) activity in the different studied groups.

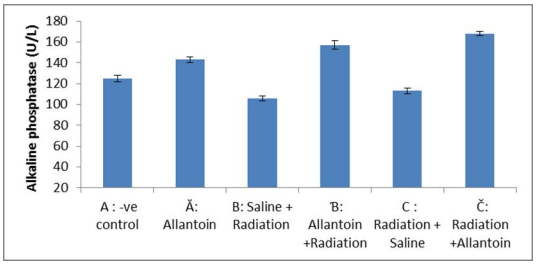
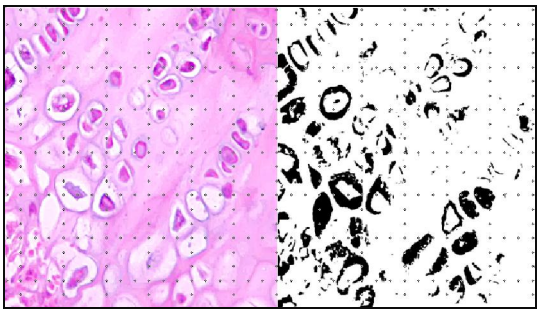


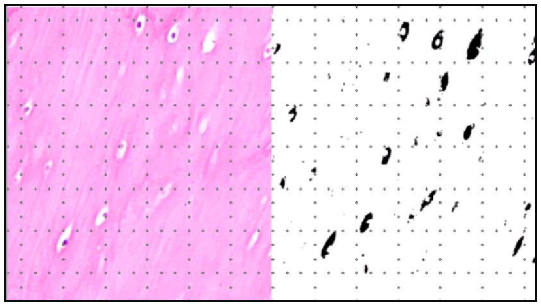
Fig 3: Plasma Alkaline phosphatase (Mean ±SE) activity in the different studied groups.

Table 1: in vitro SOD activity estimation of allantoin (Mean ±SE) activity in human serum or saline

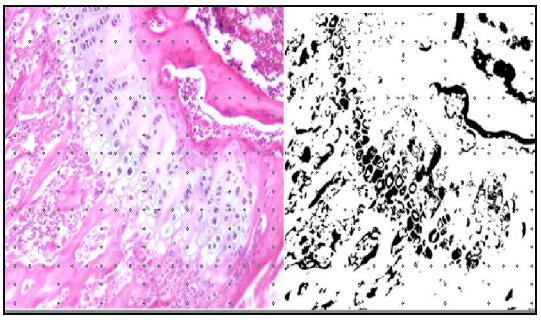
Groups	SOD activity
0.1 ml saline+ 0.9 ml Human serum	49.7 ± 2.7
0.1 ml allantoin (1% in saline) + 0.9 ml Human serum	81.6 ± 3.2
0.1 ml allantoin (1% in saline) + 0.9 ml saline.	0.0



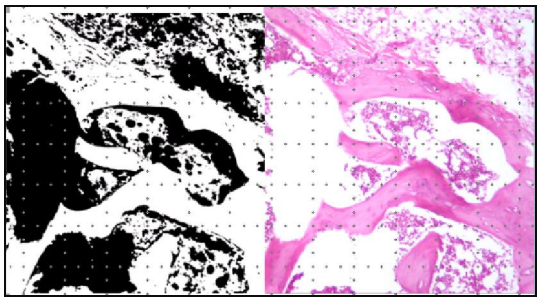
Control group



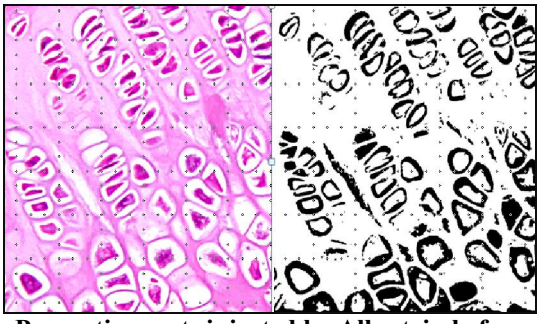
Allantoin control group



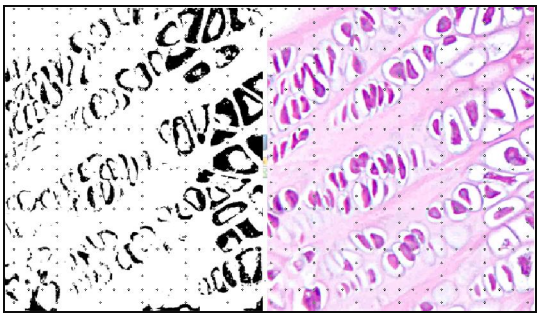
Rats: sacrificed after irradiation by week



