# Morphometric variables of offspring of *Quassia amara* treated male rats

***Obembe OO.[1], Raji Y.[2]**

**Abstract**

**Objective:** *Quassia amara* is a medicinal plant with various pharmacological properties. The bioactive compound quassin is used as flavoring in food and beverages. Reproductive toxicological action of *Q. amara* is well documented but no information exists on its effect on prenatal programming.

**Methods:** Adult male rats (180-200g, n=5) were treated daily (p.o) with *Q. amara* extract (100 mg/kg) for 6 weeks. A control group received distilled water. After 5 weeks of treatment, female rats were cohabited with the males for 7 days, at the ratio of 2:1. Mating was confirmed by presence of spermatozoa in all vaginal smear. Morphometric indices of all offspring were recorded on postnatal day one. They were also examined for any sign of abnormality or physical defect.

**Results:** Fertility was zero in four out of the five treated rats. The females that cohabited with the fertile treated male gave birth to pups of varying sizes (6 and 9). However, four of the five control male rats were fertile and the female rats they mated had 9 pups each. No visible physical defect was observed on all offspring. Anogenital distance of the male offspring of the treated rats was significantly shorter than male offspring of the control, while anogenital distance of female offspring showed no statistical difference. Head diameter and body length were also significantly lower in offspring of the treated rats. However, weight, abdominal diameter and sex ratio of offspring were not statistically different.

**Conclusion:** *Quassia amara* extract caused a reduction in the male anogenital distance, litter size, head diameter and body length of the offspring of treated male rats. Its reproductive toxicity actions may alter the developmental programming and probably transferred from one generation to another.

**Keywords:** *Quassia amara*, spermatozoa, developmental programming, anogenital distance, endocrine disruptors.

*Correspondence Author - Dr. Obembe O.O. E-mail: olawale.obembe@uniosun.edu.ng

[1]Department of Physiology, College of Health Sciences, Osun State University, Osogbo, Nigeria
[2]Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Nigeria



http://dx.doi.org/10.4314/rejhs.v5i2.3

# Les variables morphométriques de la progéniture de rats mâles traités par *Quassia amara*

***Obembe OO.[1], Raji Y.[2]**

**Resume**

**Objectif:** *Quassia amara* est une plante médicinale présentant diverses propriétés pharmacologiques. Le composé bioactif quassin est utilisé comme arôme dans les aliments et les boissons. L'action toxicologique de la reproduction de *Q. amara* est bien documentée, mais aucune information n'existe sur son effet sur la programmation prénatale.

**Méthodes:** Des rats mâles adultes (180-200g, n = 5) ont été traités quotidiennement (p.o) avec de l'extrait de *Q. amara* (100 mg / kg) pendant 6 semaines. Un groupe témoin a reçu de l'eau distillée. Après 5 semaines de traitement, les rats ont été cohabités avec les mâles pendant 7 jours, au ratio de 2: 1. L'accouplement a été confirmé par la présence de spermatozoïdes dans tous les frottis vaginaux. Les indices morphométriques de tous les descendants ont été enregistrés le premier jour post-natal. Ils ont également été examinés pour détecter tout signe d'anomalie ou de défaut physique.

**Résultats:** La fécondité était nulle sur quatre des cinq rats traités. Les femelles qui cohabitaient avec le mâle traité fertile ont donné naissance à des chiots de différentes tailles (6 et 9). Cependant, quatre des cinq rats mâles témoins étaient fertiles et les rats femelles qu'ils accouplaient avaient 9 chiots chacun. Aucun défaut physique visible n'a été observé chez tous les descendants. La distance anogenitale de la progéniture masculine des rats traités était significativement plus courte que la progéniture masculine du témoin, alors que la distance anogenitale de la progéniture féminine ne présentait aucune différence statistique. Le diamètre de la tête et la longueur du corps ont également été significativement plus faibles dans la progéniture des rats traités. Cependant, le poids, le diamètre abdominal et le rapport sexuel des descendants n'étaient pas statistiquement différents.

