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Ameliorating Effect of *Ganoderma lucidum* on Combined Oral Contraceptive-induced Cardiometabolic Syndrome in Female Guinea Pigs

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Authors' contributions

This work was carried out in collaboration among all authors. Author AIO designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors APO and OAS managed the literature searches. Authors BSA, ECC and FTG managed the analyses of the study. Author AAA performed the statistical analysis. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: This study evaluated the effect of *Ganoderma lucidum* on selected biochemical indices for cardiometabolic risk.
Study Design: Case-control study.
Place and duration of Study: This study was carried out in the Department of Medical Laboratory Science, Babcock University between December, 2018-May, 2019.
Methods: The study included 32 female guinea pigs which were assigned into 4 groups (A-D) with 8 in each group. Group A animals served as control, group B animals received only monophasic combined oral contraceptives (COC), group C animals received monophasic COC and 50mg/kg of G. lucidum, group D animals received monophasic COC and 100 mg/kg of *G. lucidum*. All the test animals received their treatment once daily through oral gavage for 12 weeks. All animals were

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sacrificed 24 hours after the last dose was given to the test groups. Blood sample was collected via cardiac puncture. Lipoprotein-associated phospholipase-A2 (Lp-PLA2), high sensitive C reactive protein (hsCRP), insulin, insulin-like growth factor-1 (IGF-1), fasting blood glucose (FBG), triglyceride (TG), total cholesterol (TC) and high density lipoprotein-cholesterol (HDL-C) were determined using spectrophotometric methods and ELISA as appropriate. HOMA-IR was calculated using homeostasis model assessment index. Data obtained were statistically analyzed using ANOVA, post hoc, all values were expressed as mean±standard deviation. *P* value less than 0.05 was considered significant.

Results: We observed that groups B (25%) and C (25%) animals had significantly higher levels of hsCRP, LpPLA2, TC, TG, LDL, FBG, IGF-1 and HOMA-IR when compared with groups A (25%) and D animals (25%) ($p<0.05$), however there was no significant statistical difference when groups B and C were compared. Also we observed higher levels of HDL in groups C and D animals when compared with groups A and B animals but not significant statistically.

Conclusion: The use of *G. lucidum* stymies the development of COC-induced cardiometabolic syndrome in a dose-dependent manner.

Keywords: Metabolic syndrome; Ganoderma lucidum; oral contraceptives; inflammation; insulin resistance; high sensitive C-reactive protein.

1. INTRODUCTION

In the past decade, there has been tremendous increase in chronic diseases like diabetes, hypertension, and obesity not only in developed nations but also in developing countries [1,2]. With the worldwide escalation of obesity, diabetes and hypertension, there has been a parallel increase in the incidence and prevalence of cardiometabolic disorder [3]. Cardiometabolic syndrome (CMS) is a cluster of interrelated metabolic disorders characterized by insulin resistance, impaired glucose tolerance, dyslipidemia, hypertension, and obesity [4]. CMS increases the risk of type 2 diabetes mellitus by fivefold and cardiovascular disease by threefold, therefore this condition has received enormous attention globally [5]. Furthermore, obesity is now known to be a major feature for increased cardiometabolic risk [3,6].

The worldwide epidemic of obesity has been attributed to consumption of high calorie diet and physical inactivity. These are believed to contribute to the manifestation of the key features of cardiometabolic syndrome which are obesity and insulin resistance.

The use of combined oral contraceptives (COC), has been associated with increased prevalence of obesity and cardiometabolic disturbances [7,8], increase in body weight has often been stated by women as the major reason for discontinuing the use of COC [9].

There is no single medication that can be used to bring CMS under control due to its multifactorial risk factors. Most therapeutic strategies focus on the combination of diet modification and exercise to control the cardiometabolic risk factors [10,11] and these however may not be sustained for a longer period. Recently, there has been focus on the use of medicinal plants as an alternative approach for the treatment of debilitating metabolic conditions and one of these plants is *Ganoderma lucidum*.

Ganoderma lucidum has been used in traditional Chinese and Japanese medicine for more than two millennia [12]. This mushroom is called "Ling Zhi" in Chinese and "Reishi" in Japanese. G. *lucidum* has been reported to exhibit many biological and pharmacological effects such as anticancer, antidiabetic, antihypertensive, antilipidemic, antimicrobial and anti-inflammatory effects [13-16].

This study was designed to assess the effect of G. *lucidum* on selected biochemical indices of cardiometabolic risk in female guinea pigs treated with combined oral contraceptives.

2. MATERIALS AND METHODS

2.1 Animals and Treatment

This study was approved by Babcock University Health and Research Ethics committee and it was carried out according to the guidelines for care and use of laboratory animals. A total of 32 female guinea pigs aged 16 weeks, weighing 250-300 g were obtained from the animal center of Babcock University. These animals were housed in plastic cages and were maintained under standard laboratory conditions

(temperature: 25±2°C; 12 h light; 12 h dark), the animals had unrestricted access to standard diet and tap water. These animals were randomly assigned into 4 groups (A-D) with 8 in each group and all treatments were initiated after 1 week of adaptation.

