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P a cd D s a de ad a fs ec fic ce a a d da e es ses a a s ec b a ea sB a e

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ABSTRACT

T e dea ad a s f e a sB acc ess d be ca ab e f e c b s da ce a e es ses, es ec a T 1ce a dc cT c e (CTL) es ses. H e e , A sed as ad a s e e a sB acc esc e c e ca ed ffe s a d c ce ed a ed es se. T e ef de, a ess e c sa a c d D (PD) f e f *Platycodon grandiflorum* as bee ed f s de a as a a e a ead a . I de c ae ead a ac A , a e s ec fic ce a a d da e es ses e e e a a ed f a d a f a c a e a sBs face a e (HBsA) ad a ed PD a d A ce. T e C A , LPS , a d HBsA d ced s e c e fe a d a d e se. HBsA s ec fic I G, I G1, I G2a, a d I G2b a b d e s e HBsA ed ce e es fica e a ced b PD ($P<0.05$, $P<0.01$ $P<0.001$). PD a s s fica ed e d c f T 1 (IL 2 a d IFN γ) a d T 2 (IL 10) c es a d e a ed e RNA e ess f T 1 c es (IL 2 a d IFN γ) s e c es f e ce ed HBsA ($P<0.001$). Bes des, PD e a ab c eased e ac es f a a e (NK) ce s a d CTLs f s e c es e HBsA ed ce ($P<0.001$), c a a e a ca s f acc a a s e a sB s. T e es s d ca ed a PD ass e a c ease b ce a a d da e es ses a d e c a ba a ced T 1/T 2 es se a s HBsA , a d a PD a be e ca d da es as ad a s f se ac ca d e a e c e a sB acc e.

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1. d c

He a sB s (HBV) s a c d s. T b e a e bee fec ed HBV, a d 360 a e c c fec d d de [1]. O ce fec ed, 5 10% ad s a d 25 90% c d e bec e c c s ca e s [2]. Ab 25% f HBV ca e s d e f c c ac e e a s, c ss, a e ca ce [3]. C c fec HBV as bec e a a d ea b e d de [4]. N ea e s c e a a ab e f ac e He a sB, a d a acc e s e effec e ea s f e e HBV fec a d s c se e ces [1]. He a sB s face a e (HBsA) ads bed A s e c sed acc e a a s HBV, a d as bee sed f a ea s d ce ec a a s HBV fec . T e ec ed as a effec e acc e, ea 10% f acc ees HBsA fa de e ec e e e s f a HBsA a b d es [5]. T s, a ac es e acc a efficac a e ac e s .

I s e acce ed a s a b d es ses a e a f c ea e HBV a d ec a a s HBV fec . T e ea e s e c eas e de ces s a e T 1ce a d CTL es se HBV a d e ass c a ed a a c es (IFN γ, TNF α a d IL 2) de e ed a a a e e s es d a a fec [6,7]. O a f acc e effec e ess c d be ac e ed b e se f a a e ad a s c c d e a ce acc e c e a e f es de s [8]. C e , a c ds (A) a e e acc e ad a s ce sed b e F d a d D Ad s a (FDA) f se a s, a d sed as ad a s e e a sB acc es c e c e ca ed. W e A ss safe, sa e a e ea ad a a ca e sed s b a e s. M e e , e A ad a sed e e a sB acc e s a d T 2 ad a a ca effec e e a ce I G1 a b d es ses, b s a e ass c a ed T 1 e e es ses [9]. F e e, A s a s a ce ed a ed e es ses, a d a ac e b c ac a a d d ffe e a f CD8$^+$ CTLs f

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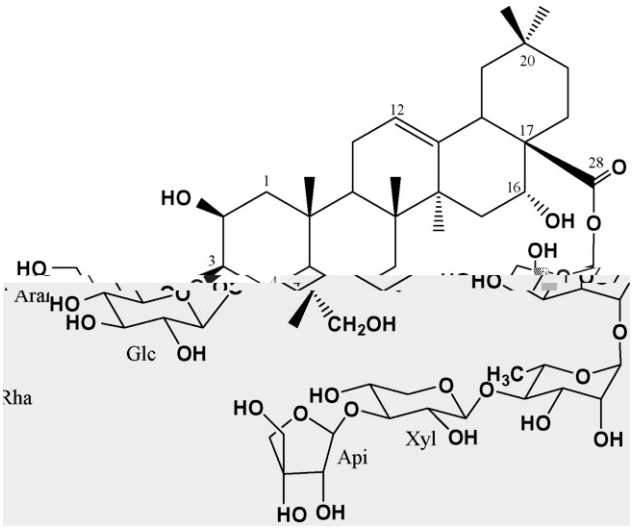


