

**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 combined oral contraceptives (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 **24 hours on the day** after the
79 last dose of contraceptive and *Ganoderma lucidum* administration. About 6ml of blood was collected via
80 cardiac puncture, 2ml was dispensed into fluoride oxalate bottle for the assay of fasting plasma glucose
81 (FPG) which was performed within 12 hours, while 4ml was collected into plain bottle and was centrifuged
82 at 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.

101

102

103

104

3. RESULTS

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

115

116

117

118

119

120

121

122

123

124

125

126

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*

129

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.

133

134

135

136

137

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.

197

198 **REFERENCES**

- 1991) Gallagher, E., Novosyadly, R., Yakar, S and LeRoith, D. (2010). The increased risk of cancer in obesity
200 and type 2 diabetes: potential mechanisms. In: Poretzky L (ed.). Principles of Diabetes Mellitus. Springer:
201 US. pp. 579– 599.
2022) Okafor, C.I. (2012). The metabolic syndrome in Africa: Current trends. *Indian Journal of Endocrinology*
203 *and metabolism*. 16(1):56-66.

- 2043) Song Qing, Wang Shaoshan, Zafari Mazair (2006). Genetics of Metabolic Syndrome. *Hospital Physician*.
205 pp 51-61.
- 2064) Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. (2009). Harmonizing the
207 metabolic Syndrome. *Circulation*.120:1640-1645.
- 2085) O'Neill,S and O'Driscoll, L (2015). Metabolic syndrome: a closer look at the growing epidemic and its
209 associated pathologies. *Obesity reviews*; 16:1-12.
- 2106) Srivastava A.K (2012). Challenges in the treatment of cardiometabolic syndrome. *Indian J.Pharmacol*.
211 44(2):155-156.
- 2127) Wei W, Li Y, Chen F, Chen C, Sun T, Sun Z, et al. (2011). Dyslipidaemia, combined oral contraceptives
213 use and their interaction on the risk of hypertension in Chinese women. *J Hum Hypertens*. 25:364–371.
- 2148) Olatunji LA, Michael OS, Adewumi FO, Aiyegboyin IJ, Olatunji, VA. (2012). Combined estrogen-
215 progesterone but not progesterone-only oral contraceptive alters glucose tolerance and plasma lipid profile
216 in female rats. *Pathophysiology*.19:29-34.
- 2179) Chukwubike U.O., Solomon, A.B., Benjamin N., Roseanne, O., Pascal C., Adebayo, A and Ngozika B
218 (2011). Comparative effects of Injectable and oral hormonal contraceptives on lipid profiles. *Eur J Cardvs*
219 *Med*; 2 (1): 20-22.
- 22010) Matfin G (2008). Challenges in developing therapies for the metabolic syndrome. *British Journal of*
221 *Diabetes and Vascular Disease*; 7: 152
- 22211) Longo-Mbenza B, Mvindu HN, On'kin JBK, Bikuku N, Phanzu BK, et al (2011). The deleterious effects of
223 physical inactivity on elements of insulin resistance and metabolic syndrome in Central Africans at high
224 cardiovascular risk. *Diabetes and Metabolic syndrome: Clinical Research and Reviews*; 5: 1-6. 24.
- 22512) C.-Y. Huang, J. Y.-F. Chen, J.-E. Wu (2010) "Ling-zhi
226 polysaccharides potentiate cytotoxic effects of anticancer drugs against drug-resistant urothelial carcinoma
227 cells," *Journal of Agricultural and Food Chemistry*, vol.58, no.15, pp.8798–8805.
- 22813) Zhang, H. N. & Lin, Z. B (2003). Effect and mechanism of *Ganoderma lucidum* polysaccharide on
229 autoimmune diabetic mice. *National Medical Journal of China*. 83(22), 1999–2000.
- 23014) Berger, A. et al (2004). Cholesterol-lowering properties of *Ganoderma lucidum* in vitro, ex vivo, and in
231 hamsters and minipigs. *Lipids in Health and Disease*. 3(2) (2004). doi: 10.1186/1476-511X-3-2.
- 23215) Paterson, R. R. (2006). *Ganoderma* - a therapeutic fungal biofactory. *Phytochemistry*. 67 (731) 1985-
233 2001
- 23416) Ojiako A.O., Chikezie P.C., Zedech U.C (2013). Serum lipid profile of hyperlipidaemic rabbits (*Lepus*
235 *townsendii*) administered with leaf extracts of *Hibiscus rosesinesis*, *Emilia coccinea*, *Acanthus montanus*
236 and *Asystasia gangetica*. *J Med Plant Res*;7:32263231.
- 23717) Friedewald WT, Levi RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein
238 cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*. 1972; 18: 499-502.
- 23918) Kaur JA (2014). Comprehensive review on metabolic syndrome. *Cardiol Res Pract*; 2014: 943162.

24019) Emokpae M.A, Udia P.O and Osadolor HB (2010). Effect of duration of use of hormonal contraceptive pills on total lipid and lipoproteins in Nigerian women. *Int.JPharmaBio*.1(3).

24220) Mahmood Isam H and Ali Hanaan J (2012). Effect of hormonal contraceptives on prevalence of metabolic syndrome in women. *Rawan Medical Journal*.37(2)

24421) George Asare, Shiela Santa, Robert A Ngala, Bernice Asiedu, Daniel Afriye, Albert Amoah (2014). Effect of hormonal contraceptives on lipid profile and the risk of cardiovascular disease in a Ghanaian Community. *International Journal of Women's Health*.;6:597-603

24722) Kemmeren JM, Algra A, Meijers JC, Tans G, Bouma BN, Curvers J (2004). Effect of second- and third-generation oral contraceptives on the protein C system in the absence or presence of the factor VLeiden mutation: a randomized trial. *Blood* 2004;103: 927–933.

