Effect of black tea extract on some immunological aspects

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Abstract

Camellia sinesis is one of the most important plants were used in the life .The important of tea plant varies with its used , antioxidant ,antibacterial ,lowering of plasma cholesterol levels and activation of leucocytes.

Oraly and injection method were used for to detect the immunological activity in rabbit . Animals were given 5 ml of extract orally and 0.5 ml of extract mixed with 0.5 ml of oil adjuvant by injection .

The effect of tea extract varies in according to method at $P \ge 0.05$. The effect was shown specific mucosal and systemic antibodies at higher titers ,32 ,16 in mucosal and 320,160 in systemic .Significant variable in orally methods than injection at $P \ge 0.05$. Lymphocyte inhibitor factor (LIF) was stimulated in systemic and mucosal by tea extract ,there some variable between orally and injection method. Total protein was increased compared with control ,while mucosal protein was decreased compared with control ,so the comparative between two methods are variable at $P \ge 0.05$.

الخلاصة

استخدمت في هذه الدراسة طريقتان لمعرفة تاثير مستخلص الشاي الاسود في بعض المعايير المناعية في الارنب ، التجريع عن طريق الفم والحقن في الوريد لمقارنة تاثيره

جرع الحيوان 5 مل من المستخلص فمويا كمجموعة اولى و 0.5 مل ممزوجة بـ0.5 مل مساعد مناعي كمجموعة ثانية. ا ختلف تاثير المستخلص باختلاف الطريقة و بمستوى احتمالية 0.05ع ضدية عالية موضعيا 32 و16 وجهازيا 320 و160 وكانت طريقة التجريع الفموي معنويا اكثر مقارنة بالحقن . و حفز المستخلص هجرة الخلايا اللمفية موضعيا وجهازيا وقد تباين تاثيره بين الطريقتين.

كما زاد المستخلص تركيز البروتين الكلي وانخفض تركيز البروتين المحاطي مقارنة بالسيطرة وكانت النتائج متباينة بالمقارنة بين الطريقتين.

Introduction

Since ancient times plants, have been an exemplary source of medicine. Many literature mention the use of plants in treatment of various human aliment .There are many plants which are having immunostimultory whereas other have immunosuppressant activity (1).

The tea plant *Camellia sinesis*, family, thecae is a perennial ever green plant that is a semi tree or shrub depending on the environment .The mild stimulant effects and sense of wellbeing produced by tea have been attributed to it's caffeine content (2) .However ,many of the pharmacological activity reported for tea extracts have been found to be due to the polyphenol content (3; 4).Some of the interesting medicinal effects of tea constituents include of leukocyte (3) antioxidant (4) and anti mutagenic (5) Activities lowering of plasma cholesterol levels (6) ,protection from the effect of radiation (7) and inhibition of angiotensin converting enzyme .Tea extract have been shown to have several useful antimicrobial effects (8)

Materials and Methods

Tea extract

1gm of tea leaves mixed with 10 ml of boiling distal water for 1hr in water bath . Aqueous extract were then filtered and cooled at room temperature (9).

Animals

Rabbits were used as experimental animals . Healthy Newzland rabbit (*Orcyctalagus cuninculus*) about 1-15 Kg. They hands at room temperature in labium condition during experimental condition .

Immunization program

A animals were divided into 4 groups, 5 animals for each group. First group were given 5ml of tea extract orally and 5 animal were given 5 ml of distal water as normal control daily for three weeks ,while second group 5 animals were given 0.5 ml of tea extract and mixed with 0.5 ml of Freund's adjuvant through injection subcutaneous only in multisided weekly for three weeks (10), normal saline for control.

Blood samples

5 ml of blood was collected from each rabbits by using sterile disposable syringes from heart , 3ml was put into AFMA disposable tubes without anticoagulant , then the serum was collected after centrifugation at 2500 rpm for 5 minutes and it was stored at-20C , other 2ml of blood was put in AFMA disposable to be with anticoagulant for LIF test and mucosal extract . The groups of animal were killed and biopsies of appendix were opened by clean scissor and laid in clean Petri dishes and the mucosa were scraped with 10 ml formal saline and laid in clean tubes centrifuged at 3500 rpm / 30 minute pellet was used in LIF test , the supernatant was collected and equal volume of PEG 6% were added to supernatant and leave 30 minute to room temperature supernatant was remove and 1 ml of saline added to pellet to form mucosal immunoglobulin.