Fig. 1. C e ca s c e f a c d D (PD, $C_{57}H_{92}O_{28}$, MW: 1224.5854) f e s f *P. grandiflorum*. I s s c e as e c da ed e bass f c e ca a d s ec a e de ces c d ass s ec e a d c ea a e c es a ce s ec sc . A , β D a f a s ; A a, α L a ab a s ; G c, β D c a s ; R a, α L a a s ; X , β D a s .

a HBsA [10]. I add , A es s ccas a ca eac s a e s e f ec a d a e a

2.6. Splenocyte proliferation assay

S ee c ec ed f e HBsA ed ce de ase c c d s, Ha s ba a ced sa s (HBSS, S a), as ced s a a fsc ss s a d assed a fi es ee es b a a e e s ce s s e s , a d e e c es e e sed a c de (0.8%, /). Af e ce f a (380 × *g* a 4 °C f 10), e e ed ce s e e as ed ee es PBS, a d es s e ded c e e ed . Ce be s e e c ed a e c e b a b e d e e c s ec — e. Ce ab e ceeded 95%. S e c es e e seeded ee f s a 96 e fla b e c e a e (N c) a 5×10^6 ce s/ 100 µ c e e ed , e e af e C A (fi a c ce a 5 µ /), LPS (fi a c ce a 10 µ /), HBsA (fi a c ce a 4 µ /), ed e e added a fi a e f 200 µ . T e a es e e c ba ed a 37 °C a d a ks e e 5% CO_2. Af e 44 (f C A a d LPS) 68 (f HBsA), 50 µ f MTT s (2 /) as added eac e a d c ba ed f f e 4 . T e a es e e ce f ed (1400 × *g*, 5) a d e a sf ed MTT as e ed ca ef b e 150 µ f a DMSO (S a, USA) s (192 µ DMSO 8 µ 1N HC) as added eac e , a d e abs ba ce as e a a ed a ELISA eade a 570 af e 15 . T e s a de (SI) as ca c a ed based e f f a: SI = e abs ba ce a e f e c es d ded b e abs ba ce a e f s a ed c es.

2.7. Measurement of HBsAg-specific antibody

HBsA s ec fic I G, I G1, I G2a, a d I G2b a b d es se a e e de ec ed d d a se sa es b a d ec ELISA. I b ef, c e a e e s e e c a ed 100 µ HBsA s (2 µ / 50 M ca b a e b ca b a e b ffe , H 9.6) f 24 a 4 °C. T e e s e e as ed ee es PBS c a 0.05% (/) T ee 20 (PBS/T ee), a d e b c ed 5% FCS/PBS a 37 °C f 2 . Af e ee as s, 100 µ f a se es f d ed se a sa e 0.5% FCS/PBS as c e added a ca e e s. T e a es e e e c ba ed f 2 a 37 °C, f ed b ee es f as .A— s f 100 µ f abb a se I G se ad s e dase c a e d ed 1:20,000, a a se I G1 e dase c a e 1:16,000, I G2a e dase c a e 1:8000, a d I G2b e dase c a e 1:8000 0.5% FCS/PBS e e added eac a e. T e a es e e f e c ba ed f 2 a 37 °C. Af e as , e e dase ac as assa ed as f : 100 µ f s bs a e s (10 f *O* e e ed a e a d 37.5 µ f 30% H_2O_2 25 f 0.1 M c a e s a e b ffe , H 5.0) as added eac e . T e a e as c ba ed f 10 a 37 °C, a d e e eac as e a ed b add 50 µ / e f 2N H_2SO_4. T e ca de s as eas ed a ELISA eade a 490 , e e se s f se a sa es a e bee s b ec ed a d be ee c a s s, ELISA assa s e e e f ed e sa e da f a f e sa es.

2.8. Assay of natural killer (NK) cell activity

T e NK ce ac f s ee as de e ed s a C 96R N Rad ac e C c Assa K (P e a). YAC 1 ce s e e sed as a e ce s a d seeded 96 e U b c e a e a 4×10^4 ce s/ e RPMI 1640. S e c es e a ed as desc bed ab e e e sed as e effec ce s a d e e added a 2×10^6 ce s/ e e E/T a 50:1. Eac es as e ea ed f e s. T e a e as ce f ed a 250 × *g* f 1 e s e effec e a d a e ce c ac . Af e 4ea e s 41157.2( )1157.2a d 37

Tab e 1
Se ˙e ces f . e sed f ea e a a e RT PCR.

