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Abstract

The present study aimed to illustrate the effect of diets enriched with onion, garlic and chicory powder by the percent of 2.5 and 5% on the immunity system parameters of rats. Forty-two (42) male albino rats ((Sprague- dawly strain) weighting (150-160g) used in this study and divided to 7 groups each (6rats). The control group received basal diet all the experimental period, the other experimental groups including 36 rats were fed on basal diet containing 2.5 and 5% of onion, garlic and chicory powder for (4 weeks). The results appeared that feed intake showed a few differences at ($p \leq 0.05$) comparing with control, body weight gain showed a few differences at ($p \leq 0.05$) comparing with control. Immunological profile (IgA, IgG and IgM) showed significant increases comparing negative control group recorded (145± 1, 184,47±0.5 and 134.5±0.5 mg/dl) . For CBC analysis (HB, HCT and PLT) were significant increase comparing with control recorded (11.6±0.1%, 47.2±0.72% and 637.33±3.05cm) . WBC and RBC were significantly increasedcomparing with controlrecorded (6.83±0.21 and 7.55±0.06 cm). Inconclusion. The tested plants in this study were effective as promoting the immunity system. We recommended more consumption of the tested plants as fresh or dried in our diets .

Keywords: IgA, IgM, IgG, HB, HCT, PLT, WBC, RBC.

Introduction

The immune system is essential for survival. Without an immune system, the bodies would be open to attack from bacteria, viruses, parasites. Immune system that keeps us healthy as we drift through a sea of pathogens **(Mohammad *et al*., 2020).**

The immune system is spread throughout the body and involves many types of cells, organs, proteins, and tissues. Crucially, it can distinguish tissue from foreign tissue — self from non-self. Dead and faulty cells are also recognized and cleared away by the immune system **(Daniel, 2018).**

White blood cells are also called leukocytes. They circulate in the body in blood vessels and thelymphatic vessels that parallel the veins and arteries. White blood cells are on constant patrol and looking for pathogens. When they find a target, they begin to multiply and send signals out to other cell types to do the same and there are two main types of leukocyte**(Lindsay,2016).**

Many dietary bioactive food components interact with the immune system with the potential to reduce the risk of chronic diseases (**Ferguson and Philpott, 2007).**

Garlic *(Allium sativum L.)* is a member of the *Liliaceae* family, is an aromatic herbaceous annual spice and its consumption has beendated back over 6000 years as a food ingredient with medicinal properties. Garlic contains organo sulfur compounds that provide a unique odor and flavor, and potential health benefits *(***Badal *et al*., 2019)** and (**Butt *et al*., 2009).**

It is a member of the lily family, which has been widely used as an ancient folk medicine in India, Egypt, Greece, Rome and China to treat various sicknesses, including abdominal pain, parasitic infections and rheumatism **(Butt *et al*., 2009)**

It is consumed worldwide as food and traditional remedy for various diseases. It has been reported to possess several biological properties including anticarcinogenic, antioxidant, antidiabetic, anti-atherosclerotic, antibacterial, antifungal, anti-hypertensive, antiviral, antifungal, antiprotozoal and anti-inflammatory activities in traditional medicines **(Gaber *et al*., 2020).**

Garlic contains many valuable compounds such as iodine salts which have positive effects on the circulatory system. Garlic also contains many vitamins such as vitamin A.C and B complex as well as linoleic acid. Garlic is rich in calcium, phosphorus, carbohydrates and generally, has a high nutritive value**(Dragan*et al*., 2008).**

It should be considered as a dietary anti-inflammatory supplement that in the long term might lead to the reduced risk of certain types of cancers, an effect known for long-term usage of aspirin **(Jacobs *et al*., 2007)**.Extracts and isolated compounds of A. sativum have been evaluated for various biological activities (**Gaber *et al*., 2020)**.