25023) Krivak TC, Zorn KK (2007). Venous thromboembolism in obstetrics and gynecology. *Obstet Gynecol*;109: 251 761–77.

25224) Cauci Sabina, Manuela Di Santolo, Jennifer F. Culhane, Giuliana Stel, Fabio Gonano, and Secondo Guaschino (2008). Effects of Third-Generation Oral Contraceptives on High-Sensitivity C-reactive Protein and Homocysteine in Young Women. *Obs&Gyn*; 111(4):857-864

25525) Sabina Cauci, Maria Pia Francescato, Francesco Curcio (2016). Combined Oral Contraceptives Increase High-Sensitivity C-Reactive Protein but Not Haptoglobin in Female Athletes. *Sports Med*. DOI 10.1007/s40279-016-0534-9.

25826) Acevedo M, Varleta P, Kramer V, Quiroga T, Prieto C, Prada J, Navarrete C (2013). Lipoprotein associated phospholipase A2 and C- reactive protein as predictors of metabolic syndrome in subjects without cardiovascular disease. *EurheartJ*;34(suppl.1)P701.doi.org/10.1093/eurheartj/ehs307.P701

26127) Deepak Y. K, Denis X, Alben S (2015). High sensitivity C-reactive protein (hsCRP) and cardiovascular disease: An Indian perspective. *Indian J Med*; 142(56):261-268

26328) vanRooijenM, HanssonLO, Frostega°rdJ, SilveiraA, HamstenA, BremmeK (2006). Treatment with combined oral contraceptives induces a rise in serum C-reactive protein in the absence of a general inflammatory response. *J Thromb Haemost*;4:77–82.

26629) Frempong, B. A., Ricks, M., Sen, S. and Sumner, A. E. (2008). Effect of Low-Dose Oral Contraceptives on Metabolic Risk Factors in African-American Women. *Journal of Clinical Endocrinology and Metabolism*, 93(6):2097–2103.

26930) Berenson, A. B., van den Berg, P., Williams, K. J. and Mahbubur R. (2011). Effect of Injectable and Oral Contraceptives on Glucose and Insulin Levels. *Obstetrics and Gynaecology*, 117(1): 41–47.

27131) Blackmore, K. M., Wong, J. and Knight, J. A. (2011). A Cross Sectional Study of Different Patterns of Oral Contraceptive use among Premenopausal Women and Circulating IGF-1: Implications for Disease Risk. *BMC Women's Health*, 11(1): 15.

27432) Rickenlund, A., Thoren, M., Nybacka, A., Frystyk, J. and Linden Hirschberg, A. (2010). Effects of Oral Contraceptives on Diurnal Profiles of Insulin, Insulin-Like Growth Factor Binding Protein-1, Growth Hormone and Cortisol in Endurance Athletes With Menstrual Disturbances. *Human Reproduction*, 1: 85-93.

27833) Friedrich, A., A.K. Ludwig, K. Jauch-Chara, M. Loebig, S. Rudolf, S. Tauchert, K. Diedrich, U. Schweiger,
279 and K.M. Oltmanns (2012). Oral contraception enhances growth hormone responsiveness to hyper- and
280 hypoglycaemia. *Diabetic Medicine*. 29: 345–50.

28134) Sitruk-Ware, Regine and Anita Nath (2013). Characteristics and metabolic effects of oestrogen and
282 progestins contained in oral contraceptive pills. *Best Practice and Research Clinical Endocrinology and*
283 *Metabolism*. 27;13-24

28435) Hassan, E. E., Ibrahim, E. Y. M., Shrif, N. M. A. and Modawe, G. (2014). Effect of Low-Dose Oral
285 Contraceptives on Lipid Profile Levels in Sudanese Women Using Oral Contraceptive Pills. *Sudan*
286 *Journal of Medical Sciences* Vol 9, No 2

28736) Naz, F., Jyoti, S., Akhtar, N., Afzal, M. and Siddique, Y. H. (2012). Lipid Profile of Women Using Oral
288 Contraceptive Pills. *Pakistan Journal of Biological Sciences*, 15(19): 947-950.

28937) Morin-Papunen L, Martikainen H, McCarthy MI, Franks S, Sovio U, Hartikainen AL,
290 Ruokonen A, Leinonen M, Laitinen J, Järvelin MR, Pouta A (2008). Comparison of metabolic and
291 inflammatory outcomes in women who used oral contraceptives and the levonorgestrel-releasing
292 intrauterine device in a general population. *Am J Obstet Gynecol*. 199:529-539.

29338) Taneepanichskul S and Phupong V (2007). Influence of a new oral contraceptive with drospirenone on
294 lipid metabolism. *Gynecological Endocrinology*. 23: 347–350.

29539) Meng G, Zhu H, Yang S, Wu F, Zheng H, Chen E, Xu J. Attenuating effects of Ganoderma lucidum
296 polysaccharides on myocardial collagen cross-linking relates to advanced glycation end product and
297 antioxidant enzymes in high-fat-diet and streptozotocin-induced diabetic rats. *Carbohydrate Polymers*
298 2011; 84: 180-185.

29940) Hüseyin Avni Eroğlu¹ and Ebru Beytut (2018). Effect of Ganoderma lucidum polysaccharides on oxidative
300 damage in liver of STZ-diabetic rats. *Biomedical Research*; 29 (18): 3436-3443.
301

30241) Kar-Lok Wong, Hung-Hsing Chao, Paul Chan, Li-Ping Chang and Chi-Feng Liu (2004). Antioxidant
303 Activity of Ganoderma lucidum in Acute Ethanol-induced Heart Toxicity. *Phytother. Res*. 18; 1024–1026.
304
305
306
307
308
309