The female guinea pigs in group A served as control and they received neither combined oral contraceptives nor G. *lucidum* but each female guinea pig in groups B, C and D received monophasic COC (a combination of 0.6 mg/kg levonogestrel and 0.12 mg/kg ethinyl estradiol) once daily through oral gavage for 12 weeks. In addition, group C animals received G. *lucidum* (50 mg/kg) and group D animals received *G. lucidum* (100 mg/kg) once daily through oral gavage for 12 weeks.

All the animals were sacrificed 24 hours after the last dose of COC and *G. lucidum* was given to the female guinea pigs in the test groups. About 6 mL of blood was collected via cardiac puncture, 2mL was dispensed into fluoride oxalate bottle for the assay of fasting plasma glucose (FPG) which was performed within 12 hours, while 4mL was collected into plain bottle and was centrifuged at 4000 rpm for 5 minutes to obtain serum which was aliquoted into small vial and stored at -20°C for the determination of lipoprotein-associated phospholipase-A_2 (Lp-PLA_2), high sensitive C reactive protein (hsCRP), insulin, insulin-like growth factor-1 (IGF-1), triglyceride (TG), total cholesterol (TC) and high density lipoprotein-cholesterol (HDL-C)

2.2 Biochemical Assay

Plasma glucose was determined by the glucose oxidase method (Randox Laboratories Ltd., UK) as previously described by Ojiako et al. [17]. Lipoprotein-associated phospholipase-A_2, hsCRP, insulin and IGF-1 were determined using ELISA kits (BT lab, China). Triglyceride (TG), TC were determined using standard enzymatic method (Randox Laboratories Ltd., UK) as previously described by Ojiako et al. [17]. HDL-C was determined by a two-step procedure using a precipitant to isolate non-HDL-C component in the plasma and this was followed by quantitative determination of HDL-C by standard enzymatic method for cholesterol determination. LDL cholesterol was determined using Friedwald equation [18], while insulin resistance (IR) was calculated using the homeostasis model assessment for insulin resistance (HOMA-IR) equation; (HOMA-IR= Fasting serum insulin (mIU/L) × Fasting plasma glucose (mg/dl)/405).

2.3 Statistical Analysis

Statistical analysis data generated from this study were analyzed using the statistical package for social sciences (SPSS 21st edition) computer software. Comparison of variables between groups was done using one-way analysis of variance (ANOVA) followed by a post-hoc test. The significant threshold was fixed at $P<0.05$. The results were expressed as mean ± standard deviations and presented in Table 1.

3. RESULTS

Table 1 shows the levels of selected cardiometabolic indices in all the groups. The values of hsCRP, LpPLA2, TC, TG, LDL, FBG, IGF and HOMA-IR were significantly higher in groups B and C subjects when compared with corresponding values in groups A and D subjects ($P<0.05$). However, the mean values of HDL were higher in groups C and D subjects when compared with groups A and B subjects but not statistically significant. Moreover, the mean values of hsCRP, LpPLA2, TC, TG, LDL, FBG, IGF and HOMA-IR were higher in group B subjects when compared with group C but not statistically significant. More so, there was reduced levels of hsCRP, LpPLA2, TC, TG, LDL, FBG, IGF and HOMA-IR when group D animals were compared with animals in both groups B and C ($P<0.05$). However, both groups A and D subjects had comparable levels of HsCRP, LpPLA2, TC, TG, LDL, FBG, IGF and HOMA-IR which are not statistically significant.

4. DISCUSSION

Cardiometabolic syndrome (CMS) is a cluster of interconnected metabolic abnormalities that include atherogenic dyslipidemia, glucose dysregulation, insulin resistance, elevated blood pressure and increased body weight (4). Epidemiological data revealed that CMS contributes significantly to mortality with approximately 1.6 fold increase globally [19].

Several studies have reported the unfavorable impact of combined oral contraceptive use on cardiometabolic disturbances in both human and experimental animal model [7-8,20-22]. Third generation COC which are the currently used hormonal contraceptives was introduced to

minimize the metabolic effects associated with the use of the first and second generation COC, however these effects still persist [23,24].

In this present study, female guinea pigs weighing 250 g-300 g in groups B, C and D were given monophasic COC once daily (in the morning) for 12 weeks. Additionally, groups C and D animals were given a single dose of *G. lucidum* 50 mg/kg and 100 mg/kg respectively once daily (in the morning) for 12 weeks.

The findings of this current study demonstrated the presence of COC-induced cardiometabolic disturbances. Our findings revealed reduced insulin sensitivity, increased levels of hsCRP, LpPLA2, TC, TG, LDL, FBG and IGF in group B animals. The elevated levels of hsCRP observed, agrees with findings of previous studies [25,26] which reported increase in low grade inflammatory status measured by hsCRP in the users of COC. Both hsCRP and LpPLA2 which are elevated in group B animals have been reported to be good predictors of metabolic syndrome and their increase is associated with higher cardiovascular risk [26,27]. The elevated hsCRP has been attributed to the oestrogen content of COCs which has the tendency to induce oxidative stress and also stimulate inflammatory mechanisms [25,28].

