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Allantoin may modulate aging impairments, symptoms and cancers

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Abstract: Allantoin increases in different stress conditions and environment such as physical activity, amniotic fluids and oxidative stress. So, we inspired to explore the role of allantoin as a metabolic by-product in health improvement and protection using irradiation as simulator for oxidative stress. Allantoin was injected i.p. (100 mg/kg) in senile male rats in irradiated and non-irradiated groups in comparison to sham operated group. The studied parameters were superoxide dismutase, Glutathione reductase, Glutathione, total antioxidant capacity, collagenase, urea, creatine kinase, alanine transaminase, aspartate aminotransferase, triglycerides, total cholesterol, and HDL and LDL cholesterol. Allantoin *in vitro* antitumor activity was MTT assayed for some age dependent cancers. Allantoin showed improvement in all *in vivo* studied oxidative stress parameters. Allantoin showed an increase in lipogenesis was recorded as a hepatic energy targeting muscles. Allantoin improves aging process indicated by its collagenase inhibitory effect. Allantoin showed cytotoxicity against prostate, colon, intestinal ovarian and breast cancers and weak inhibitory against larynx cancer. Allantoin may be the possible mysterious key factor involved in health and aging improvement and cancer protection in stress conditions such as physically activity and radiation hazards.

Keywords: Allantoin, irradiation, physical activity, cancer, radiation protection.

INTRODUCTION

Stress conditions (oxidative stress, pregnancy and physical activity) increase metabolic rate followed by excessive production of metabolic byproducts. As the metabolic pathways being activated, the biomolecules oxidation is activated by electron leaks especially through the activation of electron transport chain. The activation of electron transport chain enhances formation of metabolic oxidative by-products such as reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS and RNS collectively are named free radicals. Free radicals attack biological systems leading to cellular aging, rapid senility and peroxidative products (Zhou *et al*., 2018, Valko *et al*., 2016 and Kruk *et al*., 2019).

Free radicals oxidize different cellular components leading to oxidized products. Oxidized products predispose many symptoms such as atherosclerosis, amnesia, psychological effects, cardiovascular diseases, osteoporosis, cancer risk and disordered immunity**.** Some of stress conditions (such as physical activity and pregnancy) protect against some health impacts and many diseases. The mysterious cause of such improvement is still unknown and unclear. Many literatures attributed this mysterious phenomenon to better circulation or even to better gastric emptying (Miles, 2007 and Kruk *et al*., 2019).

All oxidative stress conditions have known and unknown protective mechanisms. Allantoin production induction occurs during such oxidative stress conditions. Allantoin was envisioned as a biomarker in certain stress conditions such as physical activity. Allantoin production increases in pregnancy and sever oxidative stress. Allantoin concentration increases in pregnancy up to ten folds and allantoin is heavily produced by placenta. Allantoin production in normal individuals is directly proportional to muscle mass and physical activities (Kanďár, 2016 and Mayneris-Perxachs *et al* 2019).

Most researches envisioned allantoin as an only oxidative stress biomarker produced by oxidation of uric acid by the action of uricase enzyme during muscle exercise and pregnancy (Kanďár, 2016). Gus'kov *et al*. mentioned that allantoin has antioxidant and cellular protection effects. Previous reports did not relate allantoin induction (physical activity and pregnancy) to possible health beneficial effects (Gus' kov *et al*., 2004 and Mayneris-Perxachs *et al* 2019). The cause of such lost link may be ascribed to the stability of allantoin structure which complicates its role in direct redox reactions through its weak redox potential. This encourages us to find out possible protective role of allantoin against oxidative stress using irradiation as a model for free radical production conditions. We were inspired to look for allantoin which could be responsible for health protection. So, our goal in this paper was to elucidate if allantoin is the radioprotector of choice because of its endogenous origin which may highlight its possible protective role in different stress conditions.

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MATERIALS AND METHODS

Allantoin (Fluka Company) was freshly dissolved in hot saline (1%) and set to cool prior to i.p. injection (100mg/ kg).

Experimental design

The study was conducted on 36 male Sprague dawely senile rats (200g±20g). They were randomly assigned to 6 groups.

Group A: was sham operated by i.p. saline injection (0.1 ml /Kg/ rat) for 7 consecutive days and used as a control for Group Ă.
Group Ă: was i.p. injected with allantoin (100 mg/ kg) for the same period.
Group B (prevention control): was i.p. injected with saline for 7 consecutive days prior to irradiation (6 Gy) and used as control for Group Ɓ.
Group Ɓ: was i.p. injected with allantoin (100 mg/ kg) for 7 days prior to irradiation (6 Gy).
Group C (treatment control): was i.p. injected with saline for 7 consecutive days after irradiation (6 Gy) and used as control for Group Č.
Group Č: This was treated with allantoin (100 mg/ kg) post irradiation (6 Gy) for 7 consecutive days prior to scarification.

