International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (1): 1049-1054
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | Open Access | MCI Approved
UGC Approved Journal

Study of Immunostimulatory Effect of Zingerone in Male Wistar Rats

[1]J. Juliet Poornamathy* and [2]C. S. Parameswari

[1]Research and Development Centre, Bharathiar University, Coimbatore.
[2]Principal (Retd) Government Arts and Science College for women, Ramanathapuram.

Received: 12 Oct 2018 / Accepted: 10 Nov 2018 / Published online: 1 Jan 2019
Corresponding Author Email: Julietpoornamathy81@gmail.com

Abstract

Aims: Inflammation is a natural response of the mammalian body to a variety of hostile agents including parasites, pathogenic microorganisms, toxic chemical substances and physical damage to tissue. The present investigation is aimed to study the immunostimulatory effect of zingerone by analysing the various hematological and immunological parameters in male wistar rats. **Methods:** Thirty healthy rats were divided into five equal groups viz. Control (Group 1), administered food and water ad libitum, (Group 2), administered with olive oil (Group 3), administered with zingerone, (Group 4), administered with concanavallin, (Group 5), administered with Cyclosporin A(csA), followed by zingerone. **Conclusion:** It was observed that the zingerone treated rats succeed in persuading as effective compound in responding to both specific and non-specific immune response.

Keywords
Cyclosporin A, Hematology, Immunology, zingerone.

*****

INTRODUCTION

The term immunostimulation comprises a prophylactic or therapeutic reactions which target at the stimulation of specific and nonspecific immune system [1]. Immunostimulatory therapy is now being recognised as an alternative therapy for various disease conditions, involving the impaired immuno-response of the host [2]. Natural products are the therapy for treatment of diseases in recent years [3]. The medicinal plants are used as treatments of many diseases along with folk medicine from different parts of the world [4]. Ginger is used as spice and food preservative in India, China, and South East Asia and probably originated in India [5]. Ginger herb used as nourishment and medicine, it has antioxidant, analgesic, antimicrobial, antiparasitic, anticancer, antiplatelet, hepatoprotective and immune stimulatory properties [6]. Medicinal properties of ginger were associated with many active compound present in ginger. The major constituent are pungent vanilloids, gingerol, paradol, shagaols and zingerone [7]. Safety precautions studies indicate that zingiber officinale even at very high dose is well tolerated without any toxicity. Thus zinger and its compound have potential for developing in the modern medicine against various diseases [8]. The principle reason for studying immunomodulatory effect of zingerone, the active component of zinger [9] is its antiemetic, anticancerous, [10] and easily metabolisable effect. [11]

MATERIALS AND METHODS

Animal Ethical Clearance:

All the studies were conducted in compliance with guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA no. 971/bc/06/CPCSEA), Government of India at Biogen Laboratory Animal Facility, Bangalore, India and approved by the Institute of Animal Ethics commission.

Experimental Design:

Treatment regimen was as follow:

Group I: Control, administered Food and water ad libitum.

Group II: Olive oil (Vehicle control) orally for 10 days

Group III: Zingerone in distilled water orally given at a dose of 10 mg/kg B wt**,** daily for 10 days.

Group IV: Concanavalin in 0.9% saline orally given at a dose of 50 mg/kg B wt**,** daily for 10 days.

Group V: Cyclosporine A (CsA) in Olive oil orally given at a dose of 20mg/Kg B wt**,** followed by Zingerone orally at a dose of 10 mg/kg B wt**,** for 10 days.

Blood Collection

At the end of the treatment period, rats were fasted overnight (water allowed) and blood was collected from ocular sinus plexus under isoflurane anesthesia. An aliquot of blood was collected in vacutainer tubes. The first sample was collected in eppendorf tubes with EDTA for haematological examination and the second blood samples were collected in clean test tubes and allowed to clot, then centrifuged for ten minutes at 3000 r.p.m. Serum was separated and stored in eppendorf tubes at – 20˚C to be used for biochemical studies. All the samples were analysed in duplicates.

