

# A COMPARATIVE STUDY OF THE EFFECT OF GREEN VERSUS ROASTED COFFEE ON THE GROWTH OF MANDIBULAR CONDYLE IN ALBINO RATS OFFSPRING

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## ABSTRACT

**BACKGROUND:** The relation between maternal coffee intake with offspring mandibular condylar growth is still inconclusive and has not been examined.

**OBJECTIVE:** To compare the effect of green versus roasted coffee on condyle growth in rats' offspring.

**MATERIALS AND METHODS:** Eighteen male Wistar albino rats' offspring were obtained from 6 adult female Wistar albino rats at birth. On the first day of gestation, the pregnant rats were divided randomly into 3 groups. Group I: Control group received 5 ml of distilled water (2 rats). Group II: Green coffee group received 5 ml of green coffee (2 rats). Group III: Roasted coffee group received 5 ml of roasted coffee. Both coffee types were used at an optimum dose equal to 2 cups in humans. The distilled water and both coffee types were daily administered during pregnancy and lactation periods. The offspring were euthanized at birth and 21 days (weaning time). The condyles were dissected and then processed for histological and histomorphometric analysis.

**RESULTS:** At birth, the control group showed well developed erosive zone. The green coffee group showed fine bone spicules in the osteogenic zone, while the roasted coffee group showed well-developed woven bone. At 21 days, the osteogenic zone in the control group showed numerous spicules of woven bone. The green coffee showed fine bone trabeculae of cancellous bone studded with numerous osteoclasts, while roasted coffee showed well-developed cancellous bone.

**CONCLUSIONS:** The roasted coffee accelerates and improves the growth of the condyle, while green coffee induces signs of osteoporosis.

**KEYWORDS:** Green coffee, Roasted coffee, Endochondral bone, Mandibular condyle.

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## INTRODUCTION

The congenital malformations are caused by hereditary and/or environmental causes. The interaction between hereditary and environmental causes occurring at a specific time of development results in the majority of congenital abnormalities (1). According to the world health organization, congenital abnormalities are defined as abnormalities in the structure, function, or metabolic anomalies that occur during intrauterine life (2,3).

Recent experiments are being directed toward altering the effect of abnormal genetic causes through changes in the environment. Since genetic changes can change which protein is made, epigenetic changes influence gene expression to turn genes "on" and "off." Hence, environmental factors, such as diet and exercise, can result in epigenetic changes. So, knowledge about teratogens as noxious environment agents and their effective time is important to understand the prevention of

these defects (1,4).

Coffee consumption nowadays is considered a regular part of daily life (5). Most pregnant women drink coffee every day. However, caffeine is a lipophilic substance that freely transfers across all biological membranes, including the blood-placental barrier. Neither fetus nor the placenta has the enzymes for its metabolism; caffeine absorbed by mothers may also accumulate in the uterine fluid (6,7).

On the other side, coffee has many beneficial health effects at minimal doses. Coffee has anti-microbial properties by acting as an antiadhesive to the bacteria on the mucous membranes (8).

Coffee has antioxidant components that suppress oxidative stress by hydrogen atoms donation to diminish free radicals. In addition, coffee increases plasma glutathione, which is a natural antioxidant in the body (8-10).

Coffee poses antiobesity properties by inhibiting fat absorption and activating fat metabolism in the liver. Also, it blocks the absorption of starch through an  $\alpha$  amylase inhibitor (11).

Coffee acts as a hypoglycemic agent by impacting glucose absorption in the intestine. It also decreases glucose output in the liver through inhibition of glucose-6-phosphate, an enzyme important in glucose transport (12).

The major components of green coffee are caffeine, chlorogenic acids, trigonelline and diterpenes such as cafestol and kahweol in addition to triacylglycerols and sterols (11,13).