Animals

Animals processed for study were purchased from Theodor Bilharz Research Institute, Cairo, Egypt. Rats were housed in poly carbonate cage with wire lids with food and water ad-libitum. The housing conditions were maintained at 24C° and 12-12-h light and dark cycle. Animals were anesthetized 10 minutes prior to scarification by i.p. injection with thiopental 50mg/kg after overnight fasting. Animals were sacrificed by blood withdrawing from retro-orbital venous plexus for further plasma separation. Blood was collected in EDTA coated tubes and set aside for 1 hour prior to 4000 rpm centrifugation for 10 min and plasma was preserved at -4°C prior to biochemical analysis.

Ethical approval

The authors declare that all experiments including animals were procured and maintained according to approval and guidelines for Egyptian Atomic Energy Authority.

Irradiation protocol

Rats were exposed to whole body gamma irradiation at a single dose of 6 Gy. The dose was delivered as one shot using 137cesiu Gamma Cell-40 belonging to the National Center for Radiation Research and Technology, Atomic Energy Authority, Cairo, Egypt (dosing rate was 0.012Gy/s).

Biochemical analysis

Superoxide dismutase (SOD) activity was assayed by using Biodiagnostic kit. Reduced glutathione (GSH) level was measured (Mavis and Stellwagen, 1968). Total antioxidant capacity was evaluated using OxiSelect™ (Total Antioxidant Capacity (TAC) Assay Kit, Cell Biolab, INC).

Plasma urea level was determined using diagnostic kit supplied by Diamond Company, Egypt (Patton and Crouch, 1977).

Evaluation of anti-aging activity of allantoin in the form of Collagenase assay

Collagenase inhibitory assay is based on spectrophotometric methods. The assay was performed in 50 mM Tricine buffer (pH 7.5 with 400 mM NaCl and 10 mM CaCl2). Collagenase from Clostridium histolyticum (ChC – EC.3.4.23.3) was dissolved in buffer for use at an initial concentration of 0.8 unit/mL according to the supplier's activity data. The synthetic substrate N-[3-(2-furyl) acryloyl]-Leu-Gly-Pro-Ala (FALGPA) was dissolved in Tricine buffer to 2mM. Samples (1000-7.81 µg/ml) were incubated with the enzyme in buffer for 15 minutes before adding substrate to start the reaction. Negative controls were performed with water. Absorbance at 335 nm was measured immediately after adding allantoin and then continuously for 20 minutes using a Cary 50 Microplate Reader in Nunc 96 well microtitre plates (Van Wart and Steinbrink, 1981). Enzyme inhibition activity (%) = [(O.D. control−O.D. sample) /O.D. control] ×10

For assessment of lipid profile, plasma triglycerides were determined according to method of Fossati *et al*. by using Biodiagnostic Kit (Fossati and Lorenzo, 1982). HDL and LDL were estimated by using fractional separation as described by Lopes-Virella *et al*. 1977 and Friedewald equation (Friedewald *et al* 1972). Total cholesterol was measured as described by Wieland and Seidel, 1983 using Biodiagnostic Kit.

Estimation of plasma aspartate aminotransferase (AST) and Alanine transaminase (ALT) activities were carried out by a kinetic method using diagnostic kit supplied by ELI Tech Company.

Creatine kinase (CPK) activity was evaluated calorimetrically using kit of Ray Biotech, Inc.

Antitumor effect of allantoin on tumor cell lines

Allantoin antitumor activity was determined on human Larynx carcinoma cells Hep-2 cell line, prostate carcinomaPc-3 cell line, CACO cells for intestinal carcinoma, CHO-k cells for ovarian carcinoma, MCF cells for breastcancer and HCT-116 for colon cancer. Different concentrations of allantoin were used. Tumor

cells (1×10^{4}cells/well) were plated in 200µL media per well in a 96 well plate and their viability was detected by using a MTT cell viability assay (4, 5-dimethylthiazol-2-yl) -2, 5-diphenyltetra-zolium bromide).

STATISTICAL ANALYSIS

Experimental data were subjected to One-Way Analysis of Variance (ANOVA) and two tailed Student's t-test (Statgraphics, Origin6.1). Data are presented as mean± S.E. and analyzed by student's T-test. Level of significance was expressed as $p<0.05$.

RESULTS

Effect of allantoin and gamma-R on the antioxidant defense system

The current study was conducted to screen the protective and healing effects of allantoin against the deleterious effect of oxidative stress caused by gamma-irradiation as a simulator in for stress conditions causing excessive free radical production. Exposure of animals to gamma radiation induced oxidative injury throughout the significant depletion of the antioxidant defense system (plasma contents of SOD, GSH, GRs and total antioxidant capacity), with respect to normal controls figs. 1- 4. These harmful effects were opposed by allantoin administration.

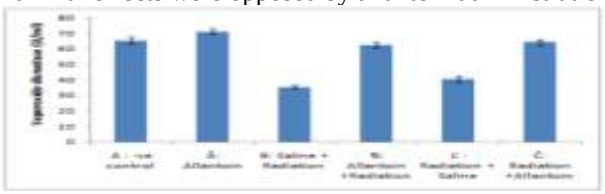

Fig. 1: Plasma SOD (Mean ±SE) activity in the experimental groups.

