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The Effect of *Ganoderma Lucidum* Powder on some Immune Parameters in Rat

Hiyam A. Altaii **Mahmood A. Altobje**
Department of Biology/ College of Science/ University of Mosul
Ali A. Dawood
Department of Anatomy/ College of Medicine/ University of Mosul

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corresponding author:
Hiyam A. Altaii
hiyamaltaii@uomosul.edu.iq

Mahmood A. Altobje
mahtsbio30@uomosul.edu.iq

Ali A. Dawood
aad@uomosul.edu.iq

ABSTRACT

The impact of *Ganoderma lucidum* powder on both chemokines, IgG and C3 complement concentrations, as well as WBC types, was investigated in 60 ester citrine rats split between two groups, one of which was injected with Carbon tetrachloride CcL4 and the other with *Ganoderma lucidum* powder. They were not given this drug; instead, two concentrations of mushroom powder, 40 mg/ per kilo-gram of body weight and 80 mg/ per kilo gram of body weight, were utilized, along with a control group. The results revealed a clear increase in all immune factors measured, with a clear difference between males and females when compared to control samples, as well as an increase in the numbers of both neutrophils and lymphocytes in males for both concentrations, with a higher increase for the second concentration than the first, while the increase in females for both concentrations was similar.

In the groups exposed to CcL4, an increase in the values of chemokines, IgG, and C3 as well as the number of neutrophils appeared in males in varying numbers for both concentrations, whereas the increase in females appeared in the second concentration higher than the first concentration of immune values, and the opposite was true for the number of white cells. The number of monocytes showed slight changes in both males and females under study with clear significant differences between C3, chemokine, white blood cell count, lymphocytes and monocytes.

Keywords: C3, Chemokine, *Ganoderma lucidum*, IgG, WBC.

INTRODUCTION

In today's world, the reishi mushroom (*Ganoderma lucidum*) is the most significant medicinal fungus. It has important medicinal properties and is used as an anti-cancer, anti-HIV, anti-heart attack (lowering cholesterol), as well as to protect the liver and kidneys, treat diabetes, and as an antioxidant, among other things (Verma, 2013). It can be used in the prevention and treatment of various diseases such as bronchitis, hepatitis, Hypertension, immune disorders, cancer (Chen and Jiang, 1980; Yuen and Gohel, 2005; Boh *et al*., 2007; Wickc *et al*., 2007).

Ganoderma has been used in traditional medicine for thousands of years in Korea, China, and Japan, and it is now being used in other parts of the world for the prevention and treatment of diseases and tumors, controlling blood sugar levels, and modifying the immune system, as well as having antibacterial and liver-protective properties (Leu *et al.*, 2002; Wachtel-Galor and Benzie, 2013). It is nearly regarded as a panacea in Chinese and Japanese medical systems. Farmers and businesspeople took it upon themselves to grow this mushroom and reap worldwide riches when the Global Research Center revealed a breakthrough in the creation of technology for reishi mushroom production (Verma, 2013).

Scientists have discovered that Ganoderma mushrooms are a good source of lignin-degrading enzymes, therefore mushroom cultivation is thought to be beneficial in both medicinal and industrial settings (Otjen *et al*., 1987).

Water and ethanol extracts of *Ganoderma lucidum* were shown to protect mice against acute hepatitis in certain experiments on laboratory animals (Leu *et al*., 1979; Lin *et al*., 1993), and other reports proofed that tri-terpenoids isolate from *Ganoderma lucidum* have a protective result against severe hepatitis caused by Ccl4 (Kim *et al*., 1999; Wan *et al*., 2000).

Park et al. revealed that polysaccharides derived from *Ganoderma lucidum* can protect mice from cirrhosis caused by cholestasis, demonstrating that Ganoderma has a liver-protective function (Park *et al*., 1997). Several tumor cells lines in vitro, such as the prostate gland cancer cell line and colorectal cancer cell lines, have indicated that Ganoderma extracts have anti-proliferative properties (Berovic *et al*., 2003).