The characteristic properties of the coffee such as flavor and smell are developed during roasting (14). During roasting, water and some volatile substances are released from the green bean. Thousand compounds are formed during the roasting process, such as melanoidins, tocopherols, caffeic acid, and serotonin, which improve bone formation (10,15).

According to several studies, high coffee intake—increases the risk of developing bone disorders, including osteoporosis and bone fractures (16-18).

Mandibular condyle cartilage is the greatest growth center in the craniofacial complex from birth until adulthood in humans. Mandibular condyle cartilage growth is associated with the morphogenesis of the maxillofacial skeleton and temporomandibular joint function (19).

As far as our knowledge, no available studies were found to show the effect of green and roasted coffee on the growth of mandibular condyle. So, the present study has been performed on Albino rats' offspring at birth and 21 days. This study was conducted with the null hypothesis that there is no significant difference in the effect of green and roasted coffee on the growth of mandibular condyle.

## MATERIALS AND METHODS

### Study sample

Eighteen male Wistar albino rat's offspring were used in this study. They were obtained from 6 adult female Wistar albino rats at birth. Animals were obtained from the animal house of the Medical Research Institute, Alexandria University. The mature female rat's weights ranged from (250-300 grams), and their age was from (2-3) months. Also, their health status were examined. Rats were housed in plastic cages under controlled lighting (12 hours light / 12 hours darkness) and temperature (21-25°C). The animals were supplied with a balanced diet and free access to water, for 30 days before the experiment (adaptation period) and during the whole experimental period (20). All animal procedures followed the National Research Council guidelines for the care and use of laboratory animals (21).

After the adaptation period, the adult female rats in the estrus period were mated overnight with males. Pregnancy was examined and inspected

in the morning by vaginal plug formation (22).

After the investigation of pregnancy, three pregnant female rats were kept in each plastic cage. On the first day of gestation, the pregnant rats were divided randomly into 3 equal groups:

Group I (Control group as placebo): 2 pregnant female rats were fed a standard diet and received 5 ml of distilled water daily.

Group II: 2 pregnant female rats were fed a standard diet and received 5 ml green coffee (Samo trading company, industrial zone, Third settlement, New Cair, <http://www.Abu-auf.com>) daily at an optimum dose equal to 2 cups in the human (23).

Group III: 2 pregnant female rats were fed a standard diet and received 5 ml roasted coffee (Samo trading company, industrial zone, Third settlement, New Cair, <http://www.Abu-auf.com>) daily at an optimum dose equal to 2 cups in the human (23).

The distilled water and both types of coffee were administered by oral gavage to the female rats at the same time (9-11 am) during pregnancy and lactation period (21 days) (24).

The dose was adjusted according to the body weight of each adult female rat (pregnant and lactating) included in this study.

The functional unit of one cup of coffee includes 7grams of coffee powder (25). Two cups were used in the present study. The average woman's weight is 62 kilograms (Average body weight globally).

So, the dose calculation of coffee consumed daily in gm =

$$\frac{\text{Coffee powder (gm) in 2 cups} \times \text{weight of rat in (kg)}}{\text{Average woman's weight}} = \frac{14 \times \text{weight of rat in (kg)}}{62}$$

### Euthanization time

Three rats' offspring from previous groups were euthanized by diethyl ether at birth and after 21 days (weaning time). The remains of rats' bodies were disposed of by burial by special authorities.

### Histological study

The mandibular condyles of each rat offspring were dissected out and were fixed in 10% neutral buffered formalin for 24h. After fixation, specimens were decalcified in 10% trichloro-acetic acid, washed in tap water overnight, and then dehydrated in ascending concentrations of alcohol. Cleared in xylene and then infiltrated and embedded in a low melting point (56°C) paraffin wax. Tissue blocks were cut sagittally at a 5  $\mu$ m thickness with a microtome. Sections were stained with hematoxylin and Eosin (H&E) for general examination (26).