Rats: sacrificed after irradiation 1 day



Prevention: rats injected by Allantoin before irradiation



Treatment profile: rats injected by Allantoin after irradiation

In **negative control** group, DIA showed about **17.7±0.8%** having no or very weak resorption. In **positive control** group taking the radiation, DIA show a percent of about **29.6±1.4% to 44.8+2.1%** having moderate resorption and may reach to advanced bone resorption. In the group taking **allantoin**, DIA show a percent of about **10.1±0.8%** having nearly no resorption. In the (**prevention**) group taking **allantoin** +**radiation**, DIA show a percent of about **24.2±1.7%** which considered as mild bone resorption. In the (TTT) group taking **radiation**+**allantoin** as a treatment, DIA show a percent of about **15±0.7%** which considered as weak bone resorption.

4. Discussion:

It was evident that the improvement of bony markers occurs at the peak time of physical activity (at time of increased production of allantoin). So we try to correlate between allantoin production and bone protection. By using different biochemical and histochemical parameters. The research focuses on alveolar bone osteoporosis and bone markers. ***Daniell 1983*** postulated that tooth integrity is strongly associated with bony diseases such as rheumatoid arthritis. Also destructive factors like Cigarette smoking disturbed osteoporosis in general body bones and teeth loss in quite similar profile.

Branca 1999 elucidated that High bone mineral density (BMD) resists bony concerns such as fractures, osteoporosis and edentulism. Also athletes have 25% greater in BMD than active people, Also physically active people have 30 % greater in BMD than sedentary people also physically active persons have lower fracture and death ratio as some fractures can lead to death. Muscular exercise participation for 3 hours exhibited significant bony improvement. ***Miles 2007*** elucidated that physically active children have improved BMD than who are less active. Improvement of bony material is always attributed to muscle mass and activity. The reason for such improvement was unknown and unclear.

Wactawski-Wende, J., 2001 mentioned that Alveolar osteoporosis strongly associated to female skeletal osteoporosis in relation to Ca and vitamin D metabolism, the lower bone mass density is strongly correlated to both osteoporosis and denture integrity as the tooth loss prevails with senility in strongly associated manner. The oral health and osteoporosis are strongly related to the quality of life.

Francis et al 1984 stated that chronic physical exercise were reported to decrease serum uric acid and alleviate gout, our results could explain such phenomena on the basis of allantoin production. Allantoin decreased uric acid formation in radiation exposed groups compared to the corresponding control groups which means body protection. Also allantoin radiation exposed groups showed decreased uric acid formation than sham control group which means cellular protection enhancement in presence of free radical stimulated production by irradiation. Uric acid

is an end product of increased DNA catabolism which reflects cellular integrity and protection in all body organs including bones. These findings explain how physical activity improves osteoporosis through allantoin production even in presence of excessive amounts of free radicals by higher metabolic rate due to physical activity induced cellular protection.

These results are confirmed with results of Acid phosphatase. Allantoin enhances potent decrease in acid phosphatase activity especially in allantoin radiation exposed groups which indicate protection against bone resorption by excessive free radicals production by irradiation which simulates free radical excessive production during physical activity. ***Folan*** found that alkaline phosphatase is decreased by irradiation which means increased bone resorption; ***Folan*** mentioned that physical activity decreased acid phosphatase due to protection against bone resorption. Also ***Folan*** stated that physical activity, antioxidants, Ca and vitamin D supplementation protects bones against osteoporosis and increases bony protein synthesis ***Samarth et al 2002.***

The Data of uric acid and alkaline phosphatase illustrates the possible role of allantoin in decrease bone resorption by physical activity in presence of metabolic oxidative stress by muscular exercise. Alkaline phosphatase is a marker of bone remodeling and formation showed different profile in different studied groups. The radiation treatment control group elucidated increase in alkaline phosphatase activity due to long term of self-tissue repair or may be due to chronic oxidative stress induced endogenous allantoin production which causes increased Alkaline phosphatase activity as an indication of bone healing, remodeling, and formation, the change in alkaline phosphatase activity in response to irradiation resembles alkaline phosphatase activity in osteoporosis. The improvement in DIA as a result of allantoin administration augment elevated osteoblast activity as the possible cause of higher Alk activity rather than cellular release of alkaline phosphatase. Decrease in Alk activity in prevention control group may be due to sudden cellular suppression which indicates initial suppression of bone formation.