Garlic derivatives and aged garlic extract (AGE), an odorless product containingS-allylcysteine (SAC) and S-allylmercaptocysteine (SAMC), have shown immunomodulatory effects byimproving the immune response, resulting in attenuation of the effects of cardiovascular disease andinflammatory-associated processes ***(Ahmadi et al., 2013).***

Onion *(Allium cepa)*is original from central Asia and is one of the oldest cultivated plants since it has been in cultivation for more than 4000 years. It is a major and popular vegetable crop product that is widely cultivated, it used as a food ingredient in the Mediterranean diet that has a high productionand consumed raw, cooked or processed into different onion products in the daily diet (**Marta and Mar,2012)**.

Onion characteristic flavor, is the third most essential horticultural spice with a substantial commercial value **(Joaheer *et al*., 2019).** Onion contain bioactive compounds such as organosulfur compounds and polyphenolic compounds with potential beneficial health effectsincluding antimicrobial, antioxidant, analgesic, anti-inflammatory, anti-diabetic, hypolipidemic, anti-hypertensive, and immuneprotective effects**(Yosuke *et al*., 2019*).*** Onion contains small quantities of fat. Sugar and vitamins A.C and B complex, it is rich in magnesium. Potassium and copper **(Gabor *et al*., 2010).**

It contains large amounts of flavonoids that are responsible for the reported antioxidant activity, immune enhancement, and anticancer property**(Elberry *et al*., 2014).**

It contains high levels of non-nutrient antioxidant compounds, flavonoids, and the alk(en)yl cysteine sulfoxides (ACSOs), which have protective effects against different degenerative pathologies such as cardiovascular disease, cancer, and other dysfunctions based on oxidative stress (**Griffiths *et al*., 2002)**

Chicory (*Cichoriumintybus L*.) is mostly known for its multiple uses in the food industry and herbal medicine. This plant has long been consumed as a vegetable by humans. Leaves and root have been used for thousands of years for nutritional purposes.Chicory was already cultivated as a medicinal plant by the ancient Egyptians and is still used in traditional dishes in various parts of the world **(Puhlmann and De- Vos, 2020).**

The whole plant is also known for its hypoglycemic and hypolipidemic properties, hypothetically due to the chlorogenic acids content of chicory **(Jackson *et al*.,2017)**. Extracts of chicory roots have also been stated to have antimicrobial **(Liu *et al* ., 2013)**,immunomodulatory, anti-inflammatory **(Lee *et al*., 2015),** and anti-cancer properties **(Behboodi *et al* .,2019).**

Chicory roots are rich in fiber, Inulin and bioactive compounds. Inulin was adopted as a functional ingredient and various studies evaluated its efficacy as prebiotic, for promoting good digestive health, for influencing lipid metabolism and for its beneficial roles in optimum plasmatic levels of glucose and insulin maintaining **(Ahmed and Rashid, 2017),** Chicory was already found responsible for various antimicrobial effects **(Puhlmann and De -Vos, 2020) ,** Chicory plays an important role in immunity and inflammation reducing by regulation the recruitment of immune cells **(Sun *et al*., 2015).**

This study aimed to illustrate the effect of diets enriched with onion, garlic and chicory powder by the percent of (2.5 and 5%) on the immunity system parameters of rats.

2.Materials and methods

Materials

Chemicals: Kits for biochemical analysis of serum were purchased from Gama Trade Company for chemicals, Cairo, Egypt.

Plant materials: Onion, Garlic and Chicory were purchased from local market in Shebin El Kom.

Rats and diets: Male albino rats, weighing 150±10g per each were purchased from Medical Insects Research Institute, Dokki, Cairo, Egypt.

Basal diet constituents: were obtained from El-Ghomhoriya Company for and Trading Drugs, Chemicals and Medicals instruments, Cairo, Egypt.