The genus Ganoderma was firstly classified on the base of phenotypic traits only (Patouillard, 1998; Smith and Ksivasithamparam, 2000), and ecological factors, variability, hybridization and phenotypic tendency can lead to inaccurate identification of *Ganoderma* sp. (Zheng *et al*., 2009), and in *Ganoderma lucidum* it is often determined, it is unclear, and the taxonomic segregation of *Ganoderma lucidum* for East Asia and Europe remains controversial (Moncalvo *et al*., 1995; Wang *et al*., 2012), and is based on analyzes of RNA gene regions, and it was found that the groups named *Ganoderma lucidum* in Asia were incompatible with European *Ganoderma lucidum* (Moncalvo *et al*., 1995), and both Pegler and Yao emphasized that on the basis of phenotypic examination the *Ganoderma lucidum* that grows in East Asia differs from that in Europe (Pegler and Yao, 1996), so Hawksworth suggested keeping the name *Ganoderma lucidum* for the Asian species and introducing a new name for the European species (Hawksworth, 2005).

This study aimed to find out the effect of *Ganoderma lucidum* powder on both chemokines, IgG and C3 concentration of complement, as well as on WBC types.

MATERIALS AND METHODS

The study was carried out at the Department of Biology's on animal house, where 60 rats of the type and ester citrine were produced, ranging in age from 4 to 3 months, with similar weights, and from both sexes. Temperature, light, targeted feeding, and proper ventilation are all ideal for animals.

The rats were separated into six groups of ten animals each, five males and five females, and kept in separate cages. The first group consisted of control animals, whereas the second group received Ccl4 injections (this material was prepared by mixing it with equal volumes of olive oil 1:1 and injected subcutaneously at two doses weekly with a concentration of 1 ml per 1 kg of body weight for a whole month).

The third group was given Ganoderma powder manufactured by DXN in Malaysia at a concentration of 40 mg per kg of weight and a dosage of 1 ml per kg, while the fourth group was given Ganoderma powder at an oral dose of 80 mg per kg daily for 30 days.

The fifth group was injected with Ccl4 and the animals were dosed with Ganoderma powder at a concentration of 40 mg/ kg daily for thirty days, the sixth group was injected with Ccl4 twice a week with Canoderma powder at a concentration of 80 mg/kg for 30 days.

Blood sample collection:

After each animal's treatment, blood was drawn from the orb vein through a capillary tube implanted in the eye stone, and blood was allowed to flow through the capillary tube to two types of test tubes, one clean and dry and free of anticoagulant to obtain serum, and the other containing a substance. EDTA anticoagulant for performing blood tests on it, dividing the blood into anticoagulant-free tubes, and storing the serum at -20°C until the analyses are completed (Timm *et al*., 2021).

Methods

-The ELISA technique was used to determine the values of chemokines, and the principle for this technique was:

The test used is a sandwich type Elisa. This test depends on the association of chemokines present in the serum with specific antibodies to it that are fixed at the bottom of the microplate. After adding a specific antibody to chemokines tagged with peroxidase on the microplate. In the appropriate incubation period, the immune complex is formed, and then all the substances not associated with washing are removed. The reducing power of the enzyme is directly proportional to the concentration of interleukin in the sample after incubation with the base substance, which is TMB. Yellow color is formed when the reaction stop solution is added, which is measured at a wavelength of 450 nm and is determined sample interleukin concentration by using the standard curve (Kasha *et al.,* 2020), and the following steps have been followed

