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IN VITRO INACTIVATION OF GONADOTROPHINS BY PSIDIUM ROOT EXTRACT

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The crude extract of psidium root inactivates human chorionic gonadotrophin or pregnant mare serum *in vitro* tested in hypophysectomized immature male and female rats. After fractionation of the crude extract of psidium root by lead acetate, the portion preciptated by lead acetate has antigonadotrophic activity *in vitro*. This portion contains a large amount of tannic acid too. Since tannic acid does not have antigonadotrophic activity *in vitro* in our experimental condition, the active principle of antigonadotrophic action of psidium root seems not to be tannic acid.

Peng *et al.* reported[1] that the crude extract of psidium root could induce sex organ atrophy in male rats and diestrus in female rats by subcutaneous injections. Chang and Peng[2] observed decreased fructose content of prostate and decreased citrate content of seminal vesicles in male Rats injected with the crude extract of psidium root. However, local necrosis and induration of the injection sites, adrenal enlargement and thymus involution[1] which were observed in the animal injected with psidium root suggest the existence of stress in the animal.

Stress can induce atrophy of reproductive organs by decreased gonadotrophin secretion at the expense of increased ACTH secretion[3]. Therefore, it is difficult to judge whether the inhibitory action of psidium root on the reproductive organ is a specific action or a nonspecific action induced by stress due to local necrosis. In order to see whether or not psidium extract can inactivate gonadotrophins directly, human chorionic gonadotrophin (HCG) or pregnant mare serum (PMS) was incubated with psidium extract *in vitro* and then injected into hypophysectomized immature male and female rats.

MATERIALS AND METHODS

Extraction of psidium root: The root of psidium guajava was collected from the drug nursery of Taipei Medical College in Autumn of 1967. After drying and slicing the psidium root, it was extracted with hot 60% alcohol three times for about six hours each time. A brown amorphous powder was obtained after evaporating the extract under reduced pressure. The powder was stored in a desiccator and the solution was freshly prepared each time just before use.

Fractionation of psidium root extract: Three fractions of psidium root were prepared by means of a series of chemical processes as following Chart 1.

The yield of Fraction A, Fraction B and Fraction C was 5 g, 0.98 g and 8 g respectively Each fraction was stored as powder in a dessicator.

Experimental rats: Rats of an inbred Long-Evans strain at 28–32 days of age (Body weight: between 50–70 gm) were hypophysectomized by parapharyngeal approach. Rats showing body weight gain less than 7 gm during postoperative 14-day period, were used in this experiment. Hypophysectomized

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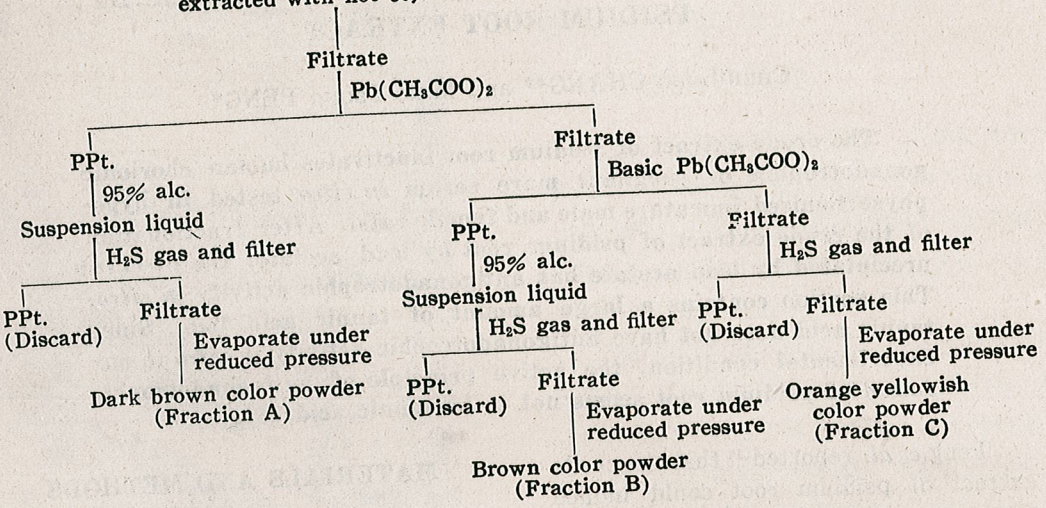
Chart 1. Dry psidium root (Total dry weight: 2115 g)
extracted with hot 60% alcohol



rats were housed in a temperature controlled room and were given Taitang Chicken food and 5% glucose solution *ad libitum*. The lighting schedule was 14-hour light and 10-hour darkness.