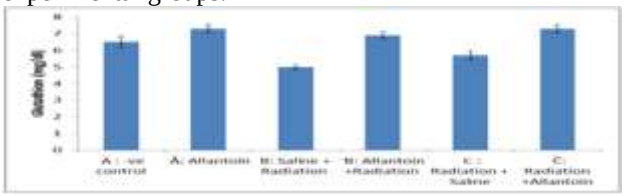

Fig. 2: Plasma glutathione (Mean ±SE) activity in the experimental groups.

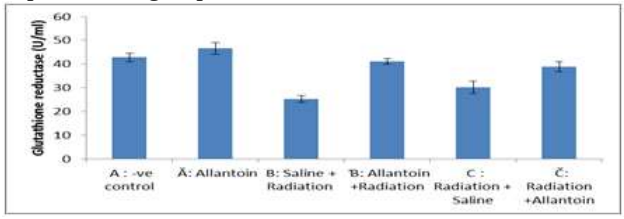

Fig. 3: Plasma Glutathione reductase (Mean ±SE) activity in the experimental groups.

Effect of allantoin on gamma-R induced liver, kidney dysfunction, myocardial infarction and lipid profile

Gamma radiation was found to affect liver and kidney functions tests as an increase in ALT, AST activities and the level of urea and creatine kinase activity. On the contrary, administration of allantoin either before or after gamma-irradiation exposure showed statistically significant improvement in the biomarkers against gamma-R injury compared with normal controls. Allantoin increases HDL with improvement in LDL besides significant increase in plasma triglycerides with higher cholesterol levels in all allantoin treated groups (figs. 5-12).

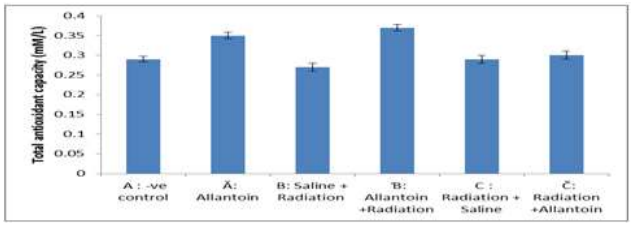

Fig. 4: Plasma total antioxidant capacity (Mean ±SE) in the experimental groups.

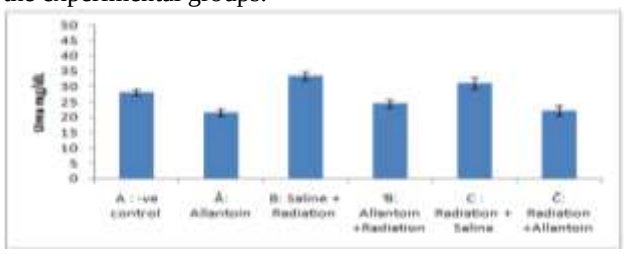

Fig. 5: Plasma urea concentration mg/dL (Mean ±SE) in the experimental groups.

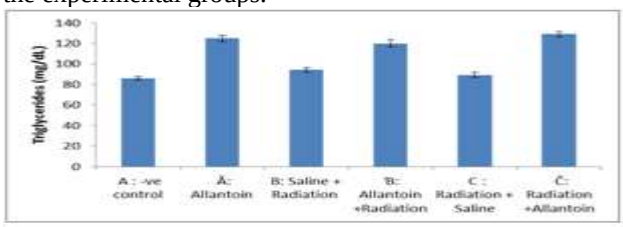

Fig. 6: Plasma triglycerides (mg/dL) (Mean ±SE) in the experimental groups.

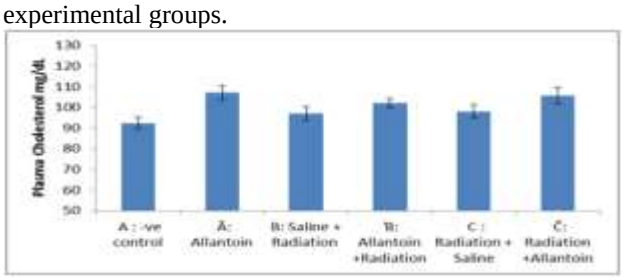

Fig. 7: Plasma cholesterol (mg/dL) (Mean ±SE) in the experimental groups.

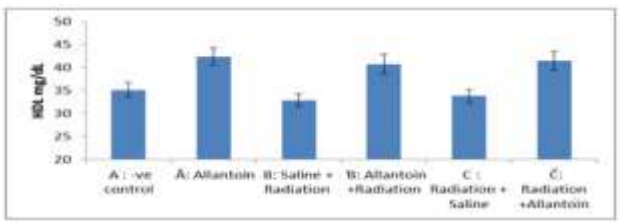

Fig. 8: Plasma HDL (mg/dL) (Mean ±SE) in the experimental groups.

