

The effect of green tea and *Hibiscus sabdruffira* aqueous extract on some immunologic aspect in lab animals

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الخلاصة

في مقارنة لدراسة تأثير المستخلص المائي لنبات الشاي الأخضر والكجرات في بعض المعايير المناعية في الحيوان المختبري جرع الحيوان المختبري به مل من المستخلص المائي فمويًا لمدة ٣ أسابيع , وفي الاسبوع الخامس تم قتل الحيوانات وتشريحها للحصول على اجزاء من الجهاز الهضمي للدراسة الموضوعية ولا اجراء بعض الاختبارات المناعية ومن النتائج اظهر مستخلص الشاي معنويًا أكثر مقارنة بمستخلص الكجرات في تحفيز الضد أجهازي والموضعي تحت مستوى احتمالية $P \geq 0.005$. واطهر نبات الكجرات زيادة في تركيز البروتين الكلي والمناعي , وحفزت النباتات مناعة خلوية متمثلاً باختبار تثبيط هجرة الخلايا البيض . في الموضع وغير تحفيزي في الجهاز ي .

Abstract

In this study compare between the effect of aqueous extract of green tea and *Hibiscus sabdroffira* on some immunological parameters in vivo . Five ml of watery extract of these was given in animal orally for three weeks after five weeks animals were killed and pieces of intestinal tract was collected to study local immunity .Green tea extract was shown higher significant at $P \geq 0.005$ in stimulation systemic and mucosal antibodies than *Hibiscus* extract . *Hibiscus sabdroffira* was shown increase in total protein and secretory immunoglobulins .Two plants were show effect on cellular immunity by increasing LIF mucosally but not systemically.

Introduction

Natural products play an important role in the field of new drugs researches and development . Since ancient times plants have been an exemplary source of medicine . In the last years there has been significant increase in the use of natural products in health care and there potential application in agriculture , pharmaceutical and food industry are being investigated . Many literature mention the use of plants in treatment of various human aliment, so there are many plants which are having immunostimulatory whereas other have immunosuppressant activity (1).

Plants extracts are potentially curative , some of these extracts can boost the humoral and cell mediated immunity against bacteria, virus ,fungi , parasite (2) Some medicinal plants have been claimed to posses immunomodulatory activity (3). *Hibiscus sabdroffira* linn (Family Malvaceae) is a herb that is cultivated for leaf , fleshy calyx ,seed or fiber . It is taken as a common local drink .

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It is medicinal herb used in treatment hypertension , cardio protective , antiseptic , aphrodisiac , astringent , diuretic and digestive (4,5) . Green produced from the leaves of *Camellia sinensis* plant , it is one of the most widely consumed beverages in the world . It contains several polyphenolic compounds , including EGCG – (-) – epigallocatechin (EGC) , epicatechin – 3- gallate (ECC) AND (-) - epicatechin EC -. Green tea has a beneficial effect on healthy , including a chemopreventive efficacy against various types of cancer (6,7) . In addition green tea has been shown to exert both antiangiogenic , antimutagen and anti-inflammatory (8) The aim of this study to compare between more popular two plants were used in many countries on some immunologic aspect in lab animals .

Materials and Methods

Animals

Rabbits were used as experimental animals . Healthy white Newzland rabbit (*Oryctolagus cuniculus*) about 1-15 kg. They housed at room temperature in labium condition during experimental condition

Tea extract

1gm of tea leaves and calyx *Hibiscus* with 10 ml of boiling tap water for 1hr at water bath . Aqueous extract were then filtered and cooled at room temperature (9).

Immunization program

Animals were divided into 3 groups , 5 animals for each group. Each group were given 5 ml of plant extract orally and 5 ml of tap water as normal control daily for three weeks ,

Blood samples

Five ml of blood was collected from each rabbits by using sterile disposable syringes from heart , 3 ml 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 tube with anticoagulant for LIF.

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 (10,11,12).

Statistical analysis

All data obtained were subjected to student s' t-test analysis . Data were expressed as mean standard deviation .Values of $P \geq 0.005$ were considered significant (13) .

