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Effect of Green Tea Supplementation on Blood Cadmium and Male Sex Hormone Levels in Automobile Workers in Emene, Enugu State, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors OECN, DCE and MSC designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors OECN, OIJ and USO managed the analyses of the study. Authors OIJ and USO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Cadmium is a toxic metal and a known endocrine disruptor capable of altering sex hormone levels especially in the occupationally exposed persons. Some phytochemicals including green tea (*Camellia sinensis*) may prove to be beneficial in reducing blood cadmium levels and the associated adverse health effects.
Aim: This study assessed the levels of blood cadmium and male sex hormones in automobile workers as well as the effect of subsequent green tea supplementation.

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Methods: The study was carried out in two parts; the cross-sectional and intervention study. 124 subjects comprising of 62 automobile workers and 62 age-matched occupationally unexposed control subjects in Emene, Enugu State, Nigeria were recruited for the cross-sectional study. Subsequently, 28 out of the 62 automobile workers received green tea supplement daily (2 green tea bags soaked in 150 ml of freshly boiled water for 5 minutes) for two months. 5mls of venous blood samples were collected from all subjects before the intervention (baseline) as well as after one and two months of green tea supplementation for the determination of blood cadmium, serum testosterone, follicle stimulating hormone (FSH) and luteinizing (LH) levels.
Results: The mean blood cadmium and serum FSH and LH levels were significantly higher in the automobile workers (2.41 ± 0.26 µg/dl, 11.64 ± 4.31 iu/l and 7.67 ± 2.82 iu/l respectively) when compared to the control (0.14 ± 0.02 µg/dl, 9.09 ± 2.90 iu/l and 5.54 ± 1.54 iu/l respectively) (p<.05) while the mean serum testosterone level was significantly lower in the automobile workers (6.96 ± 2.07 ng/ml) when compared to the control (8.99 ± 2.95 ng/ml) (p<.05). Following supplementation with green tea, blood cadmium and serum LH levels reduced significantly (2.19 ± 0.38 µg/dl and 7.67 ± 2.82 iu/l respectively), serum testosterone increased significantly (7.75 ± 2.18 ng/ml) whereas serum FSH level did not differ significantly when compared with the baseline values (p>.05).
Conclusion: This study indicates that blood cadmium level is elevated and sex hormone levels are altered in automobile workers, and two months supplementation with green tea reduced the blood cadmium and regulated serum testosterone and LH levels with no significant effect on serum FSH level.

Keywords: Cadmium; testosterone; follicle stimulating hormone; luteinizing hormone.

1. INTRODUCTION

A good number of occupations expose workers to various heavy metals as well as their oxides. Cadmium (Cd) is one of the heavy metals listed among priority metals of public health importance due to its high degree of toxicity, and as well of greater public health importance in Nigeria [1]. Exposure to this metal could result in certain health issues, especially in the occupationally exposed persons. According to the Agency for Toxic Substances and Disease Registry (ATSDR) [2], sources of exposure include working in Cd-contaminated areas or metal industries, tobacco smoking, pigments, alloys and battery manufacturing. The major routes of exposure to Cd are mainly through ingestion from contaminated hands, foods or drinks and inhalation of Cd-contaminated dust particles and fumes [3].

Apart from the general population inadvertently exposed to this toxic metal, occupations involving automobile repairs and services produce a lot of toxic substances including dusts and toxic fumes of metals including Cd which are released into the workplace and are detrimental to the health of the workers [4].

Occupational exposure to Cd may as well be enhanced by certain factors like poor industrial/occupational hygiene and lack of use of personal protective equipment at workstations. This toxic metal is capable of interfering with the endocrine system function, hence is regarded as an endocrine disruptor (ED) [5,6]. The World Health Organization in 2002 defined endocrine disruptor chemical (EDC) as “an exogenous substance or mixture that alters functions of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations” [7], these include Cd and other toxic metals or chemicals [8]. Sex hormones play a crucial role in reproductive health by influencing the development of the reproductive system as well as controlling its activities in the developed stage.

The EDCs can exert a number of detrimental effects on the male reproductive system by altering the sex hormone levels, normal testicular structure, spermatogenesis and semen quality [9]. Most of the adverse effects on male reproductive function are mainly due to alteration in the synthetic and catabolic pathways of testosterone, impairment of spermatogenesis and modification of testicular structure [10]. These EDCs act through modification of gene expression of enzymes and proteins involved in sex hormone biosynthesis, metabolism and release [11] as well as binding to hormone receptors.