1- Dilute capture Ab 1:100 in buffer, and transfer 100 µl of this working solution to each well, incubate 24/ h at room temperature.
2- Remove capture antibody and add 300 µl blocking solution to each well, incubate 1 hour at room temperature.
3- Remove block in solution, transfer 100 µl dilute standard and sample in the wells, incubate 2 hours at room temperature.
4- Wash 5/time with washing buffer.
5- Dilute detection-antibody 1:100-in reagent diluent and transfer 100µl to each well, incubate 2 hours at room temperature.
6- Wash 5/time with washing buffer.
7- Dilute poly-streptavidin 1:1000 in reagent-diluent and transfer 100µl to each well, incubate 20-30 m. at room temperature.
8- Wash 5/time with washing buffer.
9- Add 100 µl of substrate solution to each well and incubate up to 60 Min at room temperature in the dark.
10- Add 50 µl stop solution and read the microplate at wavelength 450 nm and is determined sample interleukin concentration by using the standard curve (Kasha *et al*., 2020).
- While the immunodiffusion technique was used to determine the values of both IgG and C3, and the principle for this technique was:

RID test is a type of precipitation reaction that depends on the diffusion of one towards the other (Ag or Ad) on the solid agar medium and the formation of a precipitation ring whose diameter is directly proportional to the concentration of the Ag or Ab in the sample (Parija, 2012). and the following steps have been followed:

1- Place the agar dish and samples at room temperature for 1/2 hour.
2- Added 50µl from serum to the well on the agar.

3- Incubation at room temperature for 24 hours.
4- The diameter of the zoon formed was measured with a graduated magnifying glass.
5- By using table attached to the kit, the concentrations of IgG and C3 were extracted (Parija, 2012)

- The CBC Automated Technique was used to determine the numbers of blood cells.
- Statistical analysis was carried out using SSPS Version 25.

RESULTS AND DISCUSSION

White rats of type and ester citrine were used and separated into six groups (control, Ganoderma first concentration G1, Ganoderma second concentration G2, Ccl4, G1+Ccl4, and G2+Ccl4) to determine the effect of *Ganoderma lucidum* on immunological factors and blood cell counts. As shown in (Table 1).

Table 1: Some immunological parameters of rats dosed with different concentrations of Ganoderma and exposed to CcL4

Parameter	gender	control	G1	G2	Ccl4	G1+CcL4	G2+Ccl4
chemokine pg/ml	Male	1.7	6.7	1.29	2.5	9.7	4.78
	Female	1.5	16.7	3.17	2.4	1.2	2.02
IgG mg/dl	Male	297	470	273	338	470	311
	Female	270	353	364	470	336	351
C3 g/l	Male	33	44	47	50	47	57
	Female	32	34	55	50	55	67

Table (1) shows the average values of chemokines and IgG in males and females given Ganoderma powder in both concentrations, as well as those exposed to CcL4 and given Ganoderma powder in both concentrations. In the two doses with the second concentration, the values varied between males and females, while the values for both chemokine and IgG increased among males receiving the two concentrations of Ganoderma powder and CcL4, and the values for C3 increased among males in the second concentration only without the first concentration. The values of both chemokine and IgG decreased in females exposed to Ccl4. and those given Ganoderma powder, and in both concentrations, the values of C3 increased in the higher concentration in females than in the lower concentration, with no significant differences between the concentrations of Ganoderma and the immunological values that were examined $P > 0.05$ for both sexes.

A study conducted in Singapore in 2003 on the effect LPS of *Ganoderma lucidium* on the effectiveness of the immune system

In which 1800 mg of mushroom powder was used 3 times daily for 12 weeks. The study proved the positive effect of mushroom powder on the effectiveness of the immune system if a significant increase in the production of each of IL-2, IL-6, INF-γ, while non-significant increase in the concentration of each IL-1 and TNF-α .

In China, a study was conducted in 2018 in which *Ganoderma lucidium* powder was used as a treatment for cancer caused in rats. The study found an active effect of *Ganoderma lucidium* powder to production of types of cytokines, including IL-2, TNF-α and INF- γ, In addition to increasing the effectiveness of NK cells and T cells (Chunhua *et al*., 2018).