Hematological Assay:

The following hematological parameters were determined using ADVIA 120 hematology system (Siemens) White blood cells (WBC), Red blood cells (RBC), hemoglobin (Hb), Differential Count, MCV, MCH and MCHC.

Respiratory Burst Activity of Neutrophils:

Reactive oxygen radical production was assayed by the reduction of Nitroblue Tetrazolium (NBT) Anderson (1995).

Estimation of Immunoglobulins in Plasma:

Total immunoglobulin was determined by the method described by Anderson and Siwicki (1995).

Protein Estimation:

Protein was measured using Dimension Xpand Automatic Analyzer (Siemens).

Statistical Analysis:

Parameters were analysed by analysis of variance, one way (ANOVA) using SPSS. 21 versions. The mean and standard error were calculated for each variable. Data were normally distributed; therefore, post hoc LSD multiple comparison was used to assess statistical differences among different groups. For all statistical examinations, results were considered significant at $p< 0.05$.

RESULTS AND DISCUSSION

The haematological study to analyse total erythrocyte count (RBC) and total leucocyte count (WBC) in the blood of Control rats and other treated rats were represented in Figure 1. The study revealed significantly elevated levels ($p<0.01$) of RBC and WBC in Zingerone treated rats when compared to control rats. There seems to be significantly increased ($p<0.05$) difference between Zingerone and ConA treated rats. ConA is an immunostimulant which has increased level in WBC & RBC zingerone is also having similar activity as Con A. The level of WBC in CsA + Zingerone treated animals were found to be significantly decreased ($p<0.05$) when compared to ConA and ZO treated rats,CsA is known immunosuppressor , the decreased level is restored by the immunostimulatory effect of ZO compared to control, narrating the immunostimulatory nature of ZO on CsA induced rats.

The effect of lead on haematological changes in male albino rats and its ameliorative effect by different doses of *Zingiber offcinale* extract [12]. The mean RBC and Hb values were reduced significantly ($P<0.05$) in lead treated rats but the mean WBC values were increased, whereas significant improvement was noticed in ginger treated rats, the increased RBC and Hb values and WBC levels were normal in dose dependent manner. The alteration in haematological parameters indicated decreased lifespan and fragility of RBC and damage to liver and kidney in lead poisoned male wistar albino rats.

Likewise, to evaluate the effect of Annona muricata extract on Sprague–dawley rats, even though results were significantly higher than normal, no negative effects were observed [13]. The results were found to be similar to our findings in haematological indices.

The Differential count analysis in the blood samples of Control rats and other treated rats were represented in Figure 2. The study revealed significantly elevated levels ($p<0.05$) in Lymphocytes, eosinophils and decreased level ($p<0.01$) in Neutrophils, Monocytes with Zingerone treated rats when compared to control rats. There seems to be

significantly increased (p<0.01) difference between Zingerone and ConA treated rats in lymphocytes levels. The levels of Eosinophils and basophils were found to be significantly decreased (p<0.05). The levels of Lymphocytes, neutrophils, eosinophils, basophils and monocytes in CsA+ Zingerone treated animals were found to be significantly (p<0.01) altered when compared to control.

Immunomodulatory effect of ocimum basilicum linn in *Clarius batrachus* fishes, differential count analysis revealed that, thrombocytes were found to be abundant in the blood of all treated fishes [14]. The amount of Lymphocytes, Eosinophils and Neutrophils at higher doses were decreased at the end of the experiment as compared to the control group, similar to our findings.

The values of Mean corpuscular volume (MCV), Mean cell haemoglobin (MCH) and Mean cell haemoglobin concentration (MCHC) levels in control and other treated animals were tabulated in Table 1. The MCV levels of ZO and ConA treated rats were found to show significantly (p<0.05) heightened data than control rats. The levels of CsA + ZO treated rats were found to be significantly (p<0.05, p<0.01) increased than ZO and ConA rats narrating the immunomodulatory potential of ZO comparable to ConA.