Some dietary strategies including medicinal plants and tea have been reported to play vital

roles in alleviating certain toxicities associated with heavy metal exposure [12], and green tea made from *Camellia sinensis* leaves has been reported to possess certain health promoting effects including positive effects on reproductive health [13], although some other studies have reported otherwise [14,15). Studies on occupational exposure to Cd with respect to sex hormone levels are quite limited and conflicting, moreover, there is paucity of data on the possible role of medicinal plants on blood Cd and sex hormone levels. This study therefore aims to assess the levels of blood Cd and male sex hormones in automobile workers as well as the effect of subsequent green tea supplementation.

2. METHODS

2.1 Study Population and Design

This study employed both cross-sectional and intervention approach. 62 automobile workers aged between 18 and 55 years, and 62 age-matched control subjects who are not occupationally exposed were recruited in the cross-sectional study. The automobile workers comprised of auto technicians, welders, panel beaters/spray painters and auto electricians while the control subjects consisted mainly of civil servants and students in Emene, Enugu State, Nigeria. Subsequently, 28 out of the 62 occupationally exposed workers received 150ml of green tea supplements daily at their respective workplaces for two months. The workers started receiving the supplements a day after the baseline sample collection.

2.2 Inclusion and Exclusion Criteria

Consenting male subjects aged between 18 and 55 years were recruited for the study whereas subjects with any history of diabetes, heart disease, hypertension, on any vitamin or mineral supplements or on fertility drugs as well as obese subjects were excluded from the study.

2.3 Source and Preparation of Green Tea Supplement

Green tea bags were purchased from Unilever, Nigeria with batch number 16252, and National Agency for Food and Drug Administration and Control (NAFDAC) Registration number B1-8866. The green tea supplements were prepared by soaking two green tea bags (containing 1.6 g each of dried green tea leaves) in 150 ml of freshly boiled water for 5 minutes.

2.4 Phytochemical Analysis of Green Tea

The phytochemical constituents of green tea was determined using standard method as described by Kelly and Nelson [16] and the analysis was performed on a Buck M910 Gas chromatography equipped with a flame ionization detector.

2.5 Informed Consent and Ethical Consideration

A written informed consent was obtained from all participating subjects, and ethical clearance was obtained from Nnamdi Azikiwe University Teaching Hospital Research Ethics Committee (NAUTHREC) with approval number NAUTH/CS/66/Vol. 10/ 20/2017/021.

2.6 Blood Sample Collection

Blood samples were collected from the subjects by standard venipuncture method as described by Lewis, et al. [17] between 8.00 am and 10.30 am. For the cross-sectional study, 5 mls of blood samples were collected from the occupationally exposed and control groups, and this served as the baseline samples. Subsequently, 5 mls of blood was also collected from these workers after the first and second month following green tea supplementation. 3 mls of blood were dispensed into K_2-EDTA vacutainer tubes for blood Cd analysis and stored between 2°C and 8°C while 2 mls was dispensed into plain vacutainer tubes, centrifuged at 3000 rpm for five minutes, and the sera obtained were stored at -20°C for testosterone, follicle stimulating hormone and luteinizing hormone assay. All samples were analyzed within two weeks of collection.

2.7 Biochemical Analyses

Blood metal analysis was conducted using Varian AA240 Atomic Absorption Spectrophotometer (AAS) according to the method of American Public Health Association (APHA) [18].

The serum testosterone, LH and FSH levels were estimated according to the methods described by Tateiki, et al. [19] Kosasa [20] and Odell and Parlow, [21] respectively which were based on solid phase enzyme- linked immunosorbent assay (ELISA).

2.8 Statistical Analysis

The Statistical Package for Social Sciences (SPSS) version 21.0 was used for statistical analysis. The variables were expressed as mean ± standard deviation, whereas the independent student's t-test was used to assess the mean difference between two independent variables, and the paired t-test was used to assess the mean difference between two related variables. The level of significance was considered at $p<.05$.

3. RESULTS

The mean blood Cd, serum FSH and LH levels were significantly higher in the automobile workers when compared to the control group ($p<.05$) while the mean serum testosterone level was significantly lower in the automobile workers when compared to the control ($p<.05$) (Table 1).

The mean blood Cd, serum testosterone, FSH and LH levels in the automobile workers did not differ significantly from baseline values after one month of green tea supplementation ($p>.05$). However, after two months of supplementation, the mean blood Cd and serum LH levels decreased significantly ($p<.05$) while the mean serum testosterone level increased significantly ($p<.05$), however, the mean serum FSH level did not differ significantly from the baseline after the first and second month of green tea intake ($p>.05$) (Table 2).

4. DISCUSSION

Occupational exposure to Cd is inherent in various workplaces, including auto repair workshops, battery manufacturing, welding and painting. Blood cadmium level is a known reliable marker of recent exposure to this metal [22, 23], and the elevated blood Cd level in the automobile workers observed in this study is an indication of exposure to this toxic metal. This agrees with the reports of Musa *et al*., [24] and Alli [25] who also reported elevated blood Cd levels in subjects occupationally exposed to cadmium.