### Histomorphometric analysis

Histomorphometric analysis was done to measure the percentage of the bone surface area of the mandibular condyle. The histomorphometric examination of the tissue was based on quantitative measurements of the microscopic structure by using image J software. The best prepared six slides of the histological section

were chosen from each group at two intervals. The image of each section of all groups (18 rat offspring x 2condyle (each side) = 36 samples) was captured using an X4 objective lens. A rectangle with identical measurements was drawn on chosen standardized areas of the osteogenic zone of each condyle. The surface area of the osteogenic zone bone was evaluated via choosing the region of interest manager (ROI), obtained by summation of total surface areas. Then the mean of these measurements was calculated. After that, the percentage of the surface area of the formed bone was calculated (27).

Statistical analysis: Data from the histomorphometric analysis were fed to the computer using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Normality of distribution was verified using the Shapiro-Wilk test. Minimum and maximum values, mean and standard deviation were used to describe quantitative data. A significance level of the obtained results was set at 5%. One way ANOVA test was used for normally distributed quantitative variables to compare between more than two groups. The post Hoc test (Tukey) was used for pairwise comparisons.

**RESULTS**

**Histological results**

**At birth**

**Group I (Control group) (Figures 1 & 2)**

The control group showed a normal histological structure of mandibular condyle (MC). The mandibular condyle consists of the fibrocartilaginous zone, proliferative cell zone, chondroblastic zone, hypertrophic zone, and erosive zone. The erosive zone showed numerous chondroclasts in its lacunae.

**Group II (Green Coffee group) (Figures 1 & 2)**

The green coffee group showed a reduction in the thickness of the cartilaginous zone compared to the control group. The green coffee group showed fine bone spicules of woven bone and numerous osteoclasts in the osteogenic zone.

**Group III (Roasted Coffee group) (Figures 1 & 2)**

The roasted coffee group showed well-developed woven bone in the osteogenic zone.

**At 21 days**

**Group I (Control group) (Figures 3 & 4)**

In the control group, the mandibular condyle showed the typical histological zones at 21 days. The osteogenic zone showed woven bone spicules.

**Group II (Green coffee group) (Figures 3 & 4)**

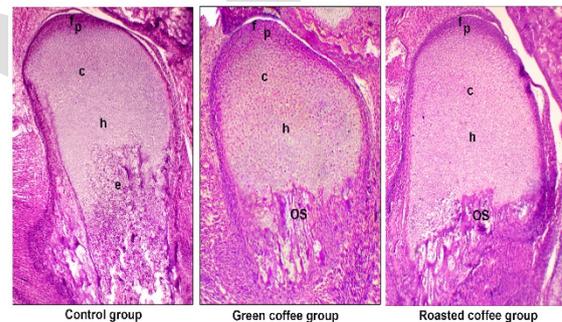
The histological sections of the green coffee group in the mandibular condyle revealed a poorly developed hypertrophic zone compared to the control group. The osteogenic zone showed the replacement of woven bone with thin bone trabeculae and wide bone marrow of the cancellous bone. Several osteoclasts inside Howship’s lacunae were observed in bone trabeculae.

**Group III (Roasted coffee group) (Figures 3 & 4)**

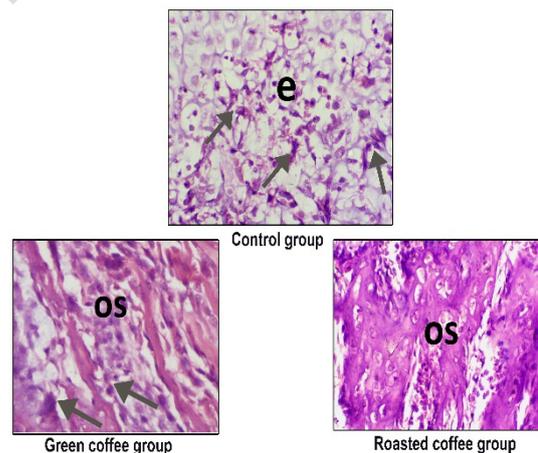
The osteogenic zone of the roasted coffee group showed smooth and well-developed bone trabeculae surrounding bone marrow tissue of cancellous bone. Histomorphometric analysis of the percentage of bone surface area

Table (1) showed a comparison between the three groups at birth; there might be a piece of evidence decrease in the percentage of bone surface area in the green coffee group compared to the roasted coffee group (p=0.016). In contrast, there was an increase in the percentage of bone surface area in the roasted coffee group compared to the control group (<0.001).