The MCH and MCHC levels of ZO treated rats were found to be on par with that of control. The levels in ConA treated rats shows significantly increase (p<0.05) difference from control rats explaining its immunomodulatory or stimulatory potential. The MCH levels of CsA + ZO treated rats were found to show significantly (p<0.001, p<0.01) increased than ZO and ConA rats narrating the immunostimulatory nature of ZO comparable to ConA. The levels of MCHC in CsA + ZO treated rats were found to show significantly (p<0.01, p<0.05) increase than ZO and ConA rats narrating the immunostimulatory nature of ZO comparable to ConA.

The comparative study of the effect of Cinnamaldehyde and ConA in gold fish and found increased level of RBC in ConA and Cinnamaldehyde treated fish, [15] our findings show similar results.

The levels of total protein, Immunoglobulin and haemoglobin in control and other treated rats were represented in Figure 4. The study revealed statistically increased (p<0.05) levels of total protein in ZO treated rats when compared to control rats. The TP levels in ConA treated groups were found to be statistically moderately increased (p<0.01) when compared to ZO levels. The TP levels in CsA + ZO treated rats were found to show significantly increased level (P<0.001) from ZO treated rats, narrating the immuostimulatory property of ZO.

There is significant increase (p<0.05) in immunoglobulin (Ig) levels of ZO treated rats when compared to control rats. The Ig levels in ConA treated groups were found to be similar -(P<0.01) with ZO treated rats. The Ig levels in ConA + ZO treated rats were found to show significantly decreased (P<0.001) from ZO treated rats, explaining the immunostimulatory nature.

There is significant increase (p<0.05) in haemoglobin (Hb) levels of ZO treated rats when compared to control rats. The Hb levels in ConA treated groups were found to be statistically increased (p<0.05) when compared to ZO levels. The Hb levels in ConA + ZO treated rats were found to show significantly moderate decreased (P<0.01) from ZO treated rats explaining the immunostimulatory nature of ZO and significant difference (p<0.05) from Control.

The total protein levels were increased in *Clarius batrachus fishes* when administered with a high dose of ocimum basilicum extract, indicating the immunomodulatory effect of ocimum basilicum linn [14].

The haematological analysis revealed the immunomodulatory nature of ZO comparable with ConA. Further the respiratory burst activity was performed to analyse, how far the innate immune system of animals was altered by the compounds.

Figure 4 represents the Respiratory burst levels of Control, Olive oil, ZO, ConA and CsA + ZO treated animals. The ZO treated groups showed significantly (p<0.01) elevated activity when compared to control animals, and showed slightly significant (p<0.05) variation from ConA adding to the immunostimulatory credential. ZO + CsA treated group animals showed significantly decreased findings with ZO and ConA treated group (p<0.001 and p<0.01 respectively).

Generally, immunostimulants increse the non-specific immunity by activating the respiratory burst activity, a good indicator of immunity, hence increased respiratory burst activity in zingerone fed rat, stipulate that it can act as an immunostimulant similar results were reported by other scientist. [16].

TABLE: 1 Mean corpuscular volume (MCV), Mean cell haemoglobin (MCH) and Mean cell haemoglobin concentration (MCHC) levels in control and other treated animals.

Parameters	Control	olive oil	Zingerone	Con A	CsA+Zingerone
MCV (fl)	60.43 ± 0.75	60.30 ± 0.48	62.05±0.6 ^{a*}	61.77±1.6 ^{b*}	63.32±0.8 $^{c*, d**}$
MCH (pg)	20.83±1.4	22.70±1.9	20.53±0.9 $^{a\ ns}$	21.43±1.8 $^{b\ *}$	23.07±0.76 $^{c***, d**}$
MCHC (g/dl)	34.52±1.2	36.27±0.98	34.15±1.4 $^{a\ ns}$	35.05±0.78 $^{b\ *}$	36.30±1.24 $^{c**, d*}$

Comparisons were made between a- control vs zingerone; b - zingerone vs Con A; c - Zingerone vs CsA + Zingerone; d-Control vs CsA +Zingerone. Results are expressed as mean ± SEM (n=6), ns –non significant *(p<0.05) ** (p<0.01) *** (p<0.001) were considered to be statistically significant. One-way ANOVA followed by student's t test using SPSS21 software package.

