

**AMELIORATING EFFECT OF *GANODERMA LUCIDUM* ON COMBINED
ORAL CONTRACEPTIVE-INDUCED CARDIOMETABOLIC SYNDROME IN
FEMALE GUINEA PIGS.**

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 COC, group C animals received monophasic COC and 50mg/kg of *Ganoderma lucidum*, group D animals received monophasic COC and 100 mg/kg of *Ganoderma lucidum*. All the test animals received their treatment once daily through oral gavage for 12 weeks. All animals were 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 that 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 *Ganoderma 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*

38 1. INTRODUCTION

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

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

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

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

58 *Ganoderma lucidum* has been used in traditional Chinese and Japanese medicine for more than two
59 millennia (12). This mushroom is called "Ling Zhi" in Chinese and "Reishi" in Japanese. *Ganoderma*
60 *lucidum* has been reported to exhibit many biological and pharmacological effects such as anticancer,
61 antidiabetic, antihypertensive, antilipidemic and anti-inflammatory effects (13-15).

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

64 2. MATERIALS AND METHODS

65 Animals and treatment

66 This study was approved by Babcock University Health and Research Ethics committee and it was
67 carried out according to the guidelines for care and use of laboratory animals. A total of 32 female guinea
68 pigs aged 16 weeks, weighing 250-300g were obtained from the animal center of Babcock University.
69 These animals were housed in plastic cages and were maintained under standard laboratory conditions
70 (temperature: 25±2°C; 12h light; 12h dark), the animals had unrestricted access to standard diet and tap

71 water. These animals were randomly assigned into 4 groups (A-D) with 8 in each group and all
72 treatments were initiated after 1 week of adaptation.

73 The female guinea pigs in group A served as control and they received neither combined oral
74 contraceptives nor *Ganoderma lucidum* but each female guinea pig in groups B, C and D received a
75 combination of 0.6mg/kg levonogestrel and 0.12mg/kg ethinyl estradiol once daily through oral gavage for
76 12 weeks. In addition, group C animals received *Ganoderma lucidum* (50mg/kg) and group D animals
77 received *Ganoderma lucidum* (100mg/kg) once daily through oral gavage for 12 weeks.

78 Both the control group animals and the test group animals were sacrificed on the day after the last dose
79 of contraceptive and *Ganoderma lucidum* administration. About 6ml of blood was collected via cardiac
80 puncture, 2ml was dispensed into fluoride oxalate bottle for the assay of fasting plasma glucose (FPG)
81 which was performed within 12 hours, while 4ml was collected into plain bottle and was centrifuged at
82 4000 rpm for 5 minutes to obtain serum which was aliquoted into small vial and stored at -20°C for the
83 determination of lipoprotein-associated phospholipase-A₂ (Lp-PLA₂), high sensitive C reactive protein
84 (HsCRP), insulin, insulin-like growth factor-1 (IGF-1), triglyceride (TG), total cholesterol (TC) and high
85 density lipoprotein-cholesterol (HDL-C)

86 **2.1 Biochemical assay**

87 Plasma glucose was determined by the glucose oxidase method (Randox Laboratories Ltd., UK) as
88 previously described by Ojiako *et al* [16]. Lipoprotein-associated phospholipase-A₂, hsCRP, insulin and
89 IGF-1 were determined using ELISA kits (BT lab, China). Triglyceride (TG), TC were determined using
90 standard enzymatic method (Randox Laboratories Ltd., UK) as previously described by Ojiako *et al* [16].
91 HDL-C was determined by a two-step procedure using a precipitant to isolate non-HDL-C component in
92 the plasma and this was followed by quantitative determination of HDL-C by standard enzymatic method
93 for cholesterol determination. LDL cholesterol was determined using Friedwald equation (17), while
94 insulin resistance (IR) was calculated using the homeostasis model assessment for insulin resistance
95 (HOMA-IR) equation; (HOMA-IR= Fasting serum insulin (mIU/L) × Fasting plasma glucose (mg/dl)/405).

96 **2.2 Statistical analysis**

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

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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 statistically significant 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.

UNDER PEER REVIEW

127 **TABLE 1: SELECTED BIOCHEMICAL PARAMETERS FOR CARDIOMETABOLIC**128 **SYNDROME IN ALL THE GROUPS**

PARAMETERS	A	B	C	D	F	P-
	n = 8	n=8	n=8	n=8		value
HsCRP (mg/L)	2.1± 0.5 ^{t†u}	3.3±0.4 ^{s†v}	3.0 ±0.3 ^{s†v}	2.4±0.1 ^{t†u}	19.38	0.00*
LpPLA2 (ng/ml)	14.4 ±2.4 ^{t†u}	24.2±3.7 ^{s†v}	20.6±3.1 ^{s†v}	15.1±1.5 ^{t†u}	32.27	0.00*
TC (mg/dl)	151.7±11.1 ^{t†u}	252.5±17.1 ^{s†v}	247.3±3.1 ^{s†v}	143.5±14.4 ^{t†u}	141.9	0.00*
TG (mg/dl)	118.8±10.6 ^{t†u}	196.9±12.9 ^{s†v}	190.7±15 ^{s†v}	107.9±17 ^{t†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†u}	131.9±53.1 ^{s†v}	127.6±39 ^{s†v}	45.1±9.6 ^{t†u}	12.18	0.00*
FBG (mg/dl)	87.9±9.7 ^{t†u}	135.0±59.0 ^{s†v}	130 ±32.3 ^{s†v}	100.5±10.0 ^{t†u}	3.69	0.03*
IGF -1 (µg/L)	35.2 ±5.3 ^{t†u}	44.5±4.9 ^{s†v}	40.3±4.1 ^{s†v}	34.0±3.2 ^{t†u}	11.85	0.00*
HOMA-IR	1.9±0.5 ^{t†u}	4.2±0.8 ^{s†v}	3.8±0.7 ^{s†v}	2.1±0.3 ^{t†u}	24.94	0.00*