-Methods:

Preparation of plant powder:

Plant materials: Garlic and onion powder were purchased from market in Shebin El-Kom, Chicory were collected, cleaned and dried at degree (45), then grinded to soft powder by electric

grinder (Moulinex, France), packed and kept in dusky Stoppard glass bottles in a cool and dry location till use according to **(Russo,2001).**

Preparation of basal diet:

The basal diet prepared from protein (10%), corn oil (10%), vitamin mixture (1%), mineral mixture (4%), choline chloride(0.2%), methionine (0.3%), cellulose (5%), and the remained is corn starch (69.5%). The used vitamin mixture component was recommended by **(Hegested *et al.*,1941),** while the salt mixture used was formulated according to**(Campbell,1963)**

Experimental Design:

Forty-two (42) male albino rats were housed in healthy condition (21-23ºC), with 40-60% humidity and fed on basal diet and water for one-week acclimatization period. Then rats were divided into 7 groups each (6 rats) as following:

- group (1): fed on standard diet only as a control (ve-).
- group (2): fed on diet containing 2.5% Onion.
- group (3): fed on diet containing 5% Onion.
- group (4): fed on diet containing 2.5% Garlic.
- group (5): fed on diet containing 5% Garlic.
- group (6): fed on diet containing 2.5% Chicory.
- group (7): fed on diet containing 5% Chicory.

At the end of the experiment period (4 weeks), animals were fasted for 12-h then rats were scarified. Blood samples were collected from the portal vein into dry clean centrifuge tubes for serum separation, blood samples centrifuged for 10 minutes at 3000 rpm to separate the serum according to **(Drury and Wallington, 1980)**. Serum samples were frozen at – 20 ºC until chemical analysis.

Feed intake (FI) was calculated every other day and body weight gain (BWG) was calculated using the following equation: BWG = Final body weight (g)- Initial body weight (g)}.

Analytical methods:

Determination of IgA, IgG and IgM:

Serum IgA, IgG and IgM were determined as mg/dl according to the method of **(Ernie, 2016), (Junqueira *et al.*, 2003)** and **(Falkenburg , 2015),** respectively.

Determination of HB, HCT and PLT:

Hemoglobin, serum hematocrits and serum platelet count were determined according to the method of **(Dacie and Lewis, 2006), (Purves *et al.*, 2004)** and **(Martina and Daly, 2011)**, respectively.

Determination of W.B.C.s and R.B.C.s:

W.B.C.s and R.B.C.s were determined according to the method of **(Koda-Kimble *et al.*, 2001)** and **(Lubsandorzhiev, 2006)**, respectively.

Statistical Analysis:

Data were expressed as mean ± standard deviation. Analysis of Variance (ANOVA) test was used. Values at ($P \leq 0.05$) were considered to be statistically significant according to **(SAS ,2006).**

Results and Discussion

Effect of onion, garlic and chicory powder on feed intake, body weight gain of normal rats

Table (1) illustrate the effect of diet supplemented with onion, garlic and chicory on feed intake (FI) and body weight gain (BWG) of normal rats. Tabulated data showed that there were no

significant differences in mean values of feed intake between most groups. For BWG, showed that there were significant differences in mean values compared with control group.

Similar results were obtained by(**Apines *et al.*,2012)**and **Amar and Faisan, 2011)**they indicated that, onion effects on enhancing growth through different mechanisms either separately or synergistically Contrary to the previous results. Sulfur-containing compounds such as methyl sulfonate methane (MSM) have immunomodulation properties .

Onion improved in body weight gain, thisattributed to Sulfer being a component of the antioxidant enzyme Glutathione peroxidase.

Agbebi *et al.*, (2013) indicated that the positive effects of administrating garlic in diets on growth.

On the other hand, these results are disagreement with **Gorjipour *et al*., *(2017)*** noted that, chicory leaf extract did not affect theweight of the rats.These results are disagreement with **Manal and Lamiaa (2012)** indicated that, onion caused significant reduction in feed intake compared to control group. **Slyranda *et al.*, (2011)** Indicated that, onion and garlic produced significant increase in body weight of the birds throughout the period of the treatment.

Table (1). Effect of onion, garlic and chicory powder on feed intake , body weight gain of normal rats.