Ge e	P e se e ce	P d c s e/b	Acc
GAPDH	5 AAATGGTGAAGGTCGGTGTG 3 5 TGAAGGGGTCGTTGATGG 3	108	NM_001001303
IL 2	5 GCACCCACTTCAAGCTCCA 3 5 AAATTTGAAGGTGAGCATCCTG 3	174	NM_008366
IFN γ	5 CGGCACAGTCATTGAAAGCCTA 3 5 GTTGCTGATGGCCTGATTGTC 3	199	NM_008337

GAPDH, ce a de de 3 as a e de d e ase.

e (ABC). Af e c ba f 30 , a es e e as ed a d de e ed e a e be d e (TMB) a 37 °C f 15 . T e eac as s ed b add f 100 µ f s s . T e abs ba ce as eas ed a ELISA eade a 450 .

2.11. Real-time RT-PCR for cytokine gene expression

S e c es f e ed ce e a ed as desc bed bef e e seeded a 24 e fla b c e a e (N c) a 5×10^6 ce / 1 c e e ed , e eaf e HBsA (fi a c ce a 4 µ /) as added a fi a e f 2 (ca e s). T e a es e e c ba ed a 37 °C a d fied a s e e 5% CO_2T e 893(a) 446.3(18.8) 109064(c 87.9()12(e) 10(09064(a) 3 5.3(3)37(7)09064(383.2(09064((4)]T f)91Tf1309064(

99 T s[(99) [(99 (d)sfie)]T.99 a 37

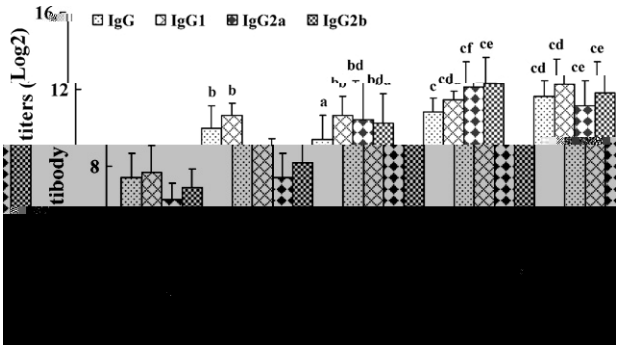


Fig. 3. Effect of acid D (PD) on HBsA specific IgG, IgG1, IgG2a, and IgG2b antibody HBsA ed ce. Sea e e ec ed 2 ee s af e e as a , and HBsA specific IgG, IgG1, IgG2a, and IgG2b a body es se a e e eas ed b a d ec ELISA as desc bed e e . T e a. es a e se ed as ea s ± S.E. (n = 5). S fica d ffe e ces HBsA a e e des a ed as ^{a}P < 0.05, ^{b}P < 0.01, a d ^{c}P < 0.001; se HBsA /A as ^{d}P < 0.05, ^{e}P < 0.01, a d ^{f}P < 0.001.

IgG1, IgG2a, and IgG2b a body es se e e ea s ed 2 ee s af e e as a s ELISA a d e es s e e s . F . 3. HBsA a e d ced e e s f HBsA specific IgG, IgG1, IgG2a, and IgG2b a body. A a d PD s fica e a ced e se HBsA specific IgG and IgG1 e s HBsA ed ce (P < 0.05, P < 0.01, P < 0.001). H e e , ec b a f HBsA PD es ec a a d se f 100 μ. ed ced e se a IgG and IgG1 es c a ed A a d HBsA c b a s. S fica e a ce e s HBsA specific se IgG2a and IgG2b e s e e se ed PD ed ce c a ed HBsA a e (P < 0.01 P < 0.001). M e e , IgG2a and IgG2b a body es e ce ed PD e e e a se e A ea ed ce. T e e e, e e, s fica d ffe e ces (P > 0.05) ese IgG2a and IgG2b e es be ee ce s ed HBsA /A a d HBsA a e. T e fi d s d ca ed a PD s fica e a ced se HBsA specific a body d c ce ed HBsA . M e e , a body es d ced b PD ad a ed HBsA af e ec s e e s e a se e c ed b A ad a ed acc e.