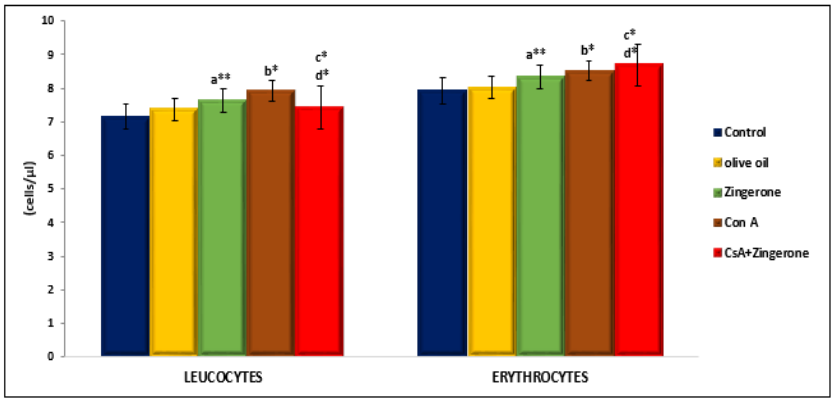

Figure 1: Total Erythrocyte and leucocyte count in control and other treated rats.

Comparisons were made between a - control vs zingerone; b - zingerone vs Con A; c - zingerone vs CsA +Zingerone; d - Control Vs CsA +Zingerone. Results are expressed as mean ± SEM (n=6), *(p<0.05) ** (p<0.01) were considered to be statistically significant. One-way ANOVA followed by student's t test using SPSS21 software package.

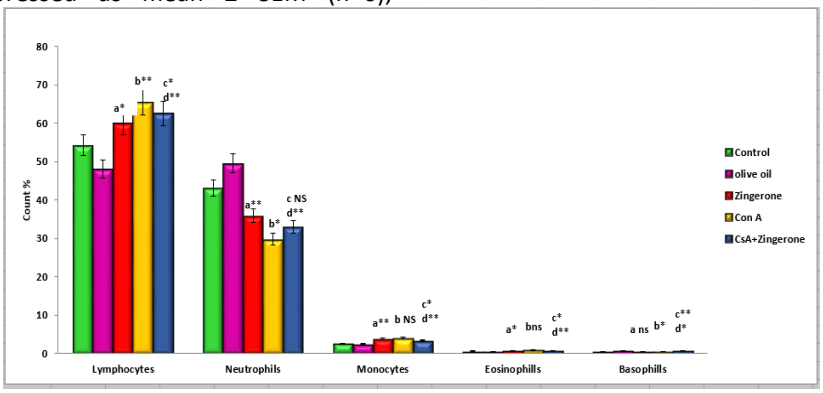

Figure 2: Differential count analysis of control and other treated rats.

Comparisons were made between a- control vs zingerone; b - zingerone vs Con A; c - Zingerone vs CsA +Zingerone; d - Control vs CsA +Zingerone. Results are expressed as mean ± SEM (n=6), ns-non significant *(p<0.05) ** (p<0.01) *** (p<0.001) were considered to be statistically significant. One-way ANOVA followed by student's t test using SPSS21 software package.