Chen and his group proved in 2014 demonstration the effect of polysaccharide extract from *Ganoderma lucidium* on the production of IL-1β in mice (Chan *et al*., 2014).

Table 2: CBC of rats dosed with various concentrations of Ganoderma and exposed to CcL4

CBC	gender	control	G1	G2	Ccl4	G1+CCL4	G2+Ccl4
WBC	**Male**	9.24	10.15	13.45	10.4	11.35	12.6
10^3/µl	**female**	8	12.9	12.35	11.3	14.3	12.65
LYM	**male**	6.6	8.45	10.3	8.1	8.8	10.3
10^3/µl	**female**	5.1	9.05	9.55	9.15	11	10.5
MON.	**male**	1.4	1.5	1.85	1.5	1.85	1.3
10^3/µl	**female**	1.2	1.8	1.3	1.3	1.8	1.25

Table (2) compares the effects of dosing with Ganoderma powder at concentrations of 40 mg and 80 mg/kg of body weight to control samples on hematological variables such as white blood cell counts, lymphocytes, and monocytes in both sexes (males and females). It was apparent in the white blood cell count in men at a dosage of 40 mg, which was 10.15, and it was much higher at an 80 mg concentration, which was 13.45, compared to the control samples, confirming Ganoderma powder's beneficial effect on raising white blood cell count for both sexes. In the current study, we didn't detect any significant correlation between patients and all parameters, $P > 0.05$.

In males, the increase was higher in the second concentration, reaching 10.3, and it was lower in the first concentration, reaching 8.45 in males compared to the control samples, and it was close in females at both concentrations, 9.05 and 9.55, respectively, with a clear increase compared to the control samples. Males had a larger rise in monocyte number in the second concentration than in the first, reaching 1.85 when compared to control samples, while females had a higher increase in the first concentration than in the second, reaching 1.8. This effect is due to the presence of polysaccharides in Ganoderma that can boost the immune system in rodents by increasing the production of white blood cells (Cao and Lin, 2006). Many studies indicate that polysaccharides greatly increase the proliferation of T and B lymphocytes.

The proliferative responses of B and T Lymphocyte and the toxic activities of T cells and NK cells were also enhanced with Ganoderma powder without any side effects (Bao *et al*., 2002). The polysaccharides in Ganoderma also allow the release of TNF-α and IFN-γ from resistant T cells in a dose-dependent manner (Zhu *et al*., 2007).

In rats exposed to Ccl4, males had higher numbers of neutrophils in the second concentration than in the first concentration, reaching 12.6 and 11.35, respectively, when compared to rats exposed to the same substance but not dosed with mushroom powder, while females had higher numbers in the concentration. The first concentration was higher than the second concentration, at 14.3 compared to the control samples, as was the number of lymphocytes, with the increase in the second concentration in males being higher than the first concentration, at 10.3, in contrast to females, who had a higher number of lymphocytes in the first concentration. It showed in numbers of 11 with a noticeable rise compared to the control samples in the second concentration, and there was a modest increase in the number of mononuclear cells in the first concentration of the powder than in the second concentration, for both sexes.

This increase is also due to the presence of polysaccharides in Ganoderma that can boost the immune system in rodents by increasing the production of WBC and help remove the effect of toxic substances and oxidizing agents in the body (Bao *et al*., 2002).

Nk cells can kill a tumor cell and are therefore considered promising tools for treating cancer (Cho and Campana, 2009) and uncontrolled proliferation is a feature of tumors. Research indicates that the polysaccharides in Ganoderma mushroom are not only a normal product that has effective functions in the immune system, but act as an adjuvant therapy that inhibits the proliferation of cell tumor (Cho and Campana, 2009). The increase in the numbers of white blood cells and lymphocytes in rats exposed to Ccl4 is symptomatic.

