



Physiological and Biochemical Study of the Impact of LLLT irradiation and GTE in Experimentally Infected laboratory Rats with Arthritis

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Abstract

The objective of this study is to investigate the effect of Green tea extracts (GTE) and Low Level Laser Therapy (L.L.L.T.) on healing of feet arthritis. Elevated free radical generation in inflamed joints and impaired antioxidant system has been implicated in arthritis. The medicinal effects of tea have a history dating back almost 5000 years. The chemical components of green tea chiefly include polyphenols, caffeine and amino acids. Tea also contains flavonoids, compounds reported to have antioxidant properties having many beneficial effects. Tea flavonoids reduce inflammation, have antimicrobial effects. Laser Therapy is active at both the cellular and systemic levels activating a variety of mechanisms including cartilage regeneration, DNA synthesis, improved microcirculation and an analgesic and anti-inflammatory effect. This study included twenty four adult male rats; the arthritis induced in all animals by using the formaldehyde then were divided into four groups (6 rats each) G1 control group, G2 treated with Low Level Laser Therapy LLLT, G3 treated with GTE and G4 treated with LLLT and GTE in physiology laboratory.

In conclusion this study investigates possible mechanisms by which laser and GTE protect joints in rat model. (By the hormonal and biochemical studying of some hormones and minerals in the serum of the animals).

Introduction

Arthritis results in the deterioration of the joint through the process of chronic inflammation. The most common form is degenerative osteoarthritis, Osteoarthritis most commonly affects the hands, feet, spine and the large weight bearing joints

such as the hips and knees (Kirkley *et al.*, 2008).

Although many medicines are prescribed for the treatment of arthritis, they are known to produce various side effects including gastrointestinal disorders, immunodeficiency and humoral disturbances. So, there is still a need to

seek therapeutic agents with lower side effects that can be used for long-term administration (Sahebari *et al.*, 2011).

The chemical components of tea leaves include polyphenols (catechins and flavonoides), alkaloids (caffeine, theobromine, theophylline, etc.), volatile oils, polysaccharides, amino acids, lipids, vitamins (e.g., vitamin C), inorganic elements (e.g., aluminium, fluorine and manganese), etc. However, the polyphenols are primarily responsible for the beneficial healthful properties of tea. The flavonoides have antioxidant, anti-inflammatory, antiallergic and antimicrobial effects (Sharangi, 2009).

Low level lasers are thought to promote healing and reduce pain possibly through

Materials and methods

Animals

Twenty four male adult white rats, named scientifically as *Rattus norvegicus*, with five to eight month aged and average weight of (350-400g.), were used in this study. They were kept in standard separate cages and had free access to tap water and were fed with standard pellets *ad libitum*. This model was selected because it provides many desired characteristics to fulfill the requirements of this study. All cages were kept in conditioned room 20-22°C with controlled lightening. The animals left for two week before the experiment for adaptation.

Extraction Procedure

the reduction of inflammation. Healing may come about by increased cell proliferation, Treatment of arthritis is very complex, and in the past years, some studies have investigated the use of low-level laser therapy (LLLT) in treatment of it (Kucuk *et al.*, 2010).

L.L.L.T promotes proliferation of multiple cells, which (especially red and near infrared light) is mainly through the activation of mitochondrial respiratory chain and the initiation of cellular signaling (Dais, 2009).

The aim of study is assessed the effects of L.L.L.T and GTE on the changing of the levels of some hormones and minerals in the rats with adjuvant-induced arthritis (AIA) as a model for arthritis.

The leaves of tea (*Camellia sinensis*) were powdered, then weighted (15 grams) of the powder and dissolved in 100 ml of 70% distilled water using a magnetic stirrer for 24 hours at room temperature (25°C). The resultant extract solution was filtered using double layers of cotton gauze. The filtered solution was dried at 40°C, after that we dissolved every 1gram in 5 ml distilled water to produce the 200 mg/ml of extracting, the dosing began in the treated groups in the treatment days in fact between day and other from fourteenth day to twenty two day (and according to concentration sentences and took the average of animals weight as 300 mg) the amount of the dosing was 1.5 ml for each animal.

Laser diode

The laser system used was semiconductor crystal with active medium is Ga Al As (gallium aluminum arsenide).

The laser used was diode 820 nm wave length, a maximum output of 200 mW, density 8 J/cm², pulsing frequency 2.5-20 Hz. Irradiation began in the irradiation groups in the treatment days in fact between day and other from fourteenth day to twenty two day with 1.20 min/session daily. The Irradiation was carried out by 1 cm. from the skin of the paws edema.

