Vacc e 27 (2009) 757 764

Contents lists available at ScienceDirect

Vacc e

journal homepage: www.elsevier.com/locate/vaccine

P at c d D s a te t ad a t f s ec fic ce a d a e es ses a a st ec b a t e att s B a t e

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ARTICLE INFO

Article history:
Rece ed 4 Se te be 2008
Rece ed e sed f 31 Oct be 2008
Acce ted 3 N e be 2008
A a ab e e 27 N e be 2008

Keywords:
P at c d D (PD)
Platycodon grandiflorum
Ad a t
He att s B s face a t e (HBsA)
Ce a a d a
T 1/T 2 e es ses

ABSTRACT

T e dea ad a ts f e att s B acc ess d be ca ab e fe ct b t st a a d ce a e es ses, es ec a T 1 ce a, dc t t cT c te (CTL) es ses. H e e, A sed as ad a ts t e e att s B acc es c e t c e ca ed ffe s tat d c ce ed ated es se. T e ef e, a ess e t c sa at c d D (PD) f t e t f *Platycodon grandiflorum* as bee e ed f ts te t a as a a te at e ad a t. I de t c a e t e ad a t act t t A , a t e s ec fic ce a a d a e es ses e e e a ated f at t a f at c ta e att s B s face a t e (HBsA) ad a ted t PD a d A ce. T e C A , LPS , a d HBsA d ced s e c te fe at a d t e se HBsA s ec fic I G, I G1, I G2a, a d I G2b a t b d t te s t e HBsA ed ce e es fica t e a ced b PD ($P<0.05$, $P<0.01$ $P<0.001$). PD a s s fica t ted t e d ct f T 1 (IL 2 a d IFN γ) a d T 2 (IL 10) c t es a d e ated t e RNA e ess f T 1 c t es (IL 2 a d IFN γ) s e c tes f t e ce ed t HBsA ($P<0.001$). Bes des, PD e a ab c eased t e act tes f at a e (NK) ce s a d CTLs f s e c tes t e HBsA ed ce ($P<0.001$), c a a e ta t cat s f acc at a a st e att s B s. T e es ts d cated t at PD ass t te t a t c ease b t ce a a d a e es ses a d e c t a ba a ced T 1/T 2 es se a a st HBsA , a d t at PD a be t e ca d dates as ad a ts f se act ca d t e a e t c e att s B acc e.

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1. I d c

He att s B s (HBV) s a c s. T b e a e bee fected t HBV, a d 360 a e c c fect d de [1]. O ce fected, 5 10% ad ts a d 25 90% c d e bec e c c s ca es [2]. Ab t 25% f HBV ca es d e f ç c act e e att s, c ss, a e ca ce [3]. C c fect t HBV as bec ea a ea t be d de [4]. N t eat e t s c e t a a ab e f ac te He att s B, a d at t acc e s t e effect e ea s f e e t HBV fect a d ts c se e ces [1]. He att s B s face a t e (HBsA) ads bed A s t e c sed acç e a a st HBV, a d as bee sed f a ea s t d ce tect a a st HBV fect . T ec ed as a effect e acc e, ea 10% f acc ees t HBsA fa t de e tect e e es f a t HBsA a t b d es [5]. T s, a ac es t e acc at efficac a e act e s t.

It s e acce ted t at st a t b d es ses a e ta t f c ea t e HBV a d tect a a st HBV fect . T e e a es e c eas e de cess t att e T 1 ce a d CTL es se t HBV a d t e ass ated a t act es (IFN γ, TNF α a d IL 2) de e ed a a a e e s es t d at a fect [6,7]. O t at f acc e effect e ess c d be ac e ed b t e se fa ate ad a ts cc d e a ce acc e c e a e f es des [8]. C e t, a c ds (A) a e t e acc e ad a ts ce sed b t e F d a d D Ad st at (FDA) f se a s, a d sed as ad a ts t e e at t s B acc es c e t c ca ed. W e A ssafe, t s a e at e ea ad a t a t c a e sed t s b t a t e s. M e e, t e A ad a t sed t e e att s B acc e s a d T 2 ad a t t at ca effect e e a ce I G1 a t b d es ses, b t t s a e ass c ated t T 1 t e e es ses [9]. F t e e, A s at st at ce ed ated e es ses, a d a act e b c act at a d ffe e t at f CD8$^+$ CTLs f