Table (2) showed a comparison between the three groups at 21 days; the percentage of bone surface area was statistically increased in the roasted coffee group compared with the control and green coffee groups (p2=0.046, p3=0.001), respectively. On the other hand, there might be a piece of evidence between the green and control groups (p1=0.001).

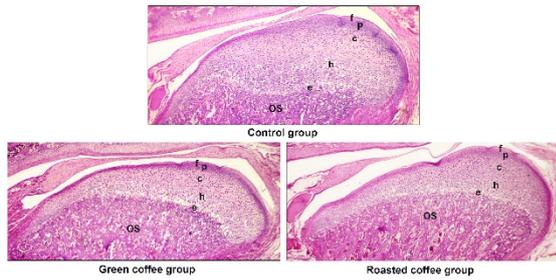


**Figure 1:** Photomicrographs show the zones of mandibular condyle at birth. Fibrocartilaginous f, proliferating p, chondroblastic c, hypertrophic h, erosive e, osteogenic zones os. (H&E. stain, original magnification x40).

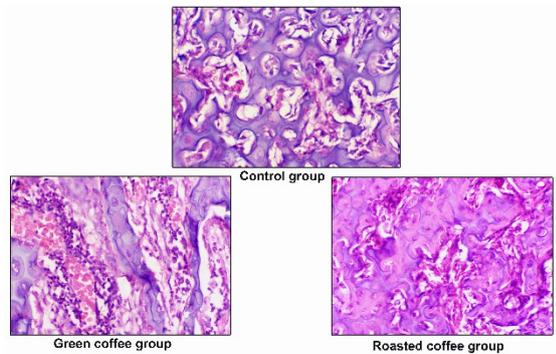


**Figure 2:** Photomicrographs of the mandibular condyle at birth. The control group shows numerous chondroclasts (arrows) in the erosive zone. The green coffee group shows fine bone spicules and osteoclasts (arrows) in the osteogenic zone. The roasted coffee group shows well-developed woven

bone in the osteogenic zone. (H&E. stain, original magnification  $\times 400$ ).



**Figure 3:** Photomicrographs show the zones of mandibular condyle at 21 days. Fibrocartilaginous f, proliferating p, chondroblastic c, hypertrophic h, erosive e, osteogenic zones os. Note in the control group the hypertrophic zone is wider than green and roasted coffee groups (H&E. stain, original magnification  $\times 40$ ).



**Figure 4:** Photomicrographs of the osteogenic zone of the mandibular condyle at 21 days. The control group shows woven bone. The green coffee group shows the poorly-developed bone trabeculae and wide bone marrow tissue of cancellous bone. The roasted coffee group shows well-developed bone trabeculae surrounding the bone marrow of the cancellous bone. (H&E. stain, original magnification  $\times 400$ )

**Table 1:** Comparison between the control, green coffee and roasted coffee groups according to the bone surface area of the condyle at birth.

	Control (n = 6)	Green coffee (n = 6)	Roasted coffee (n = 6)	p
% Bone area				
Min. – Max.	0.0 – 0.0	7.18 – 18.78	11.87 – 21.24	<0.001*
Mean $\pm$ SD.	0.0 $\pm$ 0.0	12.82 $\pm$ 4.26	18.72 $\pm$ 3.54	
Sig. bet. grps.	p <sub>1</sub> <0.001*, p <sub>2</sub> <0.001*, p <sub>3</sub> =0.016*			

SD: Standard deviation

F: F for One way ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey)

p: p value for comparing between the studied groups

p<sub>1</sub>: p value for comparing between Control and Green coffee

p<sub>2</sub>: p value for comparing between Control and Roasted coffee

p<sub>3</sub>: p value for comparing between Green coffee and Roasted coffee

\*: Statistically significant at  $p \leq 0.05$

**Table 2:** Comparison between the control, green coffee and roasted coffee groups according to the bone surface area of the condyle at 21 days.