Groups	F.I (g/d/rat) Mean± SD	% change of control	B.W.G (g) Mean± SD	% change of control
Control (-)	11.1 ± 0.1 [a]	------	34.96 ± 0. 281 [e]	------
Onion 2.5%	10.81 ± 0.23 [ab]	- 2.61	44.5 ± 0.5 [a]	27.29
Onion 5%	10. 6 ±0.27 [b]	- 4.51	41 ± 1 [b]	17.28
Garlic 2.5%	11.12 ± 0.01 [a]	0.18	35.86 ± 0.568 [de]	2.57
Garlic 5%	11.09 ± 0.075 [a]	- 0.09	38.03 ± 0.45 [c]	8.78
Chicory 2.5%	10.7 ± 0.12 [b]	- 3.60	36.9 ± 0.559 [b]	5.55
Chicory 5%	10.7 ± 0.05 [b]	- 3.06	32.98 ± 0.22 [f]	- 5.66

Means in the same column with different superscript letters are significantly different at $p \leq 0.05$.

Effect of onion, garlic and chicory powder on Serum IgA, IgG and IgM (mg\dl) of normal rats .

Table (2) illustrate the effect of diet supplemented with onion, garlic and chicory on IgA, IgG and IgM of normal rats. Data indicated that there were significant increases in mean values of IgA of all supplemented groups compared with control. For IgG, it could be noticed that there were significant increases in mean values of IgG of all supplemented groups compared with control. For IgM, data indicated that there were significant increases in mean values of IgM of all supplemented groups compared with control.

These results are supported by published by **(Hanieh *et al.*,2010) and (Jafari *et al,* 2008)** they reported that, garlic induce of antibody secretion. found that more studies with garlic and its derivatives are necessary in order to clarify the mechanism implicated in immunoglobulin production.

Garlic is rich in organo sulfur compounds containing phytoconstituents such as alliin, allicin, ajoenes, vinyldithiins, and flavonoids such as quercetin contribute to the prevention or reduction of the immunosuppressive environment during chronic inflammation (**Gaber *et al*., 2020*)*** and **(Jacobs *et al.*, 2007)**.

Mustafa and Orkide, (2020) reported that*Allium sativum* may be an acceptable preventive measure against COVID-19 infection to boost immune system cells and to repress the production and secretion of proinflammatory cytokines as well as an adipose tissue derived hormone leptin having the proinflammatory nature.

On the other hand, these results are disagreement with **Essam *et al.*,(2017)** administrated that, garlic induced a ~60% elevation in IgG levels, but had no significant effects on IgM. **Pashaki *et al.*, (2018)** reported that, garlic increased IgM in groups compared to control group, no significant effects on IgG

The immunomodulatory effects of garlic are due to garlic lectin or agglutinin proteins **(Chandrashekar and Venkatesh, 2009).** Garlic contains these compounds that exhibit a broad spectrum of beneficial effects against microbial infections as well as cardioprotective, anticancerigenic, andanti-inflammatory activity (**Fridman *et al.*,2014).**

Garlic and onion could be a promising candidate as an immune modifier, which maintains the homeostasis of immune function and the beneficial effect of both extracts can be considered as a possible means of immune system protection.

Table (2). Effect of onion, garlic and chicory powder on Serum IgA, IgG and IgM (mg\dl) of normal rats .

Groups	IgA (mg/dl) Mean±SD	% change of control	IgG (mg/dl) Mean ± SD	% change of control	IgM (mg/dl) Mean±SD	% change of control
Control (-)	96.33 ±1.53 ^{e}	------	122.66 ± 2.52^{f}	------	101. ± 1^{d}	-----
Onion 2.5%	126 ±2 ^{c}	30.89	168.67 ± 1.53^{d}	37.51	125 ± 2^{c}	23.76
Onion 5%	144.33 ±1.53 ^{a}	49.83	177.33 ± 1.53^{b}	44.69	133.98 ± 0.525^{a}	32.65
Garlic 2.5%	141.33 ±1.52 ^{b}	46.71	179 ± 1^{b}	45.93	132.83 ± 0.472 ^{a}	31.51
Garlic 5%	145 ± 1 ^{a}	50.52	184.47 ± 0.5^{a}	50.39	134.5 ± 0.5 ^{a}	33.17
Chicory 2.5%	122.93 ±0.84 ^{d}	27.61	161 ± 1^{e}	31.26	126.43 ± 0.513 ^{b} ^{c}	25.18
Chicory 5%	124.81 ± 0.23 ^{cd}	29.57	174.57 ± 0.51^{c}	42.32	128 ± 1 ^{b}	26.73

Means in the same column with different superscript letters are significantly different at $p \leq 0.05$.