3.3. Effects of PD on NK cell activity in mice immunized with HBsAg

T e effec s f PD NK ce ac ce ed HBsA e e s F . 4. PD s fica e a ced e ac f NK ce e HBsA ed ce a ee

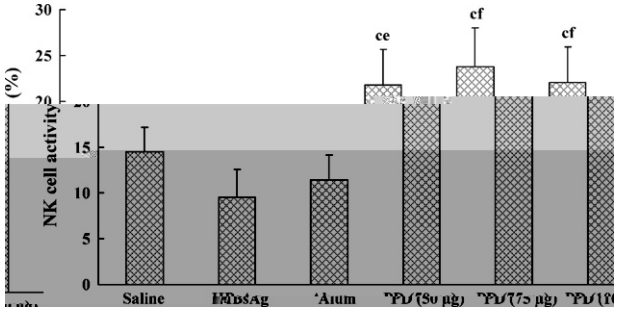


Fig. 4. Effect of acid D (PD) on NK ce ac ce ed HBsA . S e c es e e a ed 2 ee s af e e as a , and assa ed f NK ce ac b e LDH e ease e as desc bed e e . T e a. es a e se ed as ea s ± S.E. (n = 5). S fica d ffe e ces HBsA a e e des a ed as ^{c}P < 0.001; se HBsA /A as ^{e}P < 0.01 and ^{f}P < 0.001.

Fig. 5. Effect of acid D (PD) on CTL ac ce ed HBsA . S e c es e e a ed 2 ee s af e e as a , and assa ed f CTL ac b e LDH e ease e as desc bed e e . T e a. es a e se ed as ea s ± S.E. (n = 5). S fica d ffe e ces HBsA a e e and HBsA /A s e e des a ed as ^{c}P < 0.001 and ^{f}P < 0.001, es ec e .

ses (P < 0.001). T e e e, e e, s fica d ffe e ces (P > 0.05) e ac f NK ce be ee ce s ed HBsA /A a d HBsA a e. T e fi d s d ca ed a PD c d e ac a f NK ce c ac ce ed HBsA .

3.4. Effects of PD on specific CTL activity in mice immunized with HBsAg

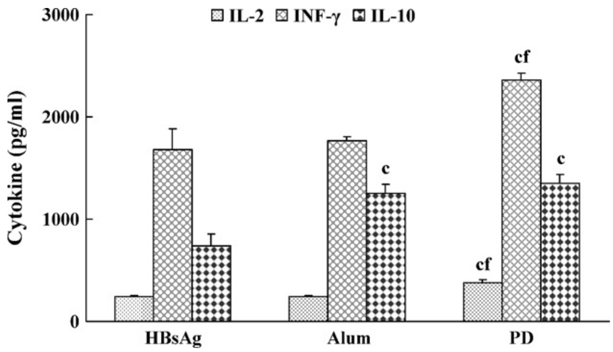
T e effec s f PD a e s ec fic CTL ac e HBsA ed ce e e s F . 5. I a f ce HBsA a e d ced e e s ec fic CTL ac 2 ee s af e e sec da a . Add f A HBsA d d es f e c ease HBsA s ec fic CTL ac ab e se see HBsA a e. I c as , PD s fica e a ced e s ec fic ac f CTL ce ed HBsA a ee ses (P < 0.001). T e fi d s d ca ed a PD c d e s ec fic ac f CTL ce ed HBsA .

3.5. Effect of PD on cytokine secretion by splenocytes from HBsAg-immunized mice

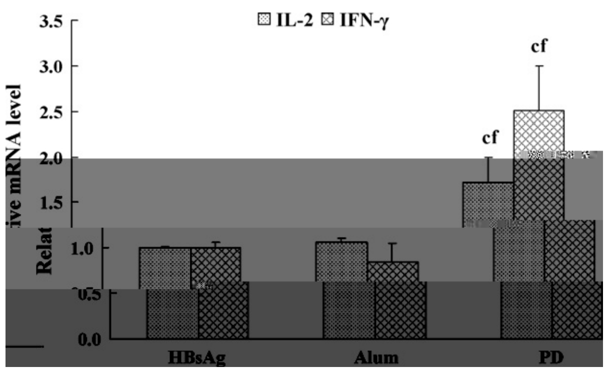
I de assess effec f PD T 1 a d T 2 c es s d HBsA , c e c s f s e c es HBsA ed ce e e de ec ed s ELISA. T e ca b a c es f IL 2, IFN γ, a d IL 10 e e c s c ed se c es a da ds, a d e c e a c e effic e e e a b e a 0.9980. As s F . 6, e c e s f c es IL 2, IFN γ, a d IL 10 e c es e a a s f HBsA s a ed s e c es f e ce ed HBsA /PD e e s fica e a se HBsA c ce (P < 0.01), s es a PD s fica e a ced e d c f e T 1 a d T 2 c es HBsA ed ce. H e e , A s fica c eased e IL 10 d c e HBsA ed ce (P < 0.001).