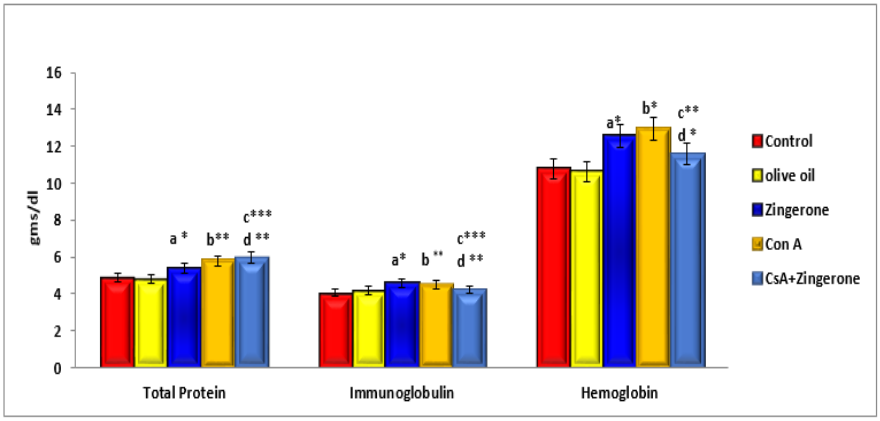

Figure 3: Total Protein, immunoglobulin and haemoglobin levels

Comparisons were made between a- control vs zingerone; b - zingerone vs Con A; c - Zingerone vs CsA + Zingerone; d - Control Vs CsA + Zingerone. Results are expressed as mean ± SEM (n=6), *(p<0.05) ** (p<0.01) *** (p<0.001) were considered to be statistically significant. One-way ANOVA followed by student's t test using SPSS21 software package.

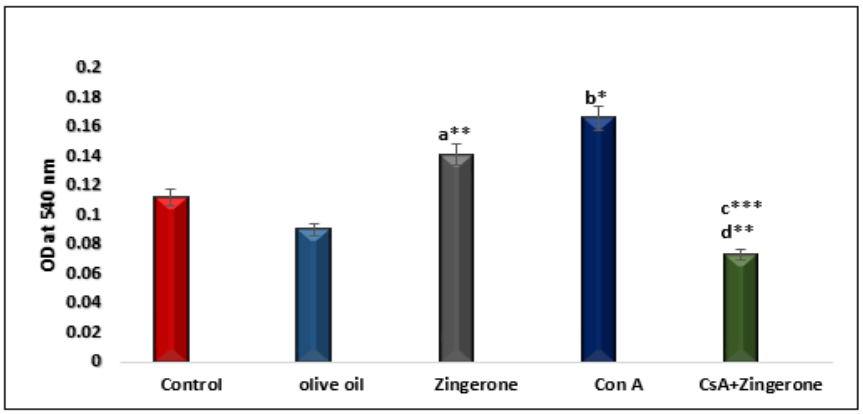

Figure 4: Respiratory burst activity in control and other treated rats.

Comparisons were made between a- control vs zingerone; b - zingerone vs Con A; c - Zingerone vs CsA + Zingerone; d - Control Vs CsA + Zingerone. Results are expressed as mean ± SE (n=6), *(p<0.05) ** (p<0.01) *** (p<0.001) were considered to be statistically significant. One-way ANOVA followed by student's t test using SPSS21 software package.

CONCLUSION

The present hematological studies prove the immunostimualtory effect of zingerone. The immuological studies authenticate that zingerone can be used to improve general immune status of the individuals. Further, molecular mechanism based studies may throw more light on the effective designing of zingerone as the common immunostimulant.

CONFLICT OF INTEREST

The authors of this manuscript have no conflict of interest.

REFERENCES

1. Kehili Houssem Eddine, Sakina Zerizer, ZahiaKabouche. (2014). Immunostimulatory Activity of Phoenix Dactylifera. International Journal of Pharmacy and Pharmaceutical Sciences ISSN- 0975-1491.Vol 6, Issue 3.