Table 3: Statistical analysis of the data

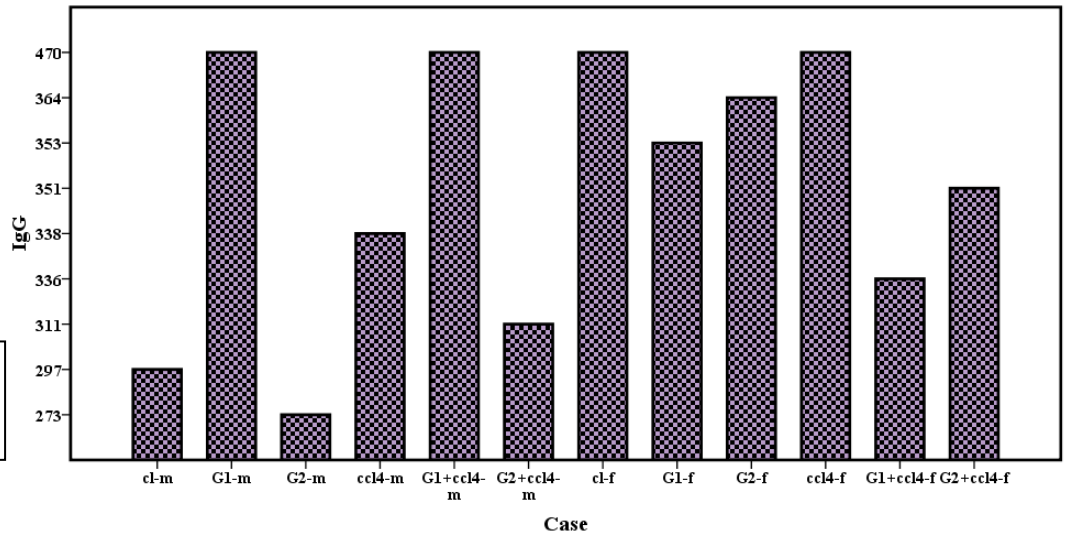
	Case	IgG	C3	CXCL-10	WBC	Lymphocyte	Monocyte
Case	1	.120	.403	-.071	.477	.448	-.041
IgG	.120	1	-.207	.202	-.481	-.390	-.325
C3	.403	-.207	1	-.725**	.070	.217	-.440
CXCL-10	-.071	.202	-.725**	1	.127	.025	.378
WBC	.477	-.481	.070	.127	1	.947**	.545*
Lymphocyte	.448	-.390	.217	.025	.947**	1	.351
monocyte	-.041	-.325	-.440	.378	.545*	.351	1

**. Correlation is significant at the 0.01 level (1-tailed).

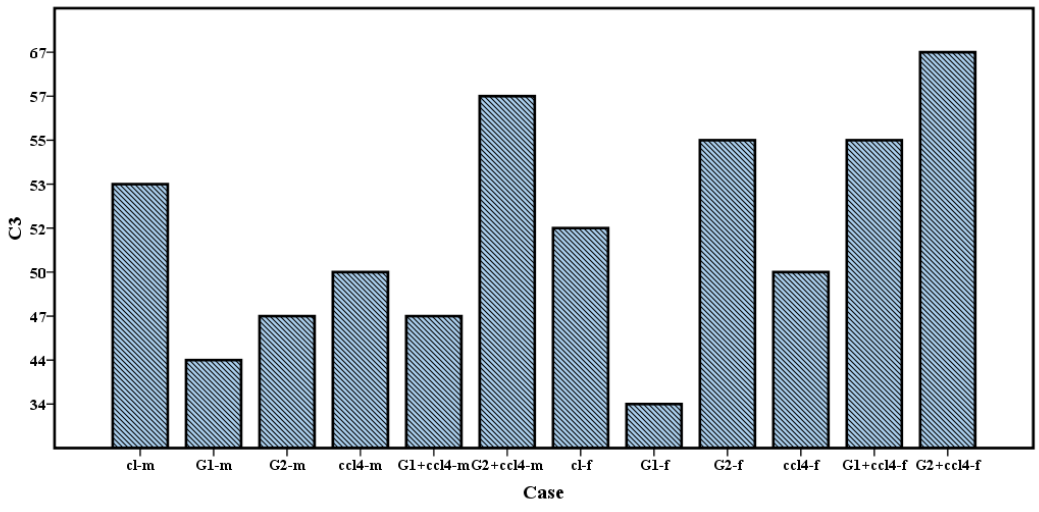
*. Correlation is significant at the 0.05 level (1-tailed).