Estimation of antigonadotrophic activity: A solution of HCG (200 I. U./ml) or PMS (40 I. U./ml) (Sigma Chemical Company) were prepared and stored at 5°C. Half of above solution was added with the extract of psidium root or tannic acid (Mallinkrodt) in Expt. IV. The volume of the mixture was adjusted so that 100 I. U. of HCG and 20 I. U. of PMS were contained in every 1 ml and pH of injection solutions was adjusted to 6.5.

It was incubated at 37°C for 2 hours before injections. A control HCG or control PMS solution was subjected to similar condition. Hypophysectomized immature rats received subcutaneous injections of the mixture of gonadotrophin with psidium root extract or control gonadotrophin solution daily for 10 days. Gonadotrophin and psidium extract were simultaneously injected into separate sites of body surface in Group B in Table 1. They were killed under ether anesthesia on the following day after the last injection. Reproductive organ such as testes, seminal vesicles, prostate, ovaries and uterus were removed and weighed. The data were tested by analysis of variance and Duncan's multiple range test[4].

RESULTS

I. Inactivation of HCG by crude psidium root extract

As shown in Group A in Table 1, the activity of HCG on seminal vesicles and prostate was inhibited by 3 mg and 5 mg of psidium root extract *in vitro*. As shown in Group B in Table 1, psidium extract can also inactivate the action of HCG on seminal vesicles and prostate when gonadotrophin and psidium extract were injected separately. pH value of psidium root extract is around 5. Although pH of the mixture of psidium root extract and HCG was adjusted to 6.5 before incubation, we checked the influence of pH value (5 vs 6.5) on HCG activity. As shown in Group C in Table 1, HCG activity showed no defference between pH 6.5 and pH 5. In contrast to the results in hypophysectomized rats, no inactivation of HCG by crude extract of psidium root was observed when it was tested in intact immature male rats (Group D in Table 1).

Table 1. Inactivation of HCG by crude extract of psidium root tested in hypophysectomized immature rats

Groups	Daily dose		No. of rats	Body weight (g)		Reproductive organ weight (mg)		
	HGG (I. U.)	Psidium root (mg)		Initial	Final	Testes	Seminal vesicle	Prostate
A. Hypophysectomized male rats								
1M	—	—	5	—	67	114± 17#	7± 2	6± 2
2M	100	—	10	60	62	282± 83	73± 8	57± 17
3M	100	5	6	62	64	185± 33	36± 16**	32± 9**
4M	100	3	3	64	71	239± 32	37± 5**	35± 4*
5M	100	0.2	3	64	70	250± 1	75± 9	52± 8
B. Hypophysectomized male rats (HCG and psidium root were injected into separate sites without incubation)								
6M	20	—	4	62	65	210± 56	78± 29	60± 20
7M	20	5	4	69	69	185± 44	35± 2*	24± 6*
C. Hypophysectomized male rats								
8M	100 pH 6.5	—	4		66	385±113	102± 6	49± 6
9M	100 pH 5	—	5		68	391± 15	106± 7	59± 20
D. Intact male rats								
10M	—	—	4		104	873± 68	24± 5	38± 10
11M	20	—	4	55	105	944±187	121± 37	76± 25
12M	20	10	3	53	82	899± 83	109± 25	87± 11
13M	20	5	6	57	96	865±155	88± 48	80± 28

Psidium root extract was mixed with HCG, adjusted to pH 6.5, and incubated for 2 hours at 37°C before subcutaneous injection in Group A and D.
M indicates males. # mean±standard error of the mean.
* and ** indicate statistically significant at the levels of 5% and 1% respectively as compared with the group injected with HCG alone.

II. Inactivation of PMS by crude psidium root extract

Table 2 shows that the activity of PMS on the testes, seminal vesicles and prostate was inhibited by 5 mg of crude psidium root extract and that of PMS on the uterus and ovaries was inhibited by 1 mg of crude psidium root extract. The action of HCG on the testes was not inhibited by psidium root extract *in vitro* as shown in Table 1, but the action of PMS on the testes was inhibited by psidium root extract *in vitro*.

III. Inactivation of HCG and PMS by fractionated pridium root extract

As shown in Table 3 and 4, the fraction precipitated by lead acetate of psidium root extract inhibited the action of HCG and PMS on the seminal vesicle, prostate, uterus and ovaries *in vitro*. As in the crude extract, fraction A inhibited the action of PMS on the testes, but not that of HCG. Other fractions of psidium root extract did not have antigonadotropic activity.