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130 Values are expressed in mean±standard deviation, *statistically significant at p< 0.05(2-tailed), s[†]-131 statistically different from A, t[†]- statistically different from B, u- statistically different from C, v- statistically

132 different from D.

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138 4. DISCUSSION

139 Cardiometabolic syndrome (CMS) is a cluster of interconnected metabolic abnormalities that include
140 atherogenic dyslipidemia, glucose dysregulation, insulin resistance, elevated blood pressure and
141 increased body weight (4). Epidemiological data revealed that CMS contributes significantly to mortality
142 with approximately 1.6 fold increase globally (18).

143 Several studies have reported the unfavorable impact of combined oral contraceptive use on
144 cardiometabolic disturbances in both human and experimental animal model (7-8; 19-21). Third
145 generation COC which are the currently used hormonal contraceptives was introduced to stymie the
146 metabolic effects associated with the use of the first and second generation COC, however these effects
147 still persist (22, 23)

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

152 The findings of this current study demonstrated the presence of COC-induced cardiometabolic
153 disturbances. Our findings revealed reduced insulin sensitivity, increased levels of hsCRP, LpPLA2, TC,
154 TG, LDL, FBG and IGF in group B animals. The elevated levels of hsCRP observed, agrees with findings
155 of previous studies (24, 25) which reported increase in low grade inflammatory status measured by
156 hsCRP in the users of COC. Both hsCRP and LpPLA2 which are elevated in group B animals have been
157 reported to be good predictors of metabolic syndrome and their increase is associated with higher
158 cardiovascular risk (25-27). The elevated hsCRP has been attributed to the oestrogen content of COCs
159 which has the tendency to induce oxidative stress and also stimulate inflammatory mechanisms (24, 28).

160 Furthermore, the decreased insulin sensitivity and glucose dysregulation measured by IGF-1, FBG and
161 HOMA-IR observed in group B animals also agrees with many previous studies (29-32). The decline in
162 insulin sensitivity induced by COC use can be attributed to both oestrogen and progestin components
163 (32-34). The mechanism by which they cause insulin resistance has not been entirely clarified but one of
164 the possible mechanisms is their antagonistic effect on insulin via accentuated adiposity that decreases
165 the affinity of tissue receptors for insulin (30, 34).

166 Additionally, the observed increase in the levels of LpPLA2, TG, TC and LDL which are predictors of
167 cardiovascular disease, is consistent with reports from previous studies (20, 35-36). The impact of COC
168 on lipids has been attributed to the androgenicity of the progestin content which has the potential to
169 induce low grade inflammation and oxidative stress (37). The present study also observed that there was

170 no significant difference in the levels of HDL-C when groups A and B animals were compared. This can
171 be attributed to the estrogen content of the COC, however the anti-inflammatory properties of HDL-C
172 appears to be overwhelmed by the androgenicity of the progestin through the induction of LDL oxidation
173 thus promoting atherogenic dyslipidemia. The observed lipid pattern in this current study is supported by
174 findings from previous studies (20, 38).

175 Furthermore, our findings revealed that group C animals (treated with a combination of 0.6mg/kg
176 levonogestrel and 0.12mg/kg ethinyl estradiol and 50mg/kg *Ganoderma lucidum*) had comparable levels
177 of reduced insulin sensitivity, increased hsCRP, LpPLA2, TC, TG, LDL, FBG and IGF with group B
178 animals, as no significant statistical difference was observed when these groups were compared. This
179 observation is consistent with the findings of previous studies that reported little or no efficacy with the
180 use of low dose of *Ganoderma lucidum* (39, 40). Additionally, our findings revealed that group D animals
181 (treated with a combination of 0.6mg/kg levonogestrel and 0.12mg/kg ethinyl estradiol and 100mg/kg
182 *Ganoderma lucidum*) had comparable levels of hsCRP, LpPLA2, TC, TG, LDL, IGF-1 and HOMA-IR with
183 group A animals (control) and there was no statistically significant difference. Our findings also revealed
184 that some of the cardiometabolic risk biochemical parameters were reduced in group D animals and the
185 favourable impact of *Ganoderma lucidum* observed in group D animals indicates that the mitigating
186 influence of *Ganoderma lucidum* is dose dependent.

187 The anti-inflammatory and antioxidant properties of *Ganoderma lucidum* have been reported by several
188 studies (12-14; 40-41). Wong *et al.* (41) also reported that *Ganoderma lucidum* has inhibitory effect on
189 free radical generation and this significantly inhibits the development of cardiovascular events.

190 **5. CONCLUSION**

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

195 **COMPETING INTERESTS**

196 Authors have declared that no competing interests exist.

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