Results

Humoral

The rabbits were immunoprimered with plants extract showed specific mucosal and systemic antibodies . Systemic antibodies was higher in two extracts 640(3 times) . 320(one times) in green tea extract and 320(4 times) ,160(one times) in *Hibiscus* extract . Mucosal antibodies appeared 64(4 times) ,32(one times) in tea extract and 32(3 times) ,16(one times) in hibiscus extract , table (1) .Comparison between two plants extract was done by using T test , we found that variable in significant , tea extract higher significant than *Hibiscus* extract in p.05 ,table (2) . The effect of two plants extract on total and immunoglobulin protein by using Biurate method compare with control . *Hibiscus* extract showed higher significant and with tea extract is significant too, table(3)

Cellular

Two plant extract was shown to stimulate LIF test compare with control ,table (4).

Green tea was 0.42 while *H. sabdraffira* was 0.40 in mucosal but not stimulate in systemic .

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

Groups	systemic		mucosal	
	Titer	Frequency	Titer	Frequency
Green tea	640	4	64	4
	320	1	32	1
<i>H. sabdraffira</i>	320	4	32	4
	160	1	16	1
<i>control</i>	10	5	1	5

Table(2) : Titer antibodiess of green tea and *Hibiscus* extract in animals

Groups	Systemic		Mucosal	
	Mean ± Standar deviations	P ≥ 0.005	Mean ± Standard deviations	P ≥ 0.005
Green tea	560.000±16.000	0.006	56.000±16.000	0.006
<i>H.sabdraffira</i>	280.000±8.000	0.006	26.000±8.000	0.006

Table(3): Comparative of protein and immunoglobulin concentrations of tea and *Hibiscus* extract with control

Groups	Total protein mg/ml		Immunoglobulin concentrations mg/ml	
	Mean ± Standar deviations	P ≥0.005	Mean ± Standard deviations	P ≥0.005
Green tea	12.208 ± 5.999	0.02	2.91 ±0 .4849	0.001
<i>H.sabdraffira</i>	.234 ± 7.1712	0.001	4.4933 ± 17.927	0.000
<i>control</i>	30.78 ± 0.0447	0.000	3.677±0 .2543	0.000

Table(4 :Comparative in LIF between plants extract and control

Animals groups				
Systemic			Mucosal	
Mean±.standard deviations	P ≥0.005	Mean ± Standard deviations	P ≥0.005	
Green tea	0. 2250 ±0.01	0.003	0.425±0.04	0.003
<i>H. sabdroffira</i>	0 .2250 ±0.01	0.003	0.4000±0 .01	0.011
Control	0 .9250 ± 0.01	0.000	0.8500 ± 0.01	0.000

Discussion

For each plants have been a good source of food and the provide essential nutritional values , medicinal properties and notable physiological effect to life .Plants extract are potentially curative . Some of these extract can boost the humoral and cell mediated immunity against some kinds of microbes (2). In this study *Hibiscus* extract was stimulate humoral response demonstrated in high titers and increased in protein and tea extract was stimulate humoral response demonstrated by high titers but the protein is decrease .

Two plants extract were effect on cellular response demonstrated by LIF there are no migration for T cells compare with control in systemic while in local was significant . Two plants are contains some compound that proved effecting on immune system varies between stimulator or suppressor . Delphinidin-3- sabu bioside (anthocyanin),flavons,glycosides, polysaccharides , protocatechuic acid ,vitamin c and other substances , which are powerful antioxidant .The antioxidants prevent cells and tissue from oxidative damage , lipid peroxidation and also improve immunity response and productivity

against stress ,including challenges of disease (14) . *Hibiscus* plant act as antiseptic ,sedative astringent. cholagogue,. Some polyphenolic compound ,flavonoids ,theaflavin ,catechins act as anti-inflammatory ,antimutagenic ,antiproliferative and antineoplastic activity (15). This study proposed two plants may be act as B dependent cells because they were stimulate higher antibodies titers and also possessed immunomodulating effect .

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