3.6. Effect of PD on mRNA expression of cytokines in splenocytes from HBsAg-immunized mice

S ce PD s fica e a ced IgG2a and IgG2b a body es ses a d T 1 c e d c , e e eas ed IL 2 a d IFN γ RNA e ess s e c es f ce ed HBsA . T es e c es f e ed ce e e s a ed HBsA , a d a RNA e e ac ed. Rea e a a e RT PCR f IL 2 a d IFN γ c e RNA e es s s e e f ed. GAPDH as sed as a a a d c f e RT PCR eac s. As s F . 7, e IL 2 a d IFN γ RNA e ess e ce ed PD e e e a



F g. 6. Effec s f a c d D (PD) HBsA d ced c e. d c s e c es f e HBsA ed ce. S e c es e e. e a ed 2 ee s af e e as a , a d c ed HBsA (fi a c ce a 4 μ /) f 48 . T e c es e a a s e e c ec ed, a d e c e s f c es IL 2, IFN γ, a d IL 10 e e de e ed b ELISA. T e a es a e ese ed as ea s ± S.E. (n = 5). S fica d ffe e ces HBsA a e a d HBsA /A . s e e des a ed as ^{c}P < 0.001 a d ^{f}P < 0.001, es ec e .



F g. 7. Effec f a c d D (PD) RNA e ess f c es IL 2 a d IFN γ s e c es f e HBsA ed ce. S e c es e e. e a ed 2 ee s af e e as a , a d c ed HBsA (fi a c ce a 4 μ /) f 24 . T e RNA e ess e e f GAPDH, IL 2, a d IFN γ e e de ec ed b ea e RT PCR s s ec fic e s. T e e a e RNA e ess e e a e ese ed as ea s ± S.E. (n = 5). S fica d ffe e ces HBsA a e a d HBsA /A . s e e des a ed as ^{c}P < 0.001 a d ^{f}P < 0.001, es ec e .

se e HBsA a e a d HBsA /A s (P < 0.001). T e e e e, e e , s fica d ffe e ces (P > 0.05) e IL 2 a d IFN γ RNA e ess s be ee ce s ed HBsA /A a d HBsA a e. T s, fi d s f e c fi ed a PD s fica d ced T 1 c e sec e b s e c es f e HBsA ed ce.

4. D c

E de ce e s s c ea s es a T 1 T 2 es ses, e e a ed a e c s a , ca be d a ed de e d e ad a sed f a [15,16]. T e d ffe e T 1 a d T 2 e es se fi es c es d e ac a f d s c a s bse s f T ce s c a ac e ed b e a e f c e d c [17]. T 1 es ses a e ass c a ed IFN γ, IL 2 a d IL 12 d c a d e a ce b s e s c I G2a, I G2b a d I G3. T e T 1 es se ca be c e a ed e d c f ce ed a ed [18], c as bee desc bed as be ca ed f e e a f s d fec [19,20]. T 2 es ses, c c e a e es se e e f B ce fe a a d d ffe e a [21], a e c a ac e ed b e e s f c c a a b d es, f a I G1 s e, a d e sec e f e c es IL 4, IL 5, IL 10 a d IL 13. F ec e ce a fec s d seases d ffe e a d c f T 1 T 2 es ses b s e ed.

A s sed as ad a s e e a s B acc es c e c e c a ed. A ads bed acc es a e bee s e c esse a T 2 e es ses, a d d ce ce ed a ed . I as e ed a a T 2 ad a , s c as A , s efficac s se c e s s es d a , e a ad a d c a ba a ced T 1/T 2 es se a a s HBsA as ab e e c e es s e ess HBsA B10.M ce. Mea e, ce a

de s a ed a PD d a ed e a f e es ses, a de c eda ba a ced T 1/T 2 e es se HBsA ce as assc a ed se s e a e a ce e f I G2a, I G2b a d I G1 e es [29].

I de c ea es ab s a T ce de ed c es e e ed e ad a ac f PD, e a a ed e T 1/T 2 c e sec e fi es HBsA ed ce s ELISA. PD s fica c eased e d c f T 2 c es IL 10, b a s s e a ced e d c f T 1 c es IL 2 a d IFN γ f s e c es e HBsA ed ce. H IL 2 sec e c ea ed e d c f a a e s ec fic ce a fe a e es se, e e e e f IFN γ s c s s e e c ease f I G2a a d I G2b a b d es. S a , e ce ed A a d PD ad e e f IL 10 a d c es ded e e e s f I G1 e s e ce. T e e , e HBsA s ec fic a b d s es a d c e fi es c fi a e PD ed a ba a ced T 1/T 2 e e es se, e A as ass c a ed ed a T 2 e e es ses. I de f e e c da e e ec a s es s b e f e c a es e a s f T 1 c es, e ed ea e RT PCR

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