Table (3) shows the statistical analysis of the results obtained, as significant differences appeared between each of the cytokine and complement, as well as between lymphocyte count and white blood cell count at the level of ≥0.01, and significant differences appeared at the level of ≥0.05, There were no clear significant differences between the white blood cell count and the mononcyte count, this indicates that the Canoderma powder does not affect these parameters

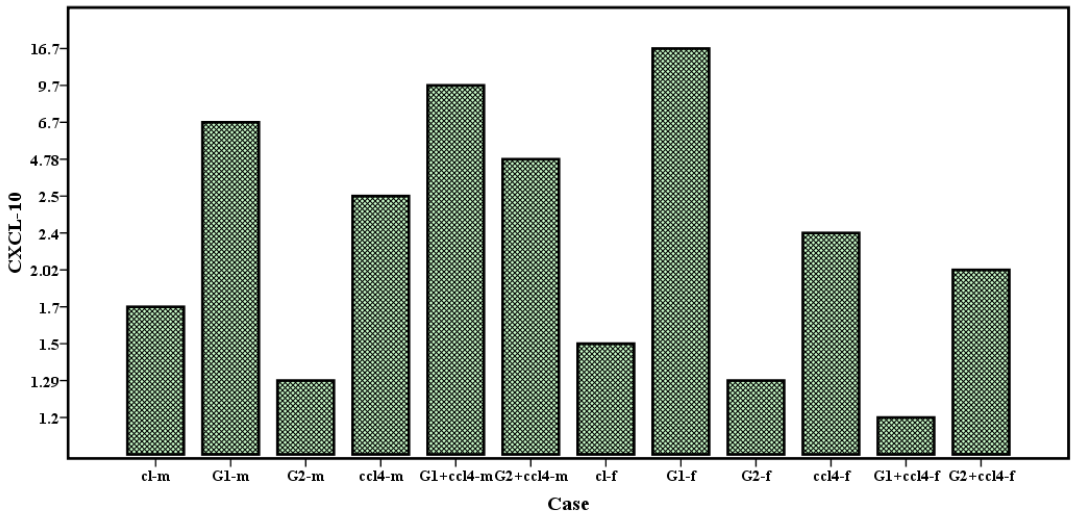
A