Effect of onion, garlic and chicory powder on serum Hb(%), HCT(%) and PLT (cm) of normal rats .

Table (3) showed the effect of diet supplemented with onion, garlic and chicory on HB, HCT and PLT of normal rats. Data indicated that there were significant increases in mean values of HB of all supplemented groups compared with control. For HCT, it could be noticed that there were significant increases in mean values of HCT of all supplemented groups compared with control. For PLT, data indicated that there were significant increases in mean values of PLT of all supplemented groups compared with control.

The present study demonstrated that administration of garlic powder induced a considerable increase in some measured blood parameters (Hb, HCT and PLT) this result coincided

with **Kalyankar *et al.* (2013) and Norhan *et al.*, (2015)** they revealed that administration of 1.5% garlic in diet induced significant increases in all blood parameters (RBCs, WBCs, Hb and PCV). Also, **Thanikachalam *et al.*,(2010)** indicated that garlic peel enhanced the hematological parameters.

This elevation in some blood constituents may be due to some constituents of garlic and onion that may play a role in the immune system stimulation and in the function oforgans related to blood cell formation such as thymus, spleen.

*Allium sativum*and its derivatives been proposed as promising candidates for maintaining the homeostasis of the immune system. The complex biochemistry of garlic makes it possible for variations in processing to yield different preparations with differences in final composition and compound proportion **(Rodrigo *et al.*, 2015).**

This result is in agreement with **Pashaki *et al.*, (2018)** reported that, garlic increase hemoglobin, hematocrit, had Significant increase compared to the control group .

On the other hand, this result disagrees with **Mahdieh *et al.*, (2016)** they revealed that, chicory significantly reduced hematocrit and hemoglobin.

Table (3). Effect of onion, garlic and chicory powder on serum HB (%), HCT (%) and PLT (cm) of normal rats .

Groups	HB (%) Mean± SD	% change of control	HCT (%) Mean± SD	% change of control	PLT (cm) Mean± SD	% change of control
Control (-)	10.4 ± 0.1 [c]	-----	38 ± 1 [c]	-----	318.67 ± 2.51 [g]	-----
Onion 2.5%	10.73± 0.252 [b]	3.17	41 ± 1 [b]	7.89	515.33 ± 1.53 [f]	61.71
Onion 5%	11. 5 ±0.2 [a]	10.58	46.33± 1.53 [a]	21.92	549.33 ± 4.04 [e]	72.38
Garlic 2.5%	11.53 ± 0.153 [a]	10.87	43 ± 1 [b]	13.16	620 ± 2 [b]	94.56
Garlic 5%	11. 6 ± 0.1 [a]	11.54	47.2 ± 0.72 [a]	24.21	637.33 ± 3.05 [a]	99.99

Chicory 2.5%	**10.45±0.098 ^{c}**	**0.48**	**40.52 ± 0.5 ^{b}**	**6.63**	**610.33 ± 1.53 ^{c}**	**91.42**
Chicory 5%	**10.9 ± 0.1 ^{b}**	**4.81**	**42 ± 1 ^{b}**	**10.53**	**596.33 ± 4.04 ^{d}**	**87.13**

Means in the same column with different superscript letters are significantly different at $p \leq 0.05$.

Effect of onion, garlic and chicory powder onSerum WBC and RBC (cm) of normal rats.

Table (4) showed the effect of diet supplemented with onion , garlic and chicory on WBC and RBC of normal rats. Data indicated that there were significant increases in mean values of WBC of all supplemented groups compared with control. For RBC, it could be noticed that there were significant increases in mean values of RBC of all supplemented groups compared with control.

Allium containing substances have antibiotic effects and antibiotics should enable the proliferation of circulating white blood cells considering that white blood cells function to protect the body from teratogens.In similar studies, **Meraiyebu *et al*., (2013)** stated that, significant increase in the Lymphocytes count in the animal treated with onion juice extract may be attributed to its major sulfur component.