Furthermore, the decreased insulin sensitivity and glucose dysregulation measured by IGF-1, FBG and HOMA-IR observed in group B animals also agrees with many previous studies [29,30]. The decline in insulin sensitivity induced by COC use can be attributed to both oestrogen and progestin components [30-32]. The mechanism by which they cause insulin resistance has not been entirely clarified but one of the possible mechanisms is their antagonistic effect on insulin via accentuated adiposity that decreases the affinity of tissue receptors for insulin [33,32].

Additionally, the observed increase in the levels of LpPLA2, TG, TC and LDL which are predictors of cardiovascular disease, is consistent with reports from previous studies [21,34-35]. The impact of COC on lipids has been attributed to the androgenicity of the progestin content which has the potential to induce low grade inflammation and oxidative stress [36]. The present study also observed that there was no significant difference in the levels of HDL-C when groups A and B animals were compared. This can be attributed to the estrogen content of the COC, however the anti-inflammatory properties of HDL-C appears to be overwhelmed by the androgenicity of the progestin through the induction of LDL oxidation thus possessing the potential to promote atherogenic dyslipidemia. The observed lipid pattern in this current study is supported by findings from previous studies [21,37].

Furthermore, our findings revealed that group C animals (treated with monophasic COC and 50mg/kg *G.lucidum*) had comparable levels of reduced insulin sensitivity, increased hsCRP, LpPLA2, TC, TG, LDL, FBG and IGF with group B animals, as no significant statistical difference was observed when these groups were compared. This observation is consistent with the findings of previous studies that reported little or no efficacy with the use of low dose of *G. lucidum* [38,39]. Additionally, our findings revealed that group D animals (treated with monophasic COC and 100 mg/kg *G. lucidum*) had comparable levels of hsCRP, LpPLA2, TC, TG, LDL, IGF-1 and HOMA-IR with group A animals (control) and there was no statistically significant difference. Our findings also revealed that some of the cardiometabolic risk biochemical parameters were reduced in group D animals and the favourable impact of *G. lucidum* observed in group D animals indicates that the mitigating influence of G. *lucidum* is dose dependent.

Table 1. Selected biochemical parameters for cardiometabolic syndrome in all the groups

Parameters	A n = 8	B n=8	C n=8	D n=8	F	P-value
HsCRP (mg/L)	2.1± 0.5$^{t\dagger u}$	3.3±0.4$^{s\dagger v}$	3.0 ±0.3$^{s\dagger v}$	2.4±0.1$^{t\dagger u}$	19.38	0.00*
LpPLA2 (mL)	14.4 ±2.4 $^{t\dagger u}$	24.2±3.7$^{s\dagger v}$	20.6±3.1$^{s\dagger v}$	15.1±1.5$^{t\dagger u}$	32.27	0.00*
TC (mg/dL)	151.7±11.1$^{t\dagger u}$	252.5±17.1$^{s\dagger v}$	247.3±3.1$^{s\dagger v}$	143.5±14.4$^{t\dagger u}$	141.9	0.00*
TG (mg/dL)	118.8±10.6$^{t\dagger u}$	196.9±12.9$^{s\dagger v}$	190.7±15$^{s\dagger v}$	107.9±17$^{t\dagger u}$	87.53	0.00*
HDL (mg/dL)	65.6±21.1	68.8±13	72.2 ±12.3	75.7±15.1	0.69	0.51
LDL (mg/dL)	62.3±30.3$^{t\dagger u}$	131.9±53.1$^{s\dagger v}$	127.6±39$^{s\dagger v}$	45.1±9.6 $^{t\dagger u}$	12.18	0.00*
FBG (mg/ dL)	87.9±9.7$^{t\dagger u}$	135.0±59.0$^{s\dagger v}$	130 ±32.3$^{s\dagger v}$	100.5±10.0$^{t\dagger u}$	3.69	0.03*
IGF -1 (µg/L)	35.2 ±5.3$^{t\dagger u}$	44.5±4.9$^{s\dagger v}$	40.3±4.1$^{s\dagger v}$	34.0±3.2 $^{t\dagger u}$	11.85	0.00*
HOMA-IR	1.9±0.5 $^{t\dagger u}$	4.2±0.8 $^{s\dagger v}$	3.8±0.7 $^{s\dagger v}$	2.1±0.3 $^{t\dagger u}$	4.94	0.00*

*Values are expressed in mean±standard deviation, *statistically significant at p< 0.05(2-tailed), $s^{\dagger}$- statistically different from A, $t^{\dagger}$- statistically different from B, u- statistically different from C, v- statistically different from D*

The anti-inflammatory and antioxidant properties of *G. lucidum* have been reported by several studies [12-14,39,40]. Wong et al. [40] also reported that *G. lucidum* has inhibitory effect on free radical generation and this significantly inhibits the development of cardiovascular events.

5. CONCLUSION

This study observed that the use of *G. lucidum* mitigates against the development of COC-induced cardiometabolic syndrome in a dose-dependent manner. This can be explored as a possible therapeutic means to stymie inflammation and oxidative stress that characterize cardiometabolic syndrome.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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