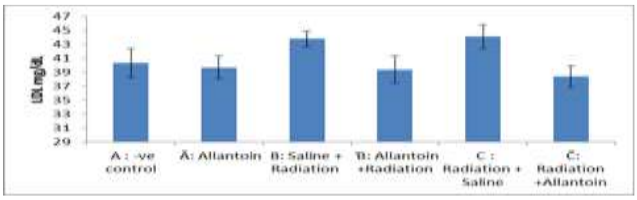

Fig. 9: Plasma LDL (mg/dL) (Mean ±SE) in the experimental groups.

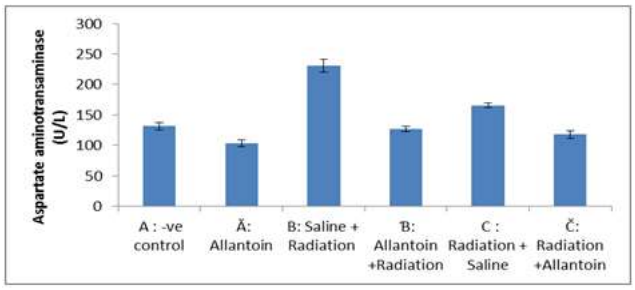

Fig. 10: Plasma Aspartate transaminase activity (U/L) (Mean ±SE) in the experimental groups.

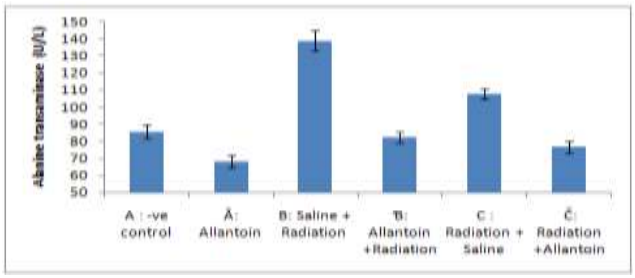

Fig. 11: Plasma Alanine transaminase activity (U/L) (Mean ±SE) in the experimental groups.

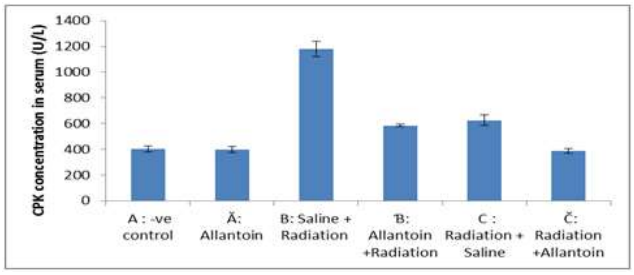

Fig. 12: Plasma creatine kinase activity (U/L) (Mean ±SE) in the experimental groups.

Allantoin in vitro inhibitory action on collagenase activity

Allantoin showed mild collagenase inhibitory effect which means improvement in aging parameters (fig. 13).

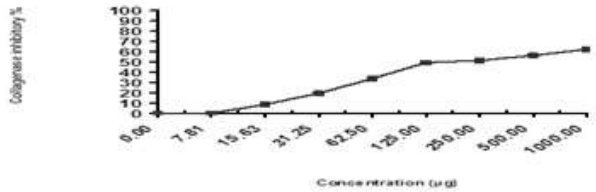

Fig. 13: *In vitro* improvement of aging parameter (collagenase inhibition) by allantoin (IC_{50}=166, 6 µl).

Anti-proliferative activity of allantoin on cancer cell lines

Allantoin have shown inhibitory effects towards different tumors including prostate carcinoma cells PC-3 with IC $_{50}$=411 µg/ml, intestinal carcinoma $CACO_2$ cell line *IC_{50} = 230 µg/ml*, ovarian carcinoma CHO-K1 cell line *with IC_{50} = 462 µg/ml*, breast carcinoma MCF-7 *with IC_{50} = 237 µg/ml*, colon HCT-116 cell line *with IC_{50} = 192 µg/ml* and weak inhibitory effect to Larynx carcinoma cells Hep-2 with IC $_{50}$> 500 µg/ml as shown in figs. 14-19.

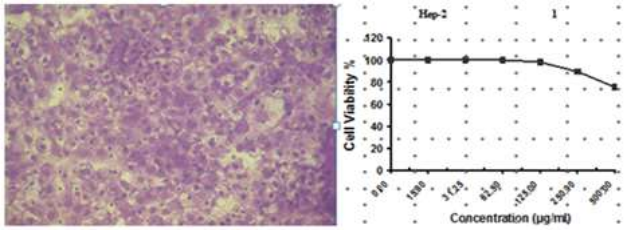

Fig. 14: Weak inhibitory activity against Larynx carcinoma cells with IC_{50}= 500ug/ml.