Immune function tests

Tube agglutination , total and immunoglobulin protein and LIF test was done as in

(11, 12).

Statistical analysis

Statistical analysis was done depending on (13).

Results

The rabbits were immunoprimed with tea extract was showed specific mucosal and systemic antibodies . Systemic titer was higher 320 in groups that orally administered and this titer was appeared in 4 rabbits while the titer 160 present in one rabbit in the same group . Mucosal antibodies was appeared titers 32 in three animals in orally administered groups and 16 titer two animal (table 1) ,while in normal (control groups) there no titers were present . Compared between two methods for administered were done by using T test , we found that significant variation between two methods at $p \ge .005$, orally administration method was show significant variable table (2).

The effect of tea extra on lymphocyte inhibition factor was shown significant stimulation compared with control no stimulation (table 3) . Injection method was shown significant than orally in systemic but orally method was significant than injection in mucosal table (3)

Total and immunoglobulin protein was increased in two methods so orally method was show significant than injection table (4).

Table(1): Specific mucosal and systemic antibodies in rabbits that administration tea
extract orally and injection

Animals groups orally administration					
Systemic	Mucosal				
Titer	Frequency	Titer	Frequency		
320	4	32	3		
160	1	16	2		
Animals groups / injected administration					
Systemic		Mu	icosal		
320	2	32	2		
160	3	16	3		

Table(2) :Compare between titers of two methods (systemic and mucosal) antibodies orally and injected

Systemic to systemic antibodies	Mean ± Standard deviations	P ≥.005
Orally	288.000 ± 71.5542	.001
Injection	224.000 ± 87.6356	.005
Mucosal to mucosal antibodies		
Orally	25.6000 ± 8.7636	.003
Injection	22.4000 ± 8.7636	.005

Table(3) :Comparative between LIF in test and control groups of animals

Animals groups /orally administration		Animals groups /injection administration			
Systemic					
Mean±.standard deviations	P ≥.005	Mean ± Standard deviations	P ≥.005		
Test .3440 ±.1426	.006	.4160 ±.1721	.006		
Control .9400 ± 8.944	.000	.9400 ± 8.944	.000		
Mucosal					
Test .5000 ±.1095	.000	.4540 ± 7/503	006		
Control .9200 ± 8.36	.000	.9200 ± 8.36	.000		

Animals groups /orally administration		Animals groups /orally administration			
Systemic					
Mean ±	Standard deviations	P ≥.005	Mean ± Standard deviations	P ≥.005	
Test	53.8892 ± 4.10	.000	33.9876 ± 17.05	.001	
Control	$31.0488 \pm .8036$.000	31.0488 ± .8035	.000	
Mucosal					
Test	$2.1638 \pm .6464$.002	3.7900 ± .8545	.001	
Control	3.755 2 ± .2932	.000	3.6370 ± .2934	.000	

 Table(4): Comparative of protein and immunoglobulin concentrations between test and control

Discussion

Tea polyphenol have been constituently shown to be the major constituents of tea leaves (8). However the xanthin , alkaloid , caffeine is also significantly present in tea and is said to be personable for the mild stimulant effect and sense of well being produced by tea(8; 2;14). In study the effect of tea extract on total leukocyte count , absolute neutrophil , monocyte ,and lymphocyte count of biologically stressed (becteria –injected) albino rats were show reduced infection – induced leucocytosis in the rats. It's currently desirable that the agent should be able to demonstrate ,confer an increase in non- specific resistant against biological , physical and chemical stressors (9;15). Some competent of tea leaves have been suppress inflammation such as polyphenol and tea pigments remain to be completely clarified but have been liked to inhibition of nuclear factor – kB (NF- kB) (16;17) . Activation of leukocyte in some of interesting medical effect of tea constituents , adaptogenic properties due to caffeine content (18).

Caffeine is also know to stimulate gastric secretion and has been incriminated in exacerbating duodenal ulcers (1;2; 19;3). However dosage, method, concentration and many factors may be affect in the variation of results, so in this study there were some clearance on effect of tea extract in some of immunologic parameter varied between stimulation and non stimulation.