	Control (n = 6)	Green coffee (n = 6)	Roasted coffee (n = 6)	p
% Bone area				
Min. – Max.	31.52 – 40.74	22.83 – 29.54	32.50 – 48.99	<0.001*
Mean $\pm$ SD.	37.26 $\pm$ 3.60	25.46 $\pm$ 2.48	43.92 $\pm$ 6.16	
Sig. bet. grps.	p <sub>1</sub> =0.001*, p <sub>2</sub> =0.046*, p <sub>3</sub> <0.001*			

SD: Standard deviation

F: F for One way ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey)

p: p value for comparing between the studied groups

p<sub>1</sub>: p value for comparing between Control and Green coffee

p<sub>2</sub>: p value for comparing between Control and Roasted coffee

p<sub>3</sub>: p value for comparing between Green coffee and Roasted coffee

\*: Statistically significant at  $p \leq 0.05$

## DISCUSSION

There is strong evidence that maternal caffeine intake causes congenital malformation and restriction of fetal growth (6,7). So the present study was designed to compare the effect of green and roasted coffee consumption on the histological structure of the growing mandibular condyle of male rats' offspring born to coffee-drinking mothers.

In this study, the rats were selected because of the similarity between the Wistar albino rat condyle and the human condyle. This is essential for the validity of the study (28).

The age of euthanization of rats' offspring selected for this study was at birth and 21 days. At birth, the time was chosen to exhibit the changes in development and calcification of condyle under the

influence of both types of coffee in intra-uterine life. Since 21 days is the weaning time for the rats, this time is suitable to observe the results of the coffee effect during lactation (6,7).

In the present study, histological examination of the H&E stain of the mandibular condyle of rats' offspring of the control group at birth showed a normal structure of the mandibular condyle. The erosive zone was well-developed, with some resorbed areas and numerous chondroclasts.

In the green coffee group at birth, the histological results revealed a reduction in cartilaginous zones and an absence of an erosive zone. This could explain that green coffee accelerates cartilage cell transition and quickens endochondral bone formation. A new layer was formed called the osteogenic zone, which formed from the poorly developed woven bone. The formation of bone was explained by the effect of the antioxidant content of green coffee. The antioxidant effect of green coffee is exerted by its rich phenolic compounds, mostly chlorogenic acid. They stimulate the differentiation of osteoblasts, maintaining the vitality of osteocytes, and the mineralization of the newly formed bone (29).

The low quality of the bone referred to the high content of caffeine compared to roasted coffee (10). These observations are in accordance with Reis et al., who found more damaging effects of caffeine on long bones and vertebrae in three-day rats' offspring of mothers treated with caffeine during pregnancy (7).

In addition, the green coffee group showed numerous osteoclasts in Howships lacunae. This is because caffeine enhances the differentiation of osteoclasts from bone marrow monocyte hematopoietic precursors. Also, caffeine stimulates osteoblasts to secrete a protein named receptor activator of nuclear factor-kappa B ligand (RANKL), which induces the differentiation of osteoclasts (11,30).

Another cause that increases the effect of maternal green coffee intake in the offspring is caffeine metabolism (16). As known, coffee passes through the placenta (6,7). The caffeine's metabolism time increases to 18 hours during the first trimester of pregnancy. Despite this, the main part of caffeine metabolites in the liver takes 3-4 hours in the human body (16).