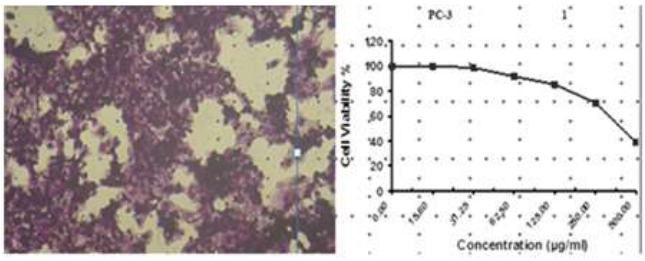

Fig. 15: Inhibitory activity against prostate carcinoma cells with IC_{50}= 411 µg/ml

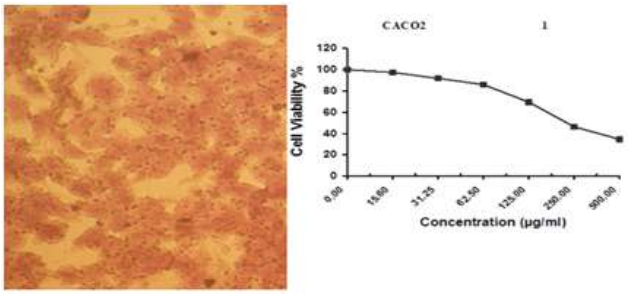

Fig. 16: Inhibitory activity against intestinal carcinoma cells with IC $_{50}$= 230 µg/ml.

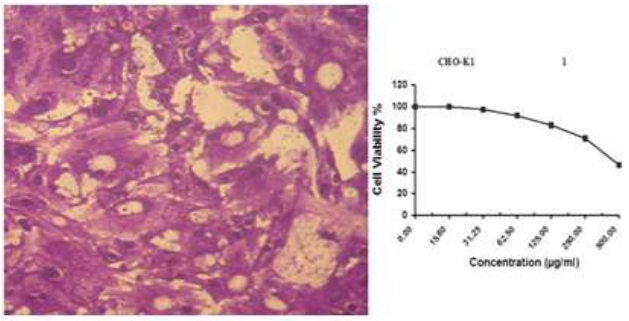

Fig. 17: Inhibitory activity against ovary carcinoma cells with IC_{50}= 462 µg/ml.

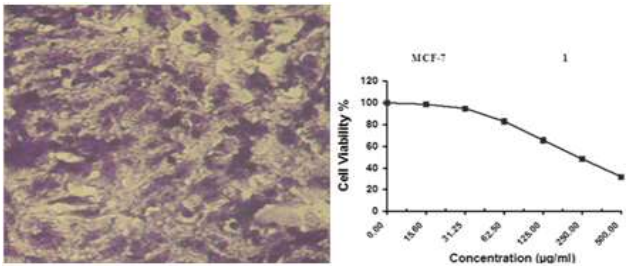

Fig. 18: Inhibitory activity against breast carcinoma cells with IC_{50}= 237 µg/ml.

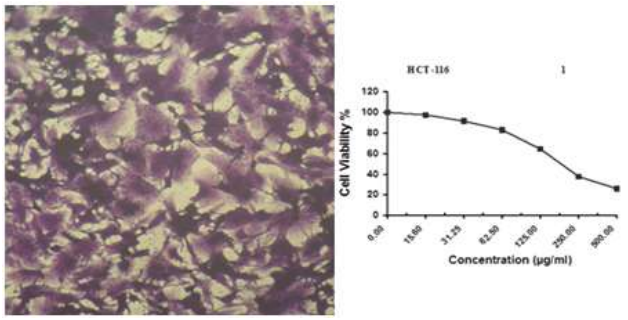

Fig. 19: Inhibitory activity against colon carcinoma cells with IC_{50}= 192 µg/ml

DISCUSSION

The overproduction of allantoin in response to physical activity may be explained by uricase induction through intracellular Ca release through muscular contraction. Allantoin showed protective action against aging process. Allantoin was envisioned as a biomarker for oxidative stress rather than biologically active molecule which can share in body protection against oxidative stress (Amirthanathan and Vijayakumar, 2011; Shestopalov, *et al*. 2006; Saso and Firuzi, 2014). We tried to find out if it is a defense mechanism against oxidative stress and to use this mechanism in radiation protection.

The results showed (As shown in fig. 1) that radiation inhibited SOD activity. This may be occurred through enzymatic denaturation. Allantoin enhances SOD profile improvement as a result of decreased protein denaturation which evidenced by improvement of urea and TAC profiles in the current work. Increased SOD activity may be due to allantoin chelates. Allantoin chelates may have SOD mimic action.

Our results showed that radiation induced oxidative stress affect reduced glutathione in the body (fig 2). GSH was attenuated by irradiation exposure which was improved by allantoin treatment. These results may be explained by either decreasing of free radicals such as hydrogen peroxide or through stimulation of glutathione reductase activity. (Niki, 2010: Milisav *et al*., 2018; Reiter *et al*., 2000; Miles, 2007). Our results elucidated that allantoin treated groups showed concomitant increase in TAC and more pronounced increase in GSH**.** Consequently, Allantoin may be a cause of DNA and cellular protection (Milisav *et al*., 2018). The body healing was evidenced by decreased plasma urea concentration in allantoin treated groups as shown in this study. Decreased urea concentration may indicate inhibition in oxidative trans deamination as a result of a decreased protein catabolism and an increase in cellular protection. This cellular protection may be due to inhibition of more potent hydroxyl radical by allantoin mediated free iron chelation (Battino *et al*., 1999: Bartges J 2019). The inhibition of electron deficient hydroxyl radical leads to inhibition of its attack for negatively charged DNA and uric acid production (Kanďár, 2016; Gus' kov *et al*., 2004; Apak *et al* 2016).