Induction of adjuvant-induced arthritis in rats

For induction of experimental arthritis, the plantar surface of the left hind paw of rat was injected with 0.1 ml of (HCHO) and with 2 % concentration in the first and third days of experimentation (Al- Fahad, 2014). The changes that have occurred in the foot (after the tenth day postoperation) which is redness, swelling and lameness,

Statistical analysis of data

The minitab statistical program was used to calculate the statistical significance of difference between groups.

The Results

1) Parathyroid Hormone (PTH)

PTH values decreasing in the all groups after the inducing of the disease put in the

as observed there a significant increase ($P < 0.05$) in the thickness of the foot in the fourteenth day (5.78 ± 0.62 mm) comparing with the first (4.01 ± 0.21 mm).

Blood sampling

Blood samples were collected under general anesthesia using a mixture of Ketamine hydrochloride (10 mg/Kg) and xylazine (3 mg/ Kg) administered i/m. , at regular intervals from the heart after disinfection using 70% alcohol, in first, fourteenth, nineteenth, twenty three and twenty eight days post operation the amount of blood was 2 cc, and blood samples were collected using syringes 3 mL, then left for twenty minutes in room temperature, after that serum was separated by centrifuge 2500 cycles/minute for 15 minutes. Serum was used to measure each of PTH and CTH values by ELISA. Ca and Mg values by Spectrophotometer.

The difference between groups at the $P \leq 0.05$ levels was considered statistically significant.

treatment groups returns to the normal values after the treatment and with significant varies between these groups and control group. (fig. 1)

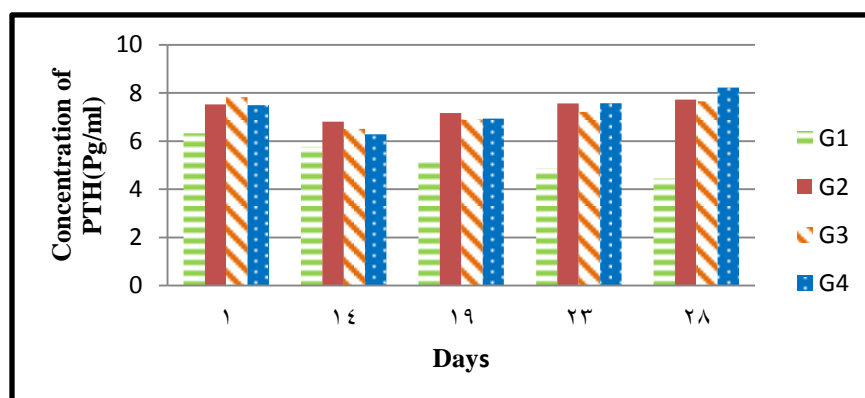


Figure 1: PTH values variations among the groups of rats during the experimentation time (inducing and treatment). There are significant variations between G1 & G2 at $P \leq 0.003$, between G1 & G3 at $P \leq 0.002$ and between G1 & G4 at $P \leq 0.004$.

2) Calcitonin hormone (CTH)

CTH values increasing in the all groups after the inducing of the disease put in the treatment groups returns to the normal

values after the treatment and with significant varies between these groups and control group. (fig. 2)

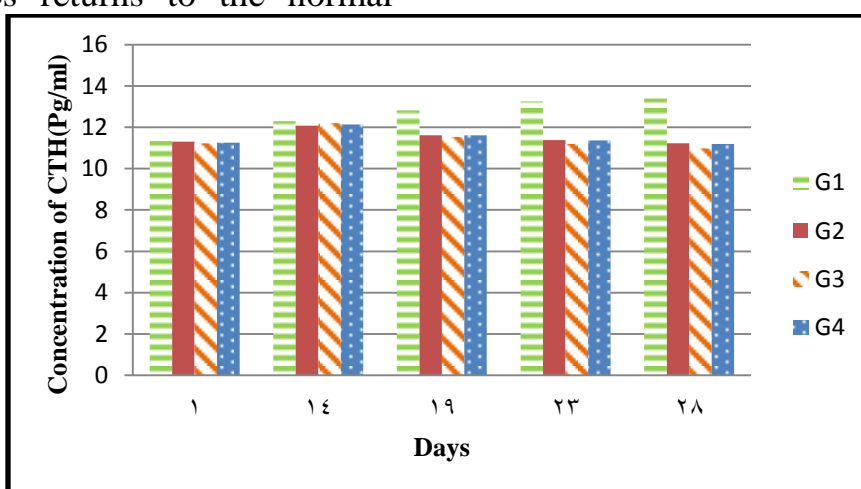


Figure 2: CTH values variations among the groups of rats during the experimentation time (inducing and treatment). There are significant variations between G1 & G2 at $P \leq 0.041$, between G1 & G3 at $P \leq 0.032$ and between G1 & G4 at $P \leq 0.043$.