**Conclusion:** L'extrait de *Quassia amara* a entraîné une réduction de la distance anogenitale masculine, de la taille de la litière, du diamètre de la tête et de la longueur du corps de la progéniture des rats mâles traités. Ses actions de toxicité pour la reproduction peuvent modifier la programmation du développement et probablement transférées d'une génération à l'autre.

**Mots-clés:** *Quassia amara*, spermatozoïdes, programmation du développement, distance anogenitale, perturbateurs endocriniens.

* Correspondance Auteur - Dr. Obembe O.O. E-mail- obembe24@yahoo.com

[1]Department of Physiology, College of Health Sciences, Osun State University, Osogbo, Nigeria
[2]Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Nigeria

## INTRODUCTION

Scientific findings have reported a decline in semen quality and male births with an increased rate in male genital abnormalities over the past 70 years. While the mechanisms and etiology is uncertain, several researchers have identified various factors which disrupt normal endocrine signaling, leading to abnormal androgen action and altered testicular development and testicular cancer (1). Investigators have used the anogenital distance (AGD) as a measure of genital development and androgen status in both experimental animals and humans in an attempt to gauge reproductive toxicities (2).

*Anogenital distance (AGD)* is a sexually dimorphic measure of genital development. The AGD has been used to sex animals, since males have longer lengths than females (3). Moreover, human studies in infants have also established that boys have longer AGD than girls (4,5). It is a marker for endocrine disruption in animal studies and may be shorter in infant males with genital anomalies (6).

*Quassia amara* is a 6-8 meters tall tree native to Suriname, Brazil, in South America and is naturally distributed in several tropical countries. It has been used as flavorings in beverages and baked foods and is reputed for its strong antimalarial and antimicrobial activities (7, 8). Raji and Bolarinwa (7) studied the antifertility activities of *Q. amara* plant and they reported that quassin was the bioactive component of the plant. It accounted for the observed decline in testosterone, Luteinizing hormone (LH) and Follicle Stimulating hormone (FSH) in treated rodents. Its effect on epididymal proteins, sperm capacitation and acrosome reaction were later reported (8). Its reported effects on these sex hormones may be inferred to mean it acts as endocrine disruptors.

Ever since Barker (9) pioneered the concept of fetal programming, the role of prenatal programming as determinant of adult diseases has become increasingly clear. Reports from experimental animals continue to provide insights into the molecular, cellular and systemic mechanisms that contribute to the several different disease conditions observed during lifetime (10). Endocrine disruptors as defined by the US Environmental Protection Agency, is an exogenous agent that interferes with the production, release, transport, metabolism, binding, action, or elimination of natural hormones in the body responsible for the maintenance of homeostasis and the regulation of developmental processes (11,12). Endocrine disruptors have been reported to decrease the AGD of male and female rats (13), impair sperm chromatin concentration and induce human sperm DNA damage (14). It is however not clear if these DNA damage is transmitted to successive generations. Since *Q. amara* plant induces reproductive hormone imbalance and reproductive toxicity in male rodents (7,8), and AGD is a reliable predictor of permanent alterations of the reproductive system, we sought to know in this study the AGD and other morphometric variables of the offspring of *Q. amara* treated male rats.

## MATERIALS AND METHODS

### Animals

Ten male and twenty female Wistar rats (180-200 g) were obtained from the central animal house, College of Medicine and housed in well ventilated animal cages in the animal house, Department of Physiology, University of Ibadan. They were fed standard rat feed and clean water *ad libitum*. All procedures in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding principles in the care and use of animals (15).

### *Quassia amara* extraction

The stem bark of *Q. amara* was obtained from the Botanical Gardens, University of Ibadan and the plant was authenticated at herbarium of Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. Voucher number 109103 was assigned to the specimen of *Q. amara*. The stem bark of *Q. amara* plant was extracted in methanol as earlier described (8). The air-dried stem bark (2.51 kg) was soaked in aqueous methanol at room temperature for 5 days with daily shaking and thereafter was decanted. The extract obtained was concentrated to a dark-green residue (24.4 g) on a rotary evaporator at 40$^{o}$C and weighed. Percentage yield was 0.97 %.