The results of this study showed improved collagenase profile (fig. 13). This was evidenced by in vitro mild collagenase inhibitory (IC_{50} =166.6) effect. So allantoin production induction conditions such as physical activity, pregnancy etc. may improve health aging process by improving cellular integrity and functions (Fisher*et al*., 1997; Acworth *et al* 2017).

Our results of urea give a glimpse about possible anabolic effects of allantoin on protein metabolism. The anabolic effectors may also show improvement against type II diabetes with enhancement of lipogenesis (Ali *et al*., 1992; Ludwig *et al*., 2012). Also our results showed an increase in triglycerides formation in all allantoin treated groups. These results indicate possible increase in hepatic lipogenesis which reflects hepatic glucose consumption to form lipids being the main lipogenic precursor. This increase in lipogenesis may explain improvement in type II diabetes through glucose consumption as a result of lipogenesis enhancement by higher levels of allantoin.

Muscles are the main extrahepatic tissue that utilizes lipids as a main source for muscular energy during prolonged exercise. Although Miles stated that there is no change in lipids level till 24 hours post severe muscular exercise (Gus' kov *et al*., 2004, Seip *et al*., 1997). Our results explained that this phenomenon by the increase in hepatic lipogenesis in allantoin treated groups. Allantoin during muscular exercise may stimulate hepatic lipogenesis while epinephrine (released by muscular exercise) stimulates peripheral lipolysis. Consequently the stimulated hepatic lipogenesis balances the peripheral lipolysis providing muscles with fatty acids. This means that liver target energy in the form of lipids for muscles (Savini *et al*., 2016). Targeting energy in the form of lipids rather than glucose during muscular exercise may decrease excessive lactate production and acidosis. These results were augmented by the finding of (Seip *et al*., 1997) who found that physical activity induces temporarily skeletal muscle lipoprotein lipase gene by short-term exercise.

The above mentioned results explain the possible cause of higher lipogenesis rate in pregnancy due to higher rate of allantoin productions (Kanďár 2016; Lawrence, 1990).Our results in plasma total cholesterol showed significant increase in all allantoin treated groups which may explain how physical activity increases cholesterol synthesis. Aleksandrow *et al* found that physical activity increases cholesterol synthesis in the liver cells for unknown cause with concomitant decrease in serum cholesterol (Aleksandrow *et al*., 1964). Cardoso *et al* found that physical activity increase cholesterol synthesis in non-significant manner (Cardoso *et al*., 1994). So the increase in cholesterol synthesis may be balanced by stimulated excretion by unknown excretion mechanisms. This also may explain the significant metabolic changes in the bile formation, including the formation of cholesterol-supersaturated bile and disturbed gallbladder motility in pregnant women. The above findings may explain the unknown cause for enhancement of cholelithogenesis in pregnant women (de Bari *et al*., 2014).

Anabolic effectors also positively affect the HDL which reflects the change in their Apolipoproteins part (Graham *et al*., 2009; Savini *et al*., 2016). The decrease in urea formation by allantoin may reflect an increase in protein synthesis (anabolic effect) which may include Apolipoproteins. Allantoin in our results significantly increases HDL reflecting more improvement in lipid profiles. This phenomenon may explain how induction of allantoin production protects against CVD. Allantoin treated groups showed a decrease of LDL in comparison to the corresponding non allantoin treated groups while HDL showed significant increase. This can be explained by the higher contents of the HDL Apolipoproteins than LDL which may be due to the increase in the synthesis of Apolipoproteins. Our results including triglycerides, HDL and LDL and urea highlight possible improvements in type II diabetes, CVD and hepatocellular protection by allantoin production (Graham *et al*., 2009).

The cellular radiation protection by allantoin was augmented by decreased plasma intracellular enzymes AST, ALT and CPK according the current study. The lower significant activity of plasma AST in all allantoin treated groups in irradiated groups indicate powerful healing effect due to inhibition of cellular destruction, negative protein turn over and positive nitrogen balance. AST is an index for cellular healing and destruction in liver, muscles, and heart. ALT showed hepatic-protection by allantoin against oxidative stress induced irradiation. CPK showed similar trend as AST. Since, it is well established that increased CPK activity is liable to occur if there is damage to cardiac muscle. These data support the concept of heart protection (Nageswari*et al*., 1999; Pal *et al*., 2015). Our data cleared that allantoin is a potent endogenous healer and its healing effect may be exaggerated in oxidative stress conditions.