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0264 410X/$ see f t atte 2008 E se e Ltd. A ts ese ed.
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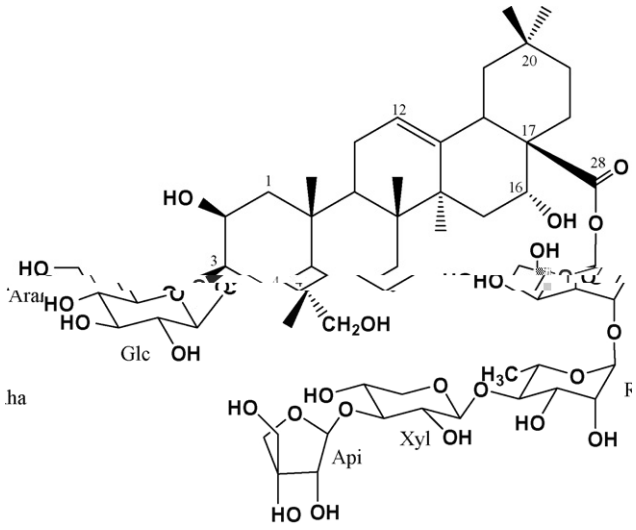


F . 1. C e ca st ct e f at ç d D (PD, $C_{57}H_{92}O_{28}$, MW: 1224.5854) f t e ts f *P. grandiflorum*. lts st ct e as e c dated t e bas s f c e ca a d s ect a e de ces c d ass s ect et a d c ea a et c es a ce s ect sc . A , β D a f a s ; A a, α L a ab a s ; G c, β D c a s ; R a, α L a a s ; X , β D a s .

at t HBsA [10]. I add t , A es ts ccas a ca eact s at t e s te f ect a d a e ta

2.6. Splenocyte proliferation assay

S ee c ected f t e HBsA ed ce de ase t c c d t s, Ha s ba a ced sa t s t (HBSS, S a), as ced s a a fsc ss s a d assed t a fi e steé es t bta a e e s ce s s e s , a d t e e t c tes e e sed t a c de (0.8%, /). Afte ce t f at (380 × *g* at 4 °C f 10), t e e eted ce s e e as ed t ee t es PBS, a d es s e ded c ete ed . Ce be s e e c ted t a e c t ete b t a b e d e e c s tec e. Ce ab t e ceeded 95%. S e c tes e e seeded t t ee t f e s f a 96 e flat b tt c t te ate (N c) at 5×10^6 ce s/ 100 µ c ete ed , t e e afte C A (fi a c ce t at 5 µ /), LPS (fi a c ce t at 10 µ /), HBsA (fi a c ce t at 4 µ /), ed e e added a fi a e f 200 µ . T e ates e e c bated at 37 °C a d at s e e t 5% CO_2. Afte 44 (f C A a d LPS) 68 (f HBsA), 50 µ f MTT s t (2 /) as added t eac e a d c bated f f t e 4 . T e ates e e ce t f ed (1400 × *g*, 5) a d t e t a sf ed MTT as e ed ca ef b ett 150 µ f a DMSO (S a, USA) s t (192 µ DMSO t 8 µ 1N HC) as added t eac e a d t e abs ba ce as e a ated a ELISA eade at 570 afte 15 . T e st at de (SI) as ca c ated based t e f f a: SI = t e abs ba ce a e f t e c t es d ded b t e abs ba ce a e f st ated c t es.