IV. Effect of tannic acid on HCG and PMS *in vitro*

Because fraction A of psidium root extract contains a large amount of tannic acid, it is worthwhile to test

Table 2. Inactivation of PMS by crude extract of psidium root *in vitro* tested in hypophysectomized immature rate

Groups	Daily dose		No. of rats	Body weight (g)		Reproductive organ weight (mg)		
	PMS (I.U.)	Psidium root (mg)		Initial	Final	Testes or Ovaries	Seminal vesicles	Prostate or uterus
1M	50	—	7	66	73	570±190#	121± 40	61± 16
2M	50	5	4	62	68	315± 34**	91± 15*	37± 8*
3F	—	—	3		56	3± 1		17± 4
4F	20	—	4	54	55	73± 43		106± 16
5F	20	1	5	57	58	6± 1**		18± 3**

Psidium root extract was mixed with PMS, adjusted to pH 6.5 and incubated for 2 hours at 37°C before subcutaneous injections. M and F indicate males and females respectively.

#: Mean±standard error of the mean.

* and ** indicate statistlcally significant at the levels of 5% and 1% respectively as compared with the group injected PMS alone.

Table 3. Inactivation of HCG by fractionated extract of psidium root *in vitro* tested in hypophysectomized immature rats

Groups	Daily dose		No. of rats	Body weignt (g)		Reproductive organ weight (mg)		
	HCG (I.U.)	Fraction of psidium root (mg)		Initial	Final	Testes or Ovaries	Seminal vesicles	Prostate or Uterus
1M	100	—	5	61	64	334± 63#	96± 7	56± 17
2M	100	(F_A) 3	5	63	66	317± 66	34± 9**	27± 6**
3M	100	(F_B) 3	5	62	65	372±104	67± 25	52± 10
4M	100	(F_C) 3	5	60	64	287± 37	93± 3	54± 6
5F	100	—	4	57	58	10± 1		111± 11
6F	100	(F_A) 3	4	56	59	6± 1**		60±15**
7F	100	(F_B) 3	3	62	64	9± 1		108± 15
8F	100	(F_C) 3	3	58	60	9± 1		104± 20

Fractionated psidium root extract was mixed with HCG, adjusted to pH 6.5 and incubated for 2 hours at 37°C before subcutaneous injection. M and F indicate males and females respectively.

#: Mean±standard error of the mean.

** indicates statistically significant at the level of 1% as compared with the group injected HCG alone.

whether or not the antigonadotrophic effect of fraction A of psidium root extract is due to the action of tannic acid. As shown in Table 5, 3 mg of tannic acid did not inactivate HCG nor PMS tested on hypophysectomized immature male and female rats.

DISCUSSION

Psidium root extract showed the ability to inactivate HCG and PMS when mixed *in vitro*. Inactivation of HCG by crude psidium root extract was observed when the mixture of HCG

Table 4. Inactivation of PMS by fractionated extract of psidium root tested *in vitro* in hypophysectomized immature rats

Groups	Daily dose		No. of rats	Body weight (g)		Reproductive organ weight (mg)		
	PMS (I. U.)	Fraction of psidium root (mg)		Initial	Final	Testes or Ovaries	Seminal vesicles	Prostate or Uterus
1M	20	—	4	58	63	569±118#	136± 4	113± 24
2M	20	(F_A) 3	4	55	60	209± 20**	35± 10**	27± 10**
3M	20	(F_B) 3	4	61	63	485± 76	108± 16	95± 22
4M	20	(F_C) 3	3	56	57	513± 25	113± 11	106± 14
5F	20	—	4	55	60	135± 46		16± 32
6F	20	(F_A) 3	4	57	58	16± 10**		72± 53**
7F	20	(F_B) 3	3	60	63	113± 8		167± 26
8F	20	(F_C) 3	3	54	57	119± 24		158± 37

Fractionated psidium root extract was mixed with PMS, adjusted to pH 6.5 and incubated for 2 hrs at 37°C before subcutaneous injection.
M and F indicate males and females respectively.
#: Mean±standard error of the mean.
* and ** indicate statistically significant at the levels of 5% and 1% respec-tively as compared with the group injected PMS alone.