### Experimental design

*Quassia amara* stem bark extract (100mg kg$^{-1}$ b.w.) was administered daily (p.o.) for 6 weeks to an experimental group while the control group received vehicle (0.5 ml distilled water) for same duration. Each group was made up of 5 male rats. After 5 weeks of treatment, virgin female rats were cohabited with the treated males for 7 days, at the ratio of 2:1. Mating was confirmed by presence of sperm cells in the vagina smear and this was determined as day 1 of

pregnancy. At the end of 6 weeks of treatment, all male rats were sacrificed by decapitation. The pregnant female rats were allowed free access to feed and water, and were nurtured till parturition. Fertility of each male in each mating was expressed as:

[Number of Pregnant rats / Total number of females in cage] X 100 (16)

**Measurement of morphometric data and anogenital distance**

On postnatal day 1, animals were held gently and firmly to allow for ease of measurement. Head diameter, abdominal diameter and body length were measured using a digital vernier caliper (17). Anogenital distance was measured by placing the animal with the base of the tail on the edge of a table. The thumb was then used to secure the tail to the side of the table and the index finger placed above the phallus to maximally stretch the skin in the perineal area. Then, a vernier caliper was used to measure the distance between the posterior base of the developing genital papilla and the anterior rim of the anus (13).

**Statistical analysis**

Data were expressed as mean ± standard error of mean (SEM). The test of significance between two groups was estimated by Student's t-test and (ANOVA). $P<0.05$ was considered significant (18).

**RESULTS**

Figure 1 showed that fertility was 20% in rats treated with *Q. amara* and significantly lower ($p<0.05$) when compared with the control (70%). Figure 2 showed that each of the female rats that successfully cohabited with the control male rats had 9 pups each. Both females that cohabited with the fertile treated male gave pups of varying sizes, 6 and 9 respectively. No visible physical defect was observed on the offspring of all rats. Anogenital distance of the male offspring of the treated rats was significantly shorter ($p< 0.05$) than the AGD of the male offspring of the control rats (Table 1), while female offspring showed no statistical difference. Head diameter and body length was also significantly lower ($p< 0.05$) in offspring of the treated rats while no effect was observed on weight and abdominal diameter (Fig 2).

**DISCUSSION**

Results from this study indicated *Q. amara* stem bark extract decreased fertility index of treated male rats (Fig 1). The observed decline in fertility may be due to the effects of this plant on androgens. Raji and Bolarinwa (7) earlier reported that the plant extract adversely affected some male reproductive functions, as serum levels of testosterone, LH and FSH were adversely affected. Also, a remarkable decrease was observed in weight of sex and accessory sex organs.

The observed decline in fertility index may not be due to decrease in libido, as mating was confirmed by the presence of sperm cells in the vagina smear of all the female rats that individually cohabited with the treated males. We earlier reported that *Q. amara* caused a remarkable decline in sperm motility and sperm count, an increase in the number of abnormal spermatozoa, a decrease in the number of sperm that successfully underwent capacitation and acrosome reaction (8) and a decline in the expression of epididymal *HongrES1* protein (19). One or a combination of these may account for the observed decline in fertility index despite successful mating.

Anogenital distance is a sexually dimorphic secondary sex characteristic in many mammalian species and can be used to measure the degree of demasculinization of males as a consequence of developmental exposure to androgen receptor antagonists, 5-alpha reductase inhibitors, or compounds that inhibit steroidogenesis (20). Swan *et al* (21) demonstrated that male infants of mothers exposed to increasing levels of known endocrine disruptors had shorter AGD, suggesting an impairment of *in utero* male genital development. We report in this study for this first time to our knowledge, the AGD of offspring of male rats treated with endocrine disruptors. In this study, the AGD of the male offspring of *Q. amara* treated rats were significantly short when compared with the male offspring of control rats (Table 1). However, AGD of female offspring were not affected. In a study in the USA, Eisenberg *et al.* (2) reported that infertile men possessed significantly shorter mean AGD. Anogenital distance was significantly correlated with sperm density and total motile sperm count, but observed no correlation between penile length and semen parameters. They therefore concluded that longer AGD is associated with fatherhood and may predict normal male reproductive potential. Likewise, AGD is useful in measuring the degree of masculinization of females exposed during sexual differentiation to androgenic compounds or anabolic growth