It is well known that physical exercise and pregnancy may improve cancer concerns (Valko *et al*., 2016; Gus' kov *et al*., 2004; Ruiz-Casado *et al*., 2017).These improvement is confused and wondered the scientists for unknown and unclear rational reason which in turn encourages us to look for the possible role of allantoin in cancer improvement. Our results of allantoin MTT assay showed weak inhibitory cytotoxic activity against Larynx carcinoma cells. But allantoin showed inhibitory cytotoxic activity against prostate carcinoma, intestinal, ovarian, breast and colon cancers cell lines. This may explains the improvement of these cancers patient in response to physical activity. Also it is well known these cancer are age and sex dependent diseases affecting elderly men at time characteristic with decreased their physical activities (Torti and Matheson, 2004; Ruiz-Casado *et al*., 2017). So, the present findings may provide an important link between physical activity and cancers improvement via an excessive allantoin secretion. So allantoin as radioprotective cytotoxic healer could be taken into consideration for clinical cancer management to alleviate cancer wasting the side effects of radiotherapy, chemotherapy and surgical approaches (figs. 14-19).

CONCLUSION

Allantoin is a powerful endogenous healer, antioxidant and radio protector increases in response to oxidative stress and physical activity which may explain some of the underlying mechanism in cellular protection in physical activity and pregnancy. Allantoin may explain the phenomena of aging and health improvement in athletes. Also allantoin modulates oxidative stress through the redox parameters such as SOD, TAC and GSH Allantoin showed an increase in hepatic lipogenesis by may be activation of glucose consumption to target energy to muscles in the form of lipid decreasing lactate formation from glucose. Further studies are needed to prove possibility of protection against type II diabetes by allantoin. Allantoin improves HDL/ LDL ratio .Allantoin showed liver protection by improvement of ALT and AST, CPK profiles. Allantoin also may inhibit protein denaturation and protein catabolism with hormonal anabolic effect. This was manifested by decreasing in transdeamination indicated by a decrease in urea formation (positive nitrogen balance to meet the anabolic demands such as pregnancy and muscular growth). Allantoin as a metabolite of physical activity improves aging parameters which is evidenced by its collagenase inhibitory effect. Allantoin treats and protects against cancer which could be as used as antitumor drug with no need for teratogenic studies because of its endogenous origin.

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REFERENCES

Aleksandrow D, KłO potowski T and Smietańska Z (1964). Effect of physical activity upon cholesterol synthesis in the rat liver. *J. Atheroscler. Res.,* **4**(4): 351-355.

Ali AM, Zhang QC and Bendtson I (1992). 2 Hypoglycaemia. *Baillieres Clin. Endocrinol. Metab.*, **6**(1): 23-39.

Amirthanathan A and Vijayakumar S (2011). Purification and optimization of uricase enzyme produced by *Pseudomonas aeruginosa*. *J. Exp. Sci.,* **2**(11): 01-04

Bartges J (2019). Pharmacotherapy of renal and lower urinary tract disease. *Pharmacother. Vet. Disp.,* **2**(Ch 13): 297-338.

Battino M, Bullon P, Wilson M and Newman H (1999). Oxidative injury and inflammatory periodontal diseases: the challenge of anti-oxidants to free radicals and reactive oxygen species. *Pharmacother. Vet. Disp.*, **10**(4): 458-476.

Cardoso GC, Posadas C, Orvananos OO, Peniche C, Zamora J, Aguilar R, Holguin JA, Raynaud AS, Morrisett JD and Guevara Jr J (1994). Long distance runners and body-builders exhibit elevated plasma levels of lipoprotein (a). *Chem. Phys. Lipids*, **67**(1): 207-221.

de Bari O, Wang TY, Liu M, Paik CN, Portincasa P and Wang DQ (2014). Cholesterol cholelithiasis in pregnant women: Pathogenesis, prevention and treatment. *Ann. Hepatol.*, **13**(6): 728-45.

Fisher GJ, Wang Z, Datta SC, Varani J, Kang S and Voorhees JJ (1997). Pathophysiology of premature skin aging induced by ultraviolet light. *NEGM*, **337**(20): 1419-1429.

Friedewald WT, Levy RI and Fredrickson DS (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.*,**18**(6): 499-502.

Graham MR, Evans P, Thomas NE, Davies B and Baker J (2009). Changes in endothelial dysfunction and associated cardiovascular disease morbidity markers in GH-IGF axis pathology. *Am. J. Cardiovasc. Drugs*, **9**(6): 371-81.

Gus' kov EP, Prokof'ev VN, Kletskii ME, Kornienko IV, Gapurenko OA, Olekhnovich LP, Chistyakov VA, Shestopalov AV, Sazykina MA, Markeev AV, Kornienko IV and Shkurat T (2004). Allantoin as a vitamin. *Dokl. Biochem. Biophys.* **398**: 320-324.

Kanďar R (2016). The ratio of oxidized and reduced forms of selected antioxidants as a possible marker of oxidative stress in humans. *Biomed Chromat,* **30**(1): 13-28.

Kruk J, Aboul-Enein HY, Kładna A and Bowser JE (2019): Oxidative stress in biological systems and its relation with pathophysiological functions: the effect of physical activity on cellular redox homeostasis. *Free Radic. Res.,* **53**(5): 497-521.