Table 5. The effect of tannic acid on HCG and PMS tested *in vitro* in hypophysectomized immature rats

Groups	Daily dose		No. of rats	Body weight (g)		Reporductive organ weight (mg)		
	Gonadotrophin (I. U.)	Tannic Acid (mg)		Initial	Final	Testes or Ovaries	Seminal vesicles	Prostate or Uterus
1M	(PMS) 20	—	5	65	67	527± 38#	138± 16	106± 12
2M	(PMS) 20	3	4	63	64	538± 26	128± 24	108± 19
3F	(PMS 20	—	4	59	60	124± 18		157± 18
4F	(PMS) 20	3	4	58	61	127± 24		162± 20
5F	(HCG) 100	—	5	61	64	10± 2		96± 10
6F	(HCG) 100	3	5	61	62	10± 2		104± 11

Tannic acid was mixed with PMS or HCG, adjusted to pH 6.5 and incubated for 2 hrs at 37°C before subcutaneous injection.
M and F indicate males and females respectively.
#: Mean±standard error of the mean.

and the extract was tested in hypophysectomized immature rats but no inactivation was noted when it was tested in intact immature rats. It seems that the pituitary of intact animals secreted gonadotrophins after the injection of the mixture and masked the inhibitory action of psidium root. Because psidium root extract could inactivate HCG even when injected at separate sites, we may exclude the possibility that the inhibitory action of psidium root extract on HCG is due to delayed absorption of HCG caused by psidium root extract. Therefore the atrophy of reproductive organs induced by the psidium root extract injection which was noted in the previous study[1] is probably due to direct gonadotrophin inactivating action of psidium root

extract in the blood stream or at the target organs. Because crude psidium root extract and Fraction A inhibited the action of PMS on the testes but not that of HCG, the inactivating effect of psidium root seems to be more potent on PMS than on HCG. We, however, can not exclude the possibility that PMS has more potent action on the testes than HCG, and the inhibitory action of psidium toot extract is easier to reveal when it is mixed with PMS than when mixed with HCG.

After fractionation of psidium root extract, the antigonadotrophic action was found in Fraction A which also contains a large amount of tannin[6]. Skelton and Grant[3] suggested that the active principle of Lithospermum which is used by American Indians as a contraceptive and has inhibitory action on the estrous cycle and temporary sterility action and can induce atrophy of sex organs in mice and rats[5,7], is a tannin-like substance. Tannin has augmentative action on pituitary powder[8] and HCG[9] but not on PMS[10] by delaying absorption of gonadotrophin, but inhibitory action of gonadotrophins was also reported[11]. We could observe neither augmentative nor inhibitory action on HCG and PMS in 3 mg of tannic acid which is the same dose as fractionated psidium root extract. Because the exact amount of tannic acid that existed in tannated gonadotrophin injected to animals in the previous studies[9-11] was not indicated, and the experimental conditions of the previous studies differ from those in our study, it is difficult to compare the present results with those reported before. Although the antigonadotropic action of Fraction A of psidium root can not be attributed to tannic acid, we can not exclude the possibility that tannic acid may participate in the activity of psidium root through a subordinate role, unless we can separate tannic acid from the antigonadotropic principle of psidium root.

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REFERENCES

(1) PENG, M. T., LEE, H. C. and LIN, H. S.: Tohoku J. Exp. Med., **62**; 287-297, 1955.
(2) CHANG, C. and PENG, M. T.: Ibid., **62**; 299-302, 1955.
(3) SKELTON, F. R. and GRAND, G. A.: Am. J. Physiol., **176**; 379-385, 1951.
(4) STEEL, R. G. D. and TOORIE, J. H.: Principles and procedures of statistics., New York, McGraw-Hill Book Co., Inc. pp. 99-114, 1960.
(5) PLANKETT, E. R. and NOBLE, R. L.: Endocrinology, **49**; 1-7, 1951.
(6) WEHMER, C.: Die Pflanzenstoffe, Jena, Fisher, **2**; 829, 1931.
(7) CRANSTON, E. M.: J. Pharmacol. Exp. Therap., **83**; 130-142, 1945.
(8) Bischoff, F.: Am. J. Physiol., **114**; 483-487, 1936.
(9) ALBERT, A., BENNETT, D. and CARL, G.: Endocrinology, **76**; 506-513, 1965.
(10) ALBERT, A., BENNETT, D. and CARL, G.: Endocrinology, **76**; 499-505, 1665.
(11) HSUI, C. T., HUANG, C. Y., PAN, S. Y. and LAI, W. C.: J. Formosan Med. Assoc., **50**; 241-250, 1951.

蕃石榴根提煉物在試管內使促性腺激素失其活性

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