In the current study at birth, the roasted coffee group showed a reduction in the cartilaginous zones, due to the rapid transformation of cartilage into bone than the control group. Woven bone formed in the roasted coffee group was well developed. This could be clarified by the high antioxidant property of roasted coffee; that is formed from the new compounds generated after roasting, such as melanoidins, tocopherols, and caffeic acid (10,15). The antioxidant activities of melanoidins have a role in maintaining osteoblast viability and function to secrete bone (29).

Tocopherols nourish the tissue by strengthening the blood vessels' walls and promoting blood circulation (10). Therefore an increase in vascularity coexists with osteoblasts differentiation and bone matrix production (31). Tocopherol also improves bone health and acts as an antiosteoporotic (32).

Caffeic acid has a role in skeletal protection; it decreases bone resorption by inhibiting osteoclastogenesis (33). Tolba et al. reported that systemic administration of caffeic acid in osteoporosis-induced rats preserves skeletal health by reducing osteolysis and bone loss (34). Caffeic acid in the same study enhanced antioxidant defense, which led to an increase in osteoblastogenesis and also osteoblasts protection. On the other hand, it suppresses osteoclastogenesis by decreasing RANKL/osteoprotegerin (OPG) ratio (34).

At 21 days, in the control group, the initiation of bone formation appeared as woven bone spicules. It may be referred to as the antioxidant components of both coffee; that reflect the normal structure of the mandibular condyle during that period.

In the green coffee group, the results revealed a decrease in the hypertrophic zone compared to the control group. The osteogenic zone showed the replacement of woven bone with poorly developed bone trabeculae. That studded with numerous osteoclasts and wide bone marrows of cancellous bone. These results are advocated by Reis et al. They reported the adverse effect of caffeine on endochondral ossification of the long bone in 21 days rats' offspring of mothers treated with caffeine during lactation (7).

As the coffee passes through the mother's milk during lactation(7). The caffeine elimination time in newborns elevates from 50 to 100 hours; due to newborns' deficiency in caffeine metabolizing enzyme (cytochrome p 450). The long caffeine' metabolism time exaggerates the caffeine effect (16).

Similar results were recorded by Kwak et al., who reported that caffeine has adverse effects on the long bones in immature and young adult rats. They also proved that chronic caffeine consumption caused an increased risk of osteoporosis (24). In the current study, the histological results of the roasted coffee group at 21 days revealed a well-developed lamellar bone trabecula surrounding bone marrow tissue. These results are explained by the healthy antioxidant and other roasted coffee contents (10,15). Melanoidins, tocopherols, and caffeic acid are components of roasted coffee that lead to the formation of good lamellar bone in the roasted coffee group (10,32,34-36).

These observations are in accordance with Herniyati et al., who proved that coffee enhances bone formation by increasing the number of osteoblasts and blood capillaries (37).

The histomorphometric results confirmed the histological results. In the current study, there was a statistically reduction in the bone surface area in the green coffee group than in the roasted coffee group at birth. These results were in accordance with previous work on the long bone of rats, which showed a decrease in the number of trabeculae accompanied by an increase in bone marrow areas with signs of osteoporosis (38).

As well as, there might be a piece of evidence difference between the control and green coffee group and between the control and roasted coffee group at birth. This difference because there was an absence of bone in the control group at birth. On the other hand, the histomorphometric results showed a increase in the bone surface area in the roasted coffee than in the other two groups because of its potent antioxidant property and a reduction of caffeine during roasting.

## CONCLUSION

It was concluded from this study that the roasted coffee had a positive influence on the condylar cartilage growth of rats' offspring, which was attributed to some caffeinic components being burned, while new beneficial compounds were created after roasting such as melanoidins, tocopherols, and caffeic acid. In contrast, green coffee had adverse effects on the endochondral bone growth because it contains more caffeine than roasted coffee, which leads to the rarefaction of the condyle bone.

## CONFLICT OF INTEREST

We declare that we have no conflicts of interest.

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