3) Calcium (Ca)

Ca values decreasing in the all groups after the inducing of the disease put in the treatment groups returns to the normal

values after the treatment and with significant varies between these groups and control group. (fig. 3)

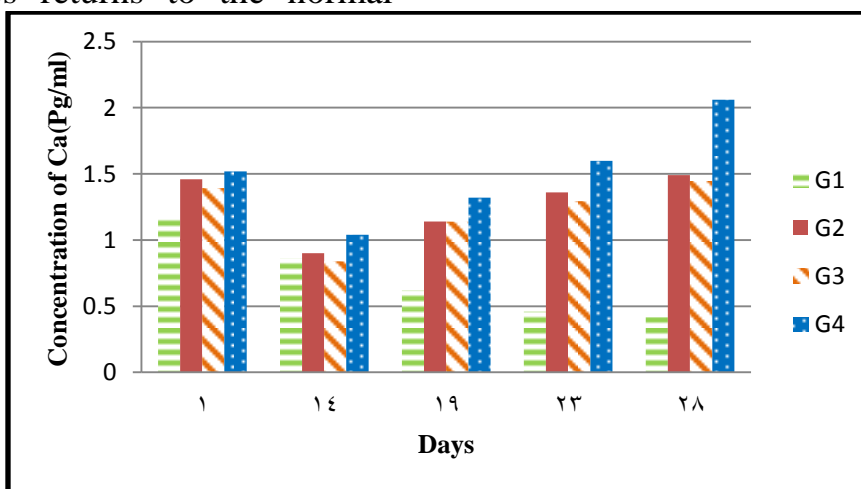


Figure 3: Ca values variations among the groups of rats during the experimentation time (inducing and treatment). There are significant variations between G1 & G2 at $P \leq 0.015$, between G1 & G3 at $P \leq 0.021$ and between G1 & G4 at $P \leq 0.008$.

4) Magnesium (Mg)

Mg values decreasing in the all groups after the inducing of the disease put in the treatment groups returns to the normal

values after the treatment and with significant varies between these groups and control group.(fig. 4).

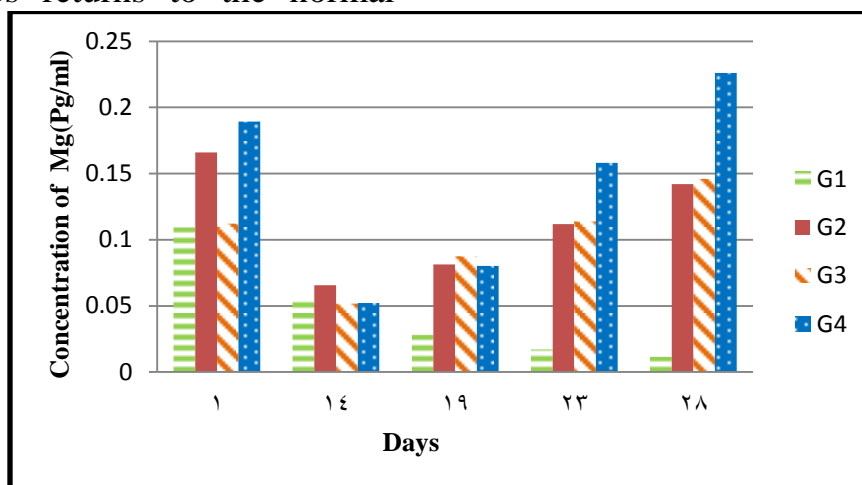


Figure 4: Mg values variations among the groups of rats during the experimentation time (inducing and treatment). There are significant variations between G1 & G2 at $P \leq 0.031$, between G1 & G3 at $P \leq 0.04$ and between G1 & G4 at $P \leq 0.039$.

Discussion

The significant decreasing at the calcium level in the serum of experimental animals after the inducing of the disease may returns to the effect of toxins that formed by the formaldehyde after the injection of the rats by which and its depressive effect on parathyroid gland function (Al- Fahad, 2014), and this effect lead to decrease of parathyroid hormone synthesis and secretion, hypocalcaemia may be have occurred as a result of hypoproteinemia or due to renal impairment and depressive effect of lead on parathyroid gland function (van Santen *et al.*, 2011). Furthermore, hypocalcaemia may occur as a result of competition absorption between lead acetate and Ca^{+2} at the level of intestinal epithelium (Walwadkar *et al.*, 2006). Inflammatory mediators,

cytokines and oxidative stress are the most likely causes corticosteroids decrease generalised BMD loss by suppression of inflammatory activity, but as aside-effect, increase BMD loss (Ramprasath *et al.*, 2006 ; Walwadkar *et al.*, 2006).