stimulant. Anogenital distance is a reliable predictor of permanent alterations of the reproductive system that are not often apparent until the animal reaches sexual maturity. Increased AGD in female pups is associated with decreased numbers of nipples (as infants and adults) and malformations of the reproductive tract (20). Decreased male AGD and increased female AGD observed in adult rats at necropsy demonstrate that endocrine disrupting compounds do permanently alter AGD.

Birth weight and abdominal diameter of the offspring of treated male rats were not affected, but litter size, body length and head diameter were significantly lower (Fig 2).

It is unclear how reproductive toxicity induced by *Q. amara* treatment is potentially transferred to the male offspring. Though we did not measure the sex hormones of these offspring, Eisenberg *et al.* (2) reported a lack of correlation between the shorter AGD observed in infertile males with their sex hormones (testosterone, LH and FSH). Also, no significant difference was seen for serum testosterone, LH, or FSH of infertile men when compared with fathers. The only conceivable possible mechanism by which *Q. amara* extract could have had a transgenerational effect that resulted in a decrease in AGD of male offspring is if it altered the sperm DNA integrity. Sperm DNA integrity represents an essential requirement for the accurate transmission of genetic information. There is limited and contradictory epidemiological evidence on whether endocrine disruptors affect human sperm DNA (14). These contradictions may be due to the different methodologies by which sperm DNA damage was characterized. Male reproductive anomalies such as cryporchidism and hypospadias were hypothesized to be positively correlated with in utero exposure to endocrine disruptors. Brucker-Davis *et al.* (22) offered a measured support for this theory. However, in a study of third-trimester serum samples from the pregnant mothers over a 6 year period, the hypothesis that endocrine disrupting chemicals are associated with cryptorchidism and/or hypospadias was not strongly supported (23).

## CONCLUSION

*Q. amara* decreased the fertility index of treated male rats. Male offspring of the treated rats also had significantly shorter AGD, which suggests a permanent alteration of the reproductive system and a possible demasculinization of the male offspring *in-utero*. Therefore, toxicity action of *Q. amara* may alter developmental programming of male rat offspring and this effect may be transferred from one generation to another.

**Acknowledgement:** This work was funded in part by UNIOSUN ETF-TETFund, Nigeria.

**Conflict of interest:** The authors declare no conflict of interest.

## REFERENCES

1. Carlsen E, Giwercman A, Keiding N, Skakkebaek NE. Evidence for decreasing quality of semen during past 50 years. BMJ 1992; 305: 609–613.
2. Eisenberg ML, Hsieh MH, Walters RC, Krasnow R, Lipshultz LI. The Relationship between Anogenital Distance, Fatherhood, and Fertility in Adult Men. PLoS ONE 2011; 6(5): e18973. doi:10.1371/journal.pone.0018973.
3. Hsieh MH, Breyer BN, Eisenberg ML, Baskin LS. Associations among hypospadias, cryptorchidism, anogenital distance, and endocrine disruption. Curr Urol Rep 2008; 9: 137–142.
4. Thankamony A, Ong KK, Dunger DB, Acerini CL, Hughes IA. Anogenital distance from birth to 2 years: a population study. Environ Health Perspect 2009; 117: 1786–1790.
5. Sathyanarayana S, Beard L, Zhou C, Grady R. Measurement and correlates of ano-genital distance in healthy, newborn infants. Int J Androl 2010; 33: 317–323.
6. Eisenberg ML and Lipshultz LI. Anogenital distance as a measure of human male fertility J Assist Reprod Genet. 2015; 32(3): 479 – 484.
7. Raji Y and Bolarinwa AF. Antifertility activity of *Quassia amara* in male rats *in vivo* study. Life Sciences 1997; 61: 1067-1074.
8. Obembe OO and Raji Y. Reproductive toxicity of Quassia amara extract: action on sperm capacitation and acrosome reaction. Acad. J. Plant Sci. 2012; 5 (3): 60-69.
9. Barker DJP. Programming the baby. In: Mothers, babies, and disease in later life. London: BMJ Publishing Group 1994; 14–36.
10. Drake AJ and Walker BR. Intergenerational consequences of fetal programming: non-genomic mechanism of the interaction of low birth weight and cardiovascular risk. *J Endocrinol* 2004; 180: 1–16.
11. Kavlock RJ, Daston GP, DeRosa C, Fenner-Crisp P, Gray LE, Kaattari S *et al*. Research needs for the risk assessment of health and environmental effects of endocrine disruptors: a report of the