The present study demonstrated that administration of garlic powder induced a considerable increase in some measured bloodparameters (WBCs) this result coincided with**Kalyankar *et al*., (2013) and Norhan *et al*., (2015)**.

These results are in agreement with **Ndong and Fall, (2011)Talpur and Ikhwanuddin, (2012)**they indicated that, garlic increased RBCs and WBCs count. On the other hand, these results disagree with **Pashaki *et al*., (2018)** and **Mikail, (2009)** they reported that, garlic not had Significant increase RBC but increase in the WBC's count.

Table (4). Effect of onion, garlic and chicory powder on Serum WBC and RBC (cm) of normal rats.

Groups	WBC (cm) Mean± SD	% change of control	RBC (cm) Mean± SD	% change of control
Control (-)	5.6 ± 0.1 [d]	-----	6.56 ± 0. 05 [e]	-----
Onion 2.5%	6.53 ± 0.15 [b]	16.61	7.07 ± 0.13 [b]	7.77
Onion 5%	6. 83 ±0.21 [a]	21.69	7.55 ± 0.06 [a]	15.09
Garlic 2.5%	6.3 ± 0. 1 [b]	12.5	6.9 ± 0.1 [c]	5.18
Garlic 5%	6.53 ± 0.1 [b]	16.61	7.48 ± 0.05 [a]	19.51
Chicory 2.5%	6.01 ± 0.08 [c]	7.32	6.69 ± 0.03 [d]	6.09
Chicory 5%	6.3 ± 0.05 [b]	12.5	6.91 ± 0.05 [c]	5.34

Means in the same column with different litters are significantly ($p \leq 0.05$) different.

Conclusion

The tested plants in this study were effective as promoting the immunity system and improvement CBC analysis. The obtained data supported our hypothesis that these plants containing several bioactive compounds which are able to enhance immunity system and blood parameters. We recommended more consumption of the tested plants as fresh or dried in our diets.

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دراسة تأثير مسحوق البصل والثوم والهندباء على بعض مقاييس المناعة في الفئران

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الملخص العربي

هدفت الدراسة الحالية إلى توضيح تأثير الأنظمة الغذائية الغنية بالبصل والثوم ومسحوق الهندباء بنسبة 2.5 و 5٪ على مقاييس المناعة لدى الفئران. اثنان وأربعون (42) من ذكور الجرذان البيضاء (Sprague– dawly) بوزن (150–160 جم) مستخدمة في هذه الدراسة وقسمت إلى 7 مجموعات لكل مجموعة (6 فئران). تلقت المجموعة الضابطة النظام الغذائي الأساسي طوال فترة التجربة، المجموعة تجريبية الأخرى تضم 36 جرذاً التي تم تغذيتها على نظام غذائي أساسي يحتوي على 2.5 و 5٪ من مسحوق البصل والثوم الهندباء لمدة (4 أسابيع) وظهرت النتائج أن المأخوذ الغذائي أوضح اختلافات قليلة عند مستوي دلاله إحصائية($p\leq 0.05$)مقارنة بالمجموعة الضابطة. أظهر اكتساب الوزن بعض الاختلافات عند مستوي دلاله إحصائية($p\leq 0.05$)مقارنة بالمجموعةالضابطة. أظهر المظهر المناعي (IgG,IgM وIgA) زيادات معنوية مقارنة بالمجموعةالضابطةالتي سجلت ±145) (mg/dl 134.5±0.5 and 1,184,47±0.5 وبالنسبة لتحليل CBC (HB, HCT and PLT) كانت هناك زيادة إحصائية مقارنةبالمجموعةالضابطةالتي سجلت(11.6±0.1%,47.2±0.72%,and 637.33±3.05cm) . بالنسبة

WBC و RBC بشكل ملحوظ مقارنة بالمجموعة الضابطة التي سجلت 6.83±0.21) and 7.55±0.06 cm).

الخلاصة: كانت النباتات المختبرة في هذه الدراسة فعالة في تعزيز نظام المناعة، لذلك أوصينا باستهلاك المزيد من النباتات المختبرة كطازجة أو مجففة في وجباتنا الغذائية بنسبة (5–10 جرام / يوم).

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