References

- 1-Hamant and Yaseen ,(2007) .Immunomodulatory Plants :Aphytopharmacological Review . Pharmacognosy Reviews .Vol.1,issue 2 .
- 2-Mark ,V.(1992) .Physyological and immunological effect of tea .In: Tea cultivation and consumption .Chmpan and Hall, London ,p.707 .
- 3-Shakagami, H.; Asano, K.; Hera, Y.; Shinamura, T. (1992). Stimulati on of human monocyte and polymorphonuclear iodination and interleukin -1 production by epigallocatachi,J. Leucocyt Boil. 54: 478-483.
- 4- Hayatsa,H. ;Inada ,N. ;Kakutani,T.;Arimota ,S. ;Negishi ,T. ;Mory , ;(1992). Suppression of genotoxicity of carcinogenes byepi(.)epigallocatechin3-0-gallate.Prev.Med .21:370-376
- 5-Xu,Y;HO,CT;Amin,SG,Han C;Chung,FL(1992). Inhibition of tobacco-specific nitrosamineinduced tung tumorigenesis in A/J mice by green tea and its major polyphenol as antioxidants ,. Cancer Res.52:3875-3879.

- 6-Ikeda, I., Imasato, Y.; Sasaki, E.; Nakay, M.; Nagno,H.; (1992). Tea catechins decrease micellar solubility and intestine absorbition of cholesterol in rats. Biochem Biophys. Acta 1127:141-145.
- 7- Uchida,S.;Ozaki,M.;Suzuki hikIta,M.(1992).Radioprotective , effects of (.)epigallocatechin3-0-gallate in mice Life.Sol .50:147-152.
- 8-Hamilton Miller, (1995): Antimicrobial properties often. Antimicrob. Chemotherapy 39 (11): 2375-2377.
- 9-Charles, O.; Michael, V.; Chukwuemeka, S.Damian,C.(2007). Adaptogenic potentials of *Camellia sinesis* leave,*Garcinia kola* and *Kola nitida* seed,.Scientific ReseaEssay.2(7):232-237
- 10-AL- Thahab , A. A. (2006). Cryptic Meningitis and Some Aspects of its Local and Systemic Immunity , Ph. D. theses . Babylon , University .
- 11- Bishop, MC; Dben-con, J.; Fody Ep and et al (1985). Clinical chemistry principles, procedure and correlation, pp.189-182. The Murry printing company Philadelphia.
- 12-Garvey, JS.; Cremev, NE. and Sussdrof, DH. (1977). Method in immunology 3th ed. PP53-267. Addison-Wesley publishing company Inc., Reading
- Dawed , K. M. and Al- Yas, ZA, (1990). Statistical method in agricultural researches. PP. 430-446. Al- Mous. Univ. (In Arabic).
- 14- Misra,A.; Chahopadhyay R.; Baneerjee S.;Chattopadhya ,D.O. ;Chatterijee,I.B
 .(2003).Black tea , prevents cigarette smoke indused oxidative damage of proteins in guines pi.J.Nutr.133:2622-2628
- 15-Kumax,A.;Dhawan,S.; Hardegen ,NJ.;Agganwal BB.;(1998). Curcumin inhibition of tumor necrosi factor mediated adhesion of monocytes to endothelial cellsby suppression of cell surface expression of adhesion molecules and of nuclear factor-kappa B activation . Biochem pharmacol 55:775-783.
- 16-Lin Y-L.;Tsai,S-H;Lin Shiau,S-Y;Hoc- T;Li(1999).Theaflarin3,3 digallate form black tea block the nitric otide synthase by down – regulation the activation of NF-KB.macrophage . Eue. J. Pharmacol, 379: -388.
- 17-Michiyo, T.; kara, I.; Zi-Jian, x., and Thoma, J.(2002). Tea pigments inhibit the production of type (TH1) and type 2(TH2) helper T cell cytokine in CD4Tcell, Phytotherapy Research. 16,36-421 Research. 16,36-421.
- 18-Ahmed N.;Mukhtar,H.(1999).Green tea polyphenol and cancer, biologyicmechanism and practice implications. Nutr. Rev, 57:78-83.
- 19- Sanbongi ,C;Suzuki ,N;Sakane ,T(1997).Polyphenols in choclate ,hhich have antioxidant activity,modulate immune function in humans in vitro .Cell Immunol. 177:129-135.