The elevation of parathyroid hormone in treatment groups (G3, G4) may be due to enhancement of parathyroid gland activity by antioxidant effect of GTE (mainly green tea catechins) (GTC) (Hajimahmoodi *et al.*, 2008), also suggest that elevation of Ca^{+2} levels in blood is due to stimulation of PTH which stimulate renal 1, α - hydroxylase system and due to increase of thyroxine hormone at these two periods which stimulate the active form of vitamin-D₃, vitamin-D₃ increase the Ca^{+2} from small intestine by

stimulation of Ca^{+2} binding protein (CBP) or by increasing their active diffusion across brush border (van Santen *et al.*, 2011), also the effect of L.L.L.T. (in G2 & G4) for reducing the level of pro-inflammatory cytokines/chemokines produced by synoviocytes. This mechanism may be more general and underlie the beneficial effects of LLLT on other inflammatory conditions (Yamaura *et al.*, 2009).

Hawkins & Abrahamse, (2005) has been reported that laser photonic energy is absorbed by the mitochondria in the cell in which this energy is converted to chemical kinetic energy and finally leads to more production of ATP. ATP is the source of energy in the cell which is necessary for cell activities such as synthesis of DNA, RNA and proteins that are important in cellular proliferation.

Mg is the most abundant divalent cation in the intracellular compartment, PTH increases Mg reabsorption; No other hormones are known to alter Mg reabsorption, (Yu ASL, 2004), therefore, the PTH, Ca and Mg connects with each other in increasing and decreasing.

(Aydm, 2013) noted that magnesium regulates PTH secretion and action and vitamin D activation through interacting

with calcium-sensing receptors on parathyroid glands and renal tubular cells.

Calcitonin has analgesic, anti-inflammatory, and antiosteoclastic actions, therefore, it is used to treat clinical and biological diseases characterized by excessive human bone remodeling, (Bulbul *et al.*, 2008; Nascimento *et al.*, 2010).

Calcitonin hormone participates in calcium (Ca^{2+}) and phosphorus metabolism. In many ways, calcitonin counteracts parathyroid hormone (PTH) ((Costoff, 2008), the main functions of calcitonin are preventing calcium release from bones and encouraging calcium to be filtered by the kidneys. The sum effect of these two processes lowers the total level of calcium in the blood, (Nascimento *et al.*, 2010), therefore these hormones values increasing in all groups of the animals after the inducing of arthritis, but in treated groups returns to the normal values because the actions of parathyroid hormone on the thyroid gland and on calcium levels in the blood.

Conclusion can be done that the treating of the arthritis with GTE and L.L.L.T was useful and efficient that the healing was promoted and accelerated because its healing stages were induced by GTE and L.L.L.T.

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دراسة فسيولوجية و كيموحيوية لتأثير أشعة الليزر واطئة الطاقة و مستخلص الشاي الأخضر في الجرذان المختبرية المصابة تجريبياً بالتهاب المفاصل

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الخلاصة

الهدف من هذه الدراسة هو دراسة تأثير مستخلص الشاي الأخضر (GTE) وأشعة الليزر واطئة الطاقة (LLLT) على الشفاء من التهاب مفاصل القدمين. تكون الجذور الحرة للالتهاب مرتفعة في التهاب المفاصل وفعالية نظام مضاد الأكسدة تتضاعف في المفصل المصاب. الآثار الطبية للشاي لها تاريخ يعود تقريبا إلى 5000 سنة. المكونات الكيميائية للشاي الأخضر تعود إلى مادة البوليفينول، والكافيين وإلى الأحماض الأمينية. يحتوي الشاي أيضا على الالفلافونويدات وكل هذه المركبات لها خصائص مضادة للأكسدة وتكون مؤثرة وفعالة ضد الميكروبات.

العلاج بالليزر يكون فعال على المستويين الخلوي والجهازي حيث يفعل مجموعة متنوعة من الآليات بما في ذلك تجديد الغضروف، تركيب الحمض النووي، وتحسين دوران الأوعية الدقيقة ومسكن وتأثير مضاد للالتهابات. وقد شملت هذه الدراسة أربع وعشرين من ذكور الجرذان البالغين. تم أستحداث الالتهاب في جميع الحيوانات بأستخدام الفورمالديهايد ثم قسمت إلى أربع مجموعات (6 جرذ لكل منها) G1 مجموعة السيطرة G2 مجموعة عوملت مع مستوى منخفض لأشعة الليزر (LLLT)، G3 عوملت مع GTE و G4 مع LLLT و GTE وتمت التجربة في مختبر الأبحاث الفسلجية.

أستنتج من هذه الدراسة أمكانية تحفيز ميكانيكيات العلاج وحماية المفاصل بأستخدام LLLT و GTE في نموذج الحيوانات المستخدم (بواسطة الدراسة الهرمونية والبيوكيميائية لبعض الهرمونات والمعادن في مصل دم الحيوانات).