2. Upadhaya SN (2013) Therapeutic Potential of Immunomodulatory Agents from Plant products. In Benmebarek A, Zerizer S, Laggoune S and Kabouche Z Immunostimulatory activity of Stachysmialhesi de Noé. Allergy, Asthma & Clinical Immunology; 9 (2): 1-4.
3. Abdullah. G. Al-Kushi1, Mohamed El-Sayed El-Boshy2,5, Naser A. ElSawy, Osama A. Shaikh Omar, Eslam A (2013). Header. Pathological Comparative Studies on Aqueous and Ethanolic Extracts of Zingiberofficinale on Antioxidants and Hypolipidemic Effects in Rats. Life Science Journal10 (2).
4. YuvaBellik, LaïdBoukra, Hasan A. Alzahrani, Balkees A. Bakhotmah, FatihaAbdellah, Si M. Hammoudi 1 and MokraneIguer-Ouada (2013). Molecular Mechanism Underlying Anti-Inflammatory and Anti-Allergic Activities of Phytochemicals: An Update. Molecules, 18, 322-353; oi: 10.3390/molecules18010322. ISSN 1420-3049.
5. Subodh Kumar, Kiran Saxena, Uday N. Singh, Ravi Saxena (2013). Anti-inflammatory action of ginger: A critical review in anemia of inflammation and its future aspects. ISSN 2321-2187. IJHM 2013; 1 (4): 16-20AkiNik Publications
6. Duke, M. J. Bogenschutz-Godwin, J. duCellier, and P. A. K. Duke, editors (2002). Handbook of medicinal herbs, 2nd edition. CRC Press, Boca Raton, Florida.
7. Ahmed elkirdasy, saad shousha, abdulmohsen h. Alrohaimi and m. Faiz arshad., (2015) Hematological and immune biochemical study of Green tea and ginger extracts in experimentally induced Diabetic rabbits., Acta poloniae pharmaceutica n drug research, vol. 72 no. 3 pp. 497-506.
8. Brinker F. Herb (1998) Contraindications and drug interactions. Edn 2. Sandy, OR: Eclectic Medical.
9. Arul jothi. M, Parameswari, C.S, Vincent. S (2016). Hematological Studies on the Effect of Zingerone on Steptozotocin Induced Diabetic Rats. International Journal of Pharmaceutical Research and Bioscience. ISSN 2277-8713 Volume 5(2):21-26.
10. Brown KE, Brunt EM, Heinecke JW, (2001) Immunohistochemical detection of myeloperoxidase and its oxidation products in kupffer cells of human liver., American Journal of Pathology., 159:2081-2088.
11. Mohammed M Safhi (2015) Zingerone protects the Tellurium toxicity in the Brain Mitochondria of Rats. Metabolomics, 5-4 do:10.4172/2153-0769.1000156.
12. Pratap mannem., (2014) lead toxicity on hematological changes and amelioration with ginger (*zingiber officinale*) extract in male albino rats., International Journal of advanced research, volume 2, issue 4, 23-28.
13. Syahida, M., Maskat, M. Y., Suri, R., Mamot, S. and Hadijah, H., *(2012)* Soursop *(Anona muricata* L.): Blood hematology and serum biochemistry of Sprague-Dawley rats., *International Food Research Journal 19 (3): 955-959*
14. Gayatri nahak and Rajani kanta sahu. (2014), Immunomodulatory activity of aqueous leaf extract of ocimum basilicum linn in clarias batrachus., International Journal of Pharmacy and Pharmaceutical sciences., vol 6, issue 6.
15. Parameswari. C.S., Dayana Janakiraman and Usha (2016). Comparative study of the effect of Cinnamaldehyde and Concanavallin A on the Hematological parameters and marker enzymes in Gold Fish. European Journal of pharmaceutical and Medical Research.ISSN 3294-3211 Volume 3(3):293-298.
16. Yueh ping chang, Chun Hung Liu, Chih Chung Wu, Chi Ming Chiang (2012). Dietary administration of zingerone to enhance growth, non-specific immune response and resistance to Vibrio alginolyticus in pacific white shrimp (Litopenaeus vannamei) juveniles. Fish and Shellfish immunology 32(2012) 284-290.