The results of DIA may reflect enhancement of protein synthesis in allantoin treated groups. This effect of allantoin explains the improvement in osteogenic adaptation in bone neighboring to the highest active muscles because of paracrine allantoin release which explain different bone matrix formation according to type of physical activity and sports. So ***Miles 2007*** mentioned that bone matrix formation site will depend on the mass of neighboring muscles and its activity according to type of sports.

Saito, M. and Marumo, K., 2016 and Roschger et al 2017 elucidated that Bone malformation and bone stiffness quality rely on bone matrix. The bone matrix strength depends on two approaches. The first approaches is related to mineral density which confers bone stiffness character and the second approach depends on collagen fibers which confers tensile strength and toughness which originated from collagen protein synthesis and its cross linking formation after its covalent modification.

Saito and Marumo 2010 mentioned that posttranslational modification of collagen affects bone mineralization and mechanical aspects of bone at matrix level. Collagen I is the most dominant collagen isoform in the bone. Collagen stabilization is related to cross linking between helical and non-helical domains ***Alford et al 2015***.

Cross linking and covalent modification depend on enzymes such as lysyl hydroxylase in presence of ascorbic acid as a coenzyme. Ascorbic acid as an antioxidant can be consumed in the presence of oxidative stress ***Saito, M. and Marumo, K., 2016***. Consequently oxidative stress can inhibit posttranslational modification decreasing the tensile strength of the bones. Also it is well known that the seeding of calcium hydroxyapatite begins in area neighboring to cross linking regions ***Saito and Marumo 2010***. Our results showed direct effect of allantoin on SOD of human serum which indicate its direct antioxidant effect which indicate the possibility of improvement in collagen covalent modification and cross linking and consequently the seeding process of calcium. So allantoin can improve bone matrix formation, maintenance and remodeling results reflect improvement in protein synthesis in all allantoin groups than their respective control group. DIA and ALK reflect the amount of osteoblast activity which is responsible for protein matrix modification (***Sato and Abe 2005***).

Allantoin production increases in hypoxia and oxidative stress conditions ***Gus'kov, et al 2001***. So our results may explain why KAATSU walk improve bone and bone markers ***Sato and Abe 2005***. In this way we can explain why bones after healing from fractures become stronger, because of inflammation induces allantoin production leading to in situ improvement of osteoblast activity and bone remodeling.

Also allantoin could explain the symmetry between physical activity and vitamin D and Ca supplementation in bone remodeling and synthesis based on two approaches. The first approach all of these increase intracellular Ca level. The second approach depends on Ca induces uricase enzyme leading to oxidation of uric acid forming allantoin ***Amirthanathan 2012.***

As the allantoin production depends on muscle mass and activity which leading to Ca release from its stores. Also allantoin could explain the basis of

physical therapy programs why is it improve osteoporosis. Also data of allantoin may explain bone demineralization in astronauts and sedentary patients based allantoin production inhibition.

The biomarkers of bone resorption and formation are compatible with data obtained by DIA which sowed decreased bone resorption and increased bone formation by allantoin against the effect of radiation exposure.

Conclusion:

1) Radiation in rats gave the same profile for osteoporosis suggesting the use of radiation as osteoporosis simulator.

2) Allantoin is a metabolic by-product increases in response to muscular exercise could be the possible key factor and missing point in the relation between physical activity and osteoporosis protection.

3) Allantoin could explain the physical therapy training improvement of bony concerns such as osteoporosis. Allantoin improved osteoporotic bony markers such alkaline phosphatase, acid phosphatase, uric acid and histochemical digital image analysis profiles.

4) Allantoin could be used in gout and demineralization treatment such as osteoporosis treatment.

5) Allantoin could be used in radiation prophylaxis and treatment with no need for studies like teratogenicity because of its endogenous origin.

6) Further studies were needed to reveal the role of allantoin in vitamin D and Calcium metabolism.

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