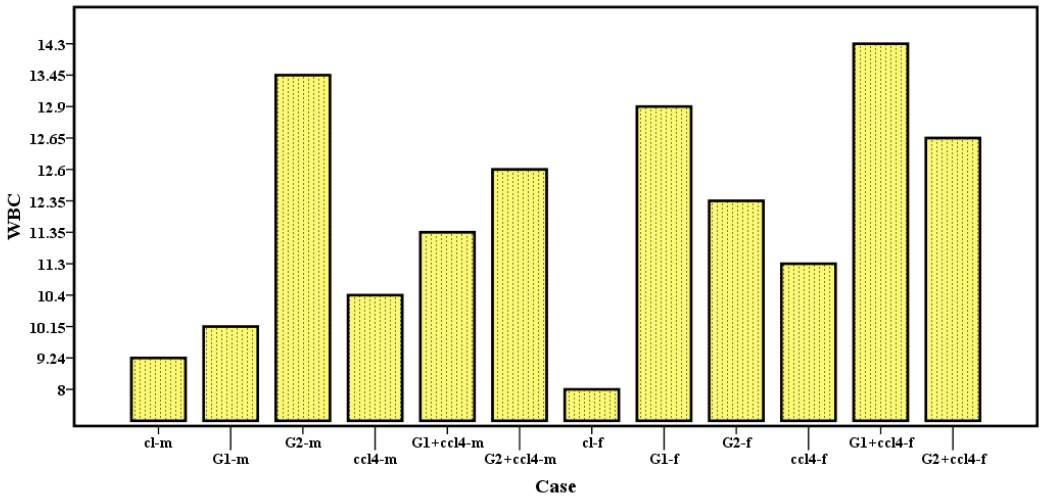
B



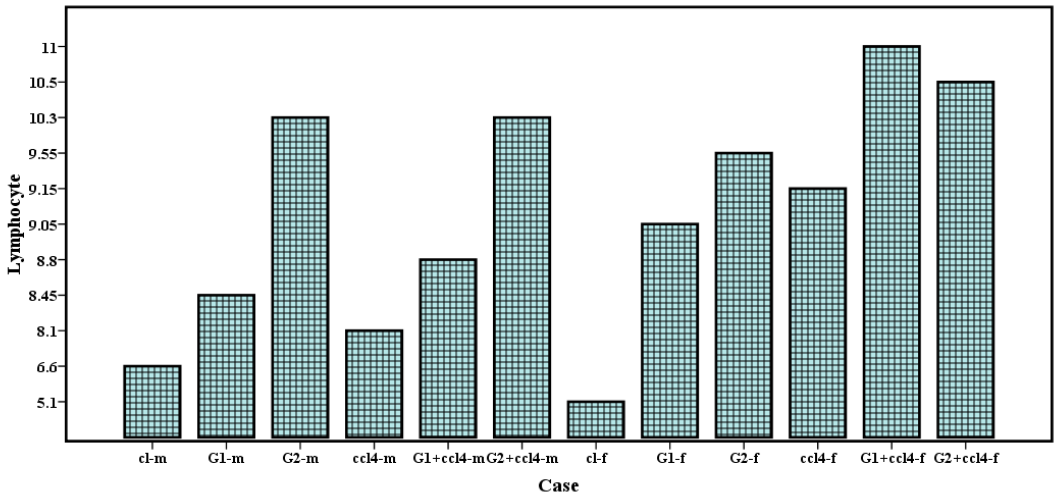
C


CXCL-10
16.7
9.7
6.7
4.78
2.5
2.4
2.02
1.7
1.5
1.29
1.2
cl-m
G1-m
G2-m
ccl4-m
G1+ccl4-m
G2+ccl4-m
cl-f
G1-f
G2-f
ccl4-f
G1+ccl4-f
G2+ccl4-f
Case

D


WBC
14.3
13.45
12.9
12.65
12.6
12.35
11.35
11.3
10.4
10.15
9.24
8
cl-m
G1-m
G2-m
ccl4-m
G1+ccl4-m
G2+ccl4-m
cl-f
G1-f
G2-f
ccl4-f
G1+ccl4-f
G2+ccl4-f
Case

E


Lymphocyte
11
10.5
10.3
9.55
9.15
9.05
8.8
8.45
8.1
6.6
5.1
cl-m
G1-m
G2-m
ccl4-m
G1+ccl4-m
G2+ccl4-m
cl-f
G1-f
G2-f
ccl4-f
G1+ccl4-f
G2+ccl4-f
Case