U.S. EPA-sponsored workshop. Environ. Health Perspect 1996; 104: 715–740.
12. Knez J. Endocrine-disrupting chemicals and male reproductive health. Reproductive BioMedicine Online 2013; 26, 440– 448.
13. Christiansen S, Axelstad M, Boberg J, Vinggaard AM, Pedersen GA, and Hass U. Low-dose effects of bisphenol A on early sexual development in male and female rats. Reproduction 2014; 147: 477-487.
14. Jeng HA. Exposure to Endocrine Disrupting Chemicals and Male Reproductive Health. Front Public Health. 2014; 2: 55: doi: 10.3389/fpubh.2014.00055 PMCID: PMC4046332.
15. World Medical Association, American Physiological Society. Guiding principles for research involving animals and human beings. Am. J. Physiol. Regul. Integr. Comp. Physiol. 2002; 283: R281-R283.
16. Ellerman DA, Veronica SB, Silvina PM, Cohen DJ, Conesa D and Cuasnicu PS. Potential Contraceptive Use of Epididymal Proteins: Immunization of Male Rats with Epididymal Protein DE Inhibits Sperm Fusion Ability1. Biology of Reproduction 1998; 59, 1029–1036.
17. Ostby JS and Gray LE. Transgenerational (InUtero/Lactational) exposure to investigate the effects of endocrine disrupting compounds (EDCs) in rats. *Current Protocols in Toxicology.* John Wiley & Sons, Inc 2004. p 16.8.1-16.8.16.
18. Snedecor GW and Cochran WG. Statistical Methods 7th Ed. Ames. Iowa State University Press 1980. p 215.
19. Obembe OO, Olaopade JO and Raji Y. Implication of *HongrES1* protein in Quassin-induced male reproductive abnormality. Endocrinology and Metabolic Syndrome. 2014; 3 (2): 128. doi:10.4172/2161-1017.1000128.
20. Fisher SJ. Environmental anti-androgens and male reproductive health: focus on phthalates and testicular dysgenesis syndrome. Reproduction 2004; 127:304-315.
21. Swan SH, Main KM, Liu F, Stewart SL, Kruse RL *et al.* Decrease in anogenital distance among male infants with prenatal phthalate exposure. Environ Health Perspect 2005; 113: 1056–1061.
22. Brucker-Davis F, Wagner-Mahler K, Delattre I, Ducot B, Ferrari P, Bongain A *et al*. Cryptorchidism at birth in Nice area (France) is associated with higher prenatal exposure to PCBs and DDE, as assessed by colostrum concentrations. Hum Reprod 2008; 23(8):1708–1810.1093/humrep/den186.
23. McGlynn KA, Guo X, Graubard BI, Brock JW, Klebanoff MA, Longnecker MP. Maternal pregnancy levels of polychlorinated biphenyls and risk of hypospadias and cryptorchidism in male offspring. Environ Health Perspect 2009; 117(9):1472–610.1289/ehp.0800389.