Cd is a well-known endocrine disruptor [1] which is capable of interfering with hypothalamus-pituitary signaling, and consequently affecting release of sex hormones including gonadotrophin releasing hormone (GnRH), FSH and LH [26]. In the present study, the reduction in testosterone level in the automobile workers may be attributed to the negative effect of cadmium on testosterone production [27], as cadmium may interfere with 3ß-hydroxysteroid dehydrogenase (3ß-HSD) or 17ß-HSD enzymes involved in testosterone production [26]. This result agrees with the report of Okoli *et al*., [28] who observed a reduction in serum testosterone level in automechanics exposed to mixed chemicals. A recent animal study by Ujah *et al*., [6] observed a reduction in the levels of 3ß-HSD, 17ß-HSD and testosterone in cadmium chloride-exposed rats. The elevated levels of FSH and LH observed in the automobile workers may be as a result of the dysregulation of pituitary gonadotrophic hormones (FSH and LH) secretion resulting from the modulatory effect of cadmium on the hypothalamus signaling of kisspeptin-1 (KiSS-1) and its G Protein-coupled receptor (GPR54) which controls GnRH secretion [26]. As a consequence of the imbalance in the

Table 1. Blood cadmium and serum testosterone, FSH and LH levels in automobile workers and control group (mean±SD)

Parameters	Automobile Workers (n=62)	Control (n=62)	*p* value
Cadmium(µg/dl)	2.41 ± 0.26	0.14 ± 0.02	<0.0001*
Testosterone(ng/ml)	6.96 ± 2.07	8.99 ± 2.95	<0.0001*
FSH(iu/l)	11.64 ± 4.31	9.09 ± 2.90	<0.0001*
LH(iu/l)	7.67 ± 2.82	5.54 ± 1.54	<0.0001*

*Key: * = Results compared are significant at p < .05*

Table 2. Blood cadmium, serum testosterone, FSH and LH levels of the automobile workers at different stages of green tea supplementation (mean±SD) n=28

Parameters	A	B	C	A/B	B/C	A/C
Cadmium(µg/dl)	2.57 ± 0.41	2.38 ± 0.38	2.19 ± 0.38	0.120	0.007*	0.005*
Testosteron(ng/ml)	7.19 ± 1.94	7.36 ± 2.09	7.75 ± 2.18	0.129	0.002*	0.001*
FSH(iu/l)	11.33 ± 4.09	11.28 ± 4.24	11.00 ± 4.22	0.617	0.131	0.138
LH(iu/l)	7.44 ± 2.27	7.20 ± 1.93	6.88 ± 1.35	0.138	0.063	0.034*

*Key: * = Results compared are significant at p < .05). A represents Baseline, B represents One Month, C represents Two Months*

hypothalamus pituitary testicular (HPT) axis, the pituitary cells may release inappropriate levels of FSH and LH which may as well alter the negative feedback loop. Few other reports in this regard have been conflicting, Algafari *et al*., [29] in their study on car customizers and welders reported high level of LH with no change in testosterone and FSH levels, Telisman *et al*., [30] reported increased testosterone with no change in LH and FSH levels in male industrial workers exposed to cadmium while Chikezie *et al*., [31] observed increased testosterone and FSH levels with no change in LH level in automechanics in Ibadan, Nigeria.

In the present study, green tea supplementation for one month had no significant effect on blood Cd and serum hormone levels, and this may be attributed to the dose-response relationship. In other words, it may be asserted that the green tea dose taken for one month was not sufficient enough to cause any significant effect on the measured biochemical parameters. Additionally, green tea intake for two months reduced the blood cadmium level in these workers, and this may be attributed to the abundant catechins present in green tea which may affect absorption as well as metabolism of cadmium ions, probably by inhibiting their absorption and promoting their excretion [32]. These flavonoids present in green tea tend to bind with these metal ions to form a non-toxic insoluble complex enhancing their elimination [33] as well as reducing lipophilicity, and consequently reducing intestinal absorption [34].

The elevated testosterone level observed after two months of green tea intake may as well be attributed to the inhibitory effect of green tea on the enzyme aromatase [35, 36] which converts testosterone to estrogen or inhibition of the enzyme 5α-reductase which converts testosterone to dihydrotestosterone (DHT) [37]. Additionally, the reduced LH level after two months of green tea intake may probably be mediated through the feedback mechanism.

5. CONCLUSION

The results of this study show that automobile workers have a higher level of blood cadmium as well as altered sex hormone levels, and two months supplementation with green tea reduced the blood cadmium levels as well as regulated serum testosterone and LH levels with no significant effect on FSH level.

CONSENT

All authors declare that 'written informed consent was obtained from the subjects and other approved parties for publication of this paper.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee (Nnamdi Azikiwe University Teaching Hospital Research Ethics Committee), and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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