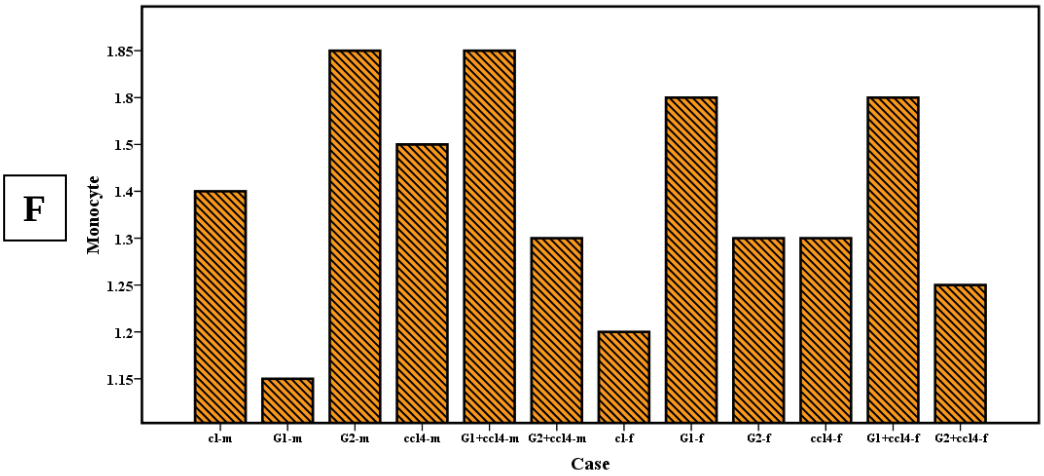


Fig. 1: The relationship between groups of exposed rats and each of the: A: IgG, B: C3, C: CXCL-10, D: WBC, E: Lymphocyte, F: Monocyte.

CONCLUSION

This research proved that Ganoderma powder has a positive effect on some immune factors and the number of immune cells in rats, especially in high concentrations of it, in addition to it reduces the effect of the carcinogen Ccl4 on the body and restores the immune values to values close to the normal values compared to the control samples.

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تأثير مسحوق الكانوديرما لوسيدوم على بعض المتغيرات المناعية في الجرذان

الملخص

شملت الدراسة تأثير مسحوق فطر الكانوديرما لوسيديوم على كل الكيموكين، IgG، وتركيز C3 من المتمم، بالإضافة الى تعداد الخلايا الدموية في 60 جرذ استرالي، قسمت الى عدة مجاميع جزء منها جرعت بمادة رابع كلوريد الكاربون والمجاميع الاخرى تركت بدون تجريع، وتم استخدام تركيزين من مسحوق الفطر، 40 ملغ/ كغم من وزن الجسم، و 80 ملغ/ كغم من وزن الجسم، بالإضافة الى مجموعة السيطرة. اظهرت النتائج زيادة واضحة في جميع العوامل المناعية التي تم تحديدها مع وجود فروقات معنوية واضحة بين الاناث والذكور مقارنة مع عينات السيطرة، بالإضافة الى زيادة واضحة في تعداد كل من الخلايا العدلة والخلايا اللمفاوية في الذكور لكلا التركيزين مع زيادة اعلى للتركيز الثاني عنه في التركيز الاول، في حين كانت الزيادة متساوية لكلا التركيزين في الاناث مقارنة مع عينات السيطرة. ولم تظهر أي زيادة واضحة في تعداد الخلايا الخلايا وحيدة النواة في كل من الاناث والذكور ولكلا التركيزين.

اما في المجاميع المجرعة بمادة رابع كلوريد الكاربون لوحظ زيادة في مستوى الكيموكين وجزئ المتمم وتركيزIgG، وكذلك زيادة في تعداد الخلايا العدلة عند الذكور لكلا التركيزين.

في حين ظهرت الزيادة عند الاناث في التركيز الثاني اعلى من التركيز الاول للعوامل المناعية المدروسة على عكس تعداد الخلايا البيضاء، وكذلك في حالة تعداد الخلايا اللمفاوية التي كانت اعلى في التركيز الثاني للذكور عنها في الاناث والتي لوحظ فيها ارتفاعا في التركيز الاول مقارنة مع عينات السيطرة. وظهر تعداد الخلايا وحيدو النواة بتغيرات طفيفة في كل من الاناث والذكور قيد الدراسة. مع فروقات معنوية واضحة بين كل من جزئ المتمم والكيموكين والتعداد الكلي لخلايا الدم.