**Table 1**: Sex ratio and anogenital distance of offspring of *Q. amara* treated rats.

| Group | Male: Female ratio | Male AGD (cm) | Female AGD (cm) |
|---|---|---|---|
| Control | 2:1 | 0.30±0.01 | 0.12±0.01 |
| Treated | 9:5 | 0.23±0.02*, | 0.16±0.01 |

*P<0.05, indicates significantly shorter AGD of male offspring of treated rats when compared with control

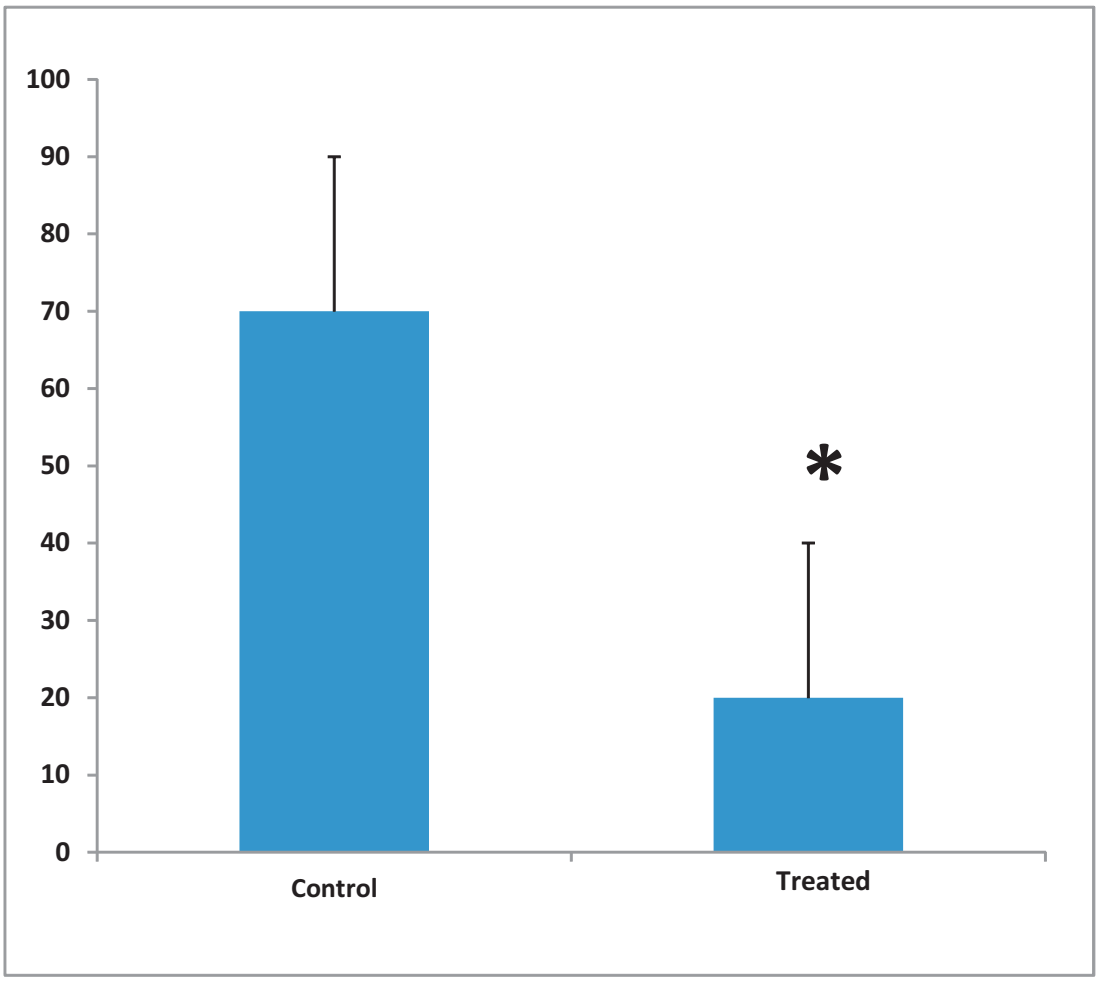


**Figure 1**: Fertility index (%) of *Q. amara* treated male rats
*P<0.05, indicates significantly lower fertility index of *Q. amara* treated male rats.