Lawrence LM (1990): Nutrition and fuel utilization in the athletic horse. *Vet. Clin. North Am. Equine. Pract,* **6**(2): 393-418.

Lopes-Virella MF, Stone P, Ellis S and Colwell JA (1977). Cholesterol determination in high-density lipoproteins separated by different methods. *Clin. Chem*, **23**(5): 882-884.

Ludwig B, Rotem A, Schmid J, Weir GC, Colton CK, Brendel MD, Neufeld T, Block NL, Yavriyants K, Steffen A and Ludwig S (2012). Improvement of islet function in a bioartificial pancreas by enhanced oxygen supply and growth hormone releasing hormone agonist. *PNAS*, **109**(13): 5022-5027.

Mavis RD and Stellwagen E (1968). Purification and subunit structure of glutathione reductase from bakers' yeast. *J. Biol. Chem.,* **243**(4): 809-814.

Mayneris-Perxachs J and Swann JR (2019). Metabolic phenotyping of malnutrition during the first 1000 days of life. *Eur. J. Nutr.,* **58**(3): 909-930.

Miles L (2007): Physical activity and the prevention of cancer: A review of recent findings. *Nut. Bull.* **32**(3): 250-82.

Milisav I, Ribaric S, Poljsak B. Antioxidant Vitamins and Ageing (2018): *In*: Biochemistry and Cell Biology of Ageing: Part I Biomed. Sci. Springer, Singapore, pp.1-23.

Nageswari K, Banerjee R and Menon VP (1999). Effect of saturated, ω-3 and ω-6 polyunsaturated fatty acids on myocardial infarction. *J. Nut. Biochem.,* **10**(6): 338-344.

Niki E (2010). Assessment of antioxidant capacity *in vitro* and *in vivo*. *Free Radic. Biol. Med*, **49**(4): 503-515.

Pal PB, Ghosh S and Sil PC (2015).Beneficial effect of naturally occurring antioxidants against oxidative stress–mediated organ dysfunctions. *Bioact. Nat. Prod.* 20. Wiley-VCH.

Patton CJ and Crouch SR (1977). Spectrophotometric and kinetics investigation of the Berthelot reaction for the determination of ammonia. *Anal. Chem.,* **49**(3): 464-469.

Pierro F and Prencipe L (1982). Serum triglycerides determined calorimetrically with an enzyme that produces hydrogen peroxide. C*lin. Chem.,* **28**(10): 2077-2080.

Reiter RJ, Tan DX, Osuna C and Gitto E (2000). Actions of melatonin in the reduction of oxidative stress. *J. Biomed. Sci.,* **7**(6): 444-458.

Ruiz-Casado A, Martin-Ruiz A, Perez LM, Provencio M, Fiuza-Luces C and Lucia A (2017). Exercise and the hallmarks of cancer. *Trends in Cancer*, **3**(6): 423-441.

Saso L and Firuzi O (2014). Pharmacological applications of antioxidants: Lights and shadows. *Curr. Drug Targets*, **15**(13): 1177-1199.

Savini I, Gasperi V and Catani MV (2016).Oxidative stress and obesity. *In*: Obesity Springer, Cham., pp.65-86.

Seip RL, Mair K, Cole TG and Semenkovich CF (1997). Induction of human skeletal muscle lipoprotein lipase gene expression by short-term exercise is transient. *Am. J. Physiol-Endoc. M.*, **272**(2): E255-E261.

Shestopalov AV, Shkurat TP, Mikashinovich ZI, Kryzhanovskaya IO, Bogacheva MA, Lomteva SV, Prokof'ev VN and Gus'kov EP (2006). Biological functions of allantoin. *Biol Bull*, **33**(5): 437-440.

Torti DC and Matheson GO (2004). Exercise and prostate cancer. *Sports Med.*, **34**(6): 363-369.

Valko M Jomova K, Rhodes CJ, Kuca K and Musilek K. (2016). Redox-and non-redox-metal-induced formation of free radicals and their role in human disease. *Arch. Toxicol*, **90**(1): 1-37.

Valko M, Rhodes C, Moncol J. Izakovic MM and Mazur M (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem. Biol. Interact.*, **160**(1): 1-40.

Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M and Telser J (2007). Free radicals and antioxidants in normal physiological functions and human disease. *Int. J. Biochem. Cell B*, **39**(1): 44-84.

Van Wart HE and Steinbrink DR (1981). A continuous spectrophotometric assay for Clostridium histolyticum collagenase. *Anal Biochem*, **113**(2): 356-365.

Wieland H and Seidel D (1983). A simple specific method for precipitation of low density lipoproteins. *J. Lipid Res.*, **24**(7): 904-909.

Zhou D, Chen L, Li J and Wu F (2018). Transition metal catalyzed sulfite auto-oxidation systems for oxidative decontamination in waters: A state-of-the-art mini-review. *Chem. Eng. J.* **346**(1): 726-38.