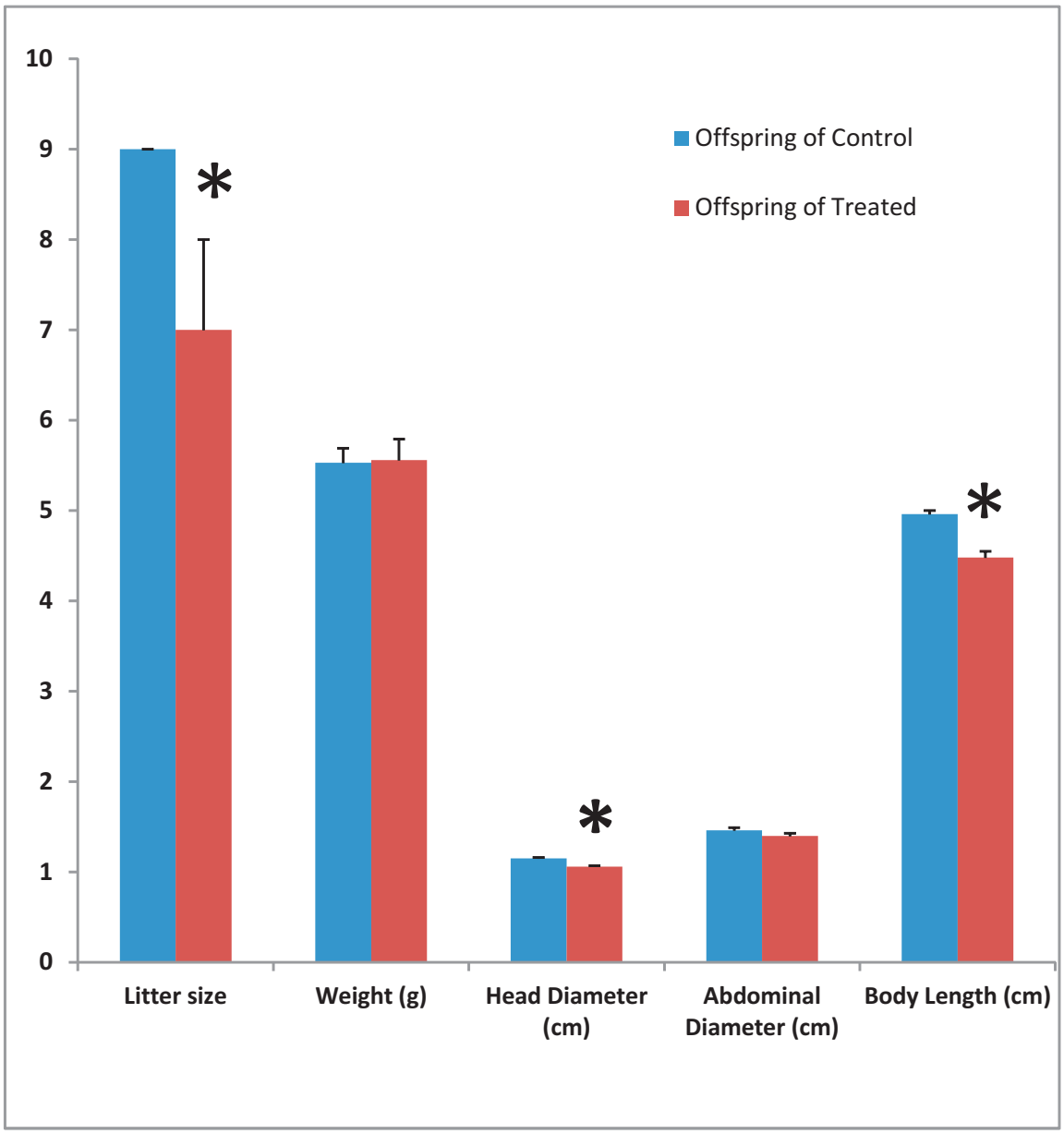


**Figure 2**: Morphometric variables of offspring of *Q. amara* treated rats.
*P<0.05, indicates decline in litter size, head diameter and body length of offspring of treated rats.