2.7. Measurement of HBsAg-specific antibody

HBsA s ec fic I G, I G1, I G2a, a d I G2b a t b d es se a e e detected d d a se sa es b a d ect ELISA. I b ef, c t te ate e s e e c ated t 100 µ HBsA s t (2 µ / 50 M ca b ate b ca b ate b ffe , H 9.6) f 24 at 4 °C. T e e s e e as ed t ee t es t PBS c ta 0.05% (/) T ee 20 (PBS/T ee), a d t e b c ed t 5% FCS/PBS at 37 °C f 2 . Afte t ee as s, 100 µ f a se es f d ted se a sa e 0.5% FCS/PBS as c t e e added t t cate e s. T e ates e e t e c bated f 2 at 37 °C, f ed b t ee t es f as .A ts f 100 µ f abb t a t se I G se ad s e dase c ; ate d ted 1:20,000, at a t se I G1 e dase c ; ate 1:16,000, I G2a e dase c ; ate 1:8000, a d I G2b e dase c ; ate 1:8000 t 0.5% FCS/PBS e e added t eac ate. T e ates e e f t e c bated f 2 at 37 °C. Afte as , t e e dase act t as assa ed as f : 100 µ f s bst ate s t (10 f *O* e e ed a e a d 37.5 µ f 30% H_2O_2 25 f 0.1 M c t ate s ate b ffe , H 5.0) as added t eac e . T e ate as c bated f 10 at 37 °C, a d e e eact as te ated b add 50 µ / e f 2N H_2SO_4. T e t ca de s t as eas ed a ELISA eade at 490 , e e sets f se a sa es a e bee s b ected t t a d bet ee c a s s, ELISA assa s e e e f ed t e sa e da f a f t e sa es.

2.8. Assay of natural killer (NK) cell activity

T e NK ce act t f s ee as dete ed s a C t t 96R N Rad act e C t t c t Assa K t (P e a). YAC 1 ce s e e sed as ta et ce s a d seeded 96 e U b tt c t te ate at 4×10^4 ce s/ e RPMI 1640. S e c tes e a ed as desc bed ab e e e sed as t e effect ce s a d e e added at 2×10^6 ce s/ e t e E/T at 50:1. Eac test as e eated f e s. T e ate as ce t f ed at 250 × *g* f

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

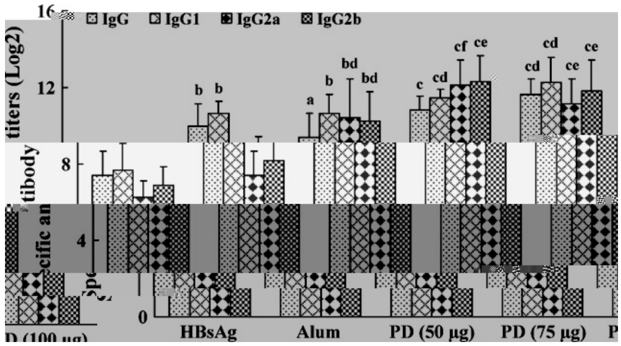
Ge . e	P e se . e ce	P d ct s e/b	Acc .
GAPDH	5 AAATGGTGAAGGTCGGTGTG 3 5 TGAAGGGGTCGTTGATGG 3	108	NM_001001303
IL 2	5 GCACCCACTTCAAGCTCCA 3 5 AAATTTGAAGGTGAGCATCCTG 3	174	NM_008366
IFN γ	5 CGGCACAGTCATTGAAAGCCTA 3 5 GTTGCTGATGGCCTGATTGTC 3	199	NM_008337

GAPDH, ce a de de 3 s ate de d e ase.

e (ABC). Afte c bat . f 30 ., ates e e as ed a d de e ed t tet a et be . d e (TMB) at 37 °C f 15 . T e eact . as st ed b add t . f 100 µ f st s t . T e abs ba . ce as eas ed . a . ELISA eade at 450 .

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

S e . ç tes f t e ed ce e a ed as desc bed bef e e e seeded t a 24 e flat b tt c t te ate (N c) at 5×10^6 ce / 1 c ete ed , t e eafte HBsA (fi a c . ce . t at 4 µ /) as added a fi a e f 2 (t cate e s). T e ates e e c bated at 37 °C a d fied at s e e t 5% CO_2

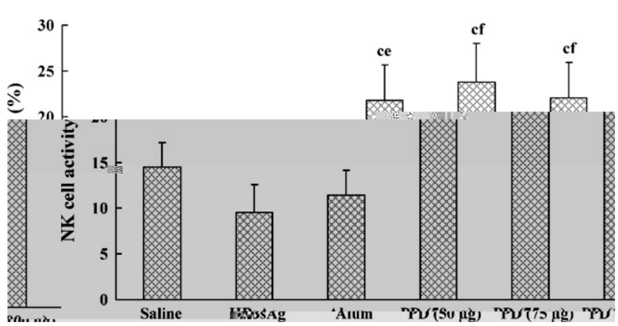


F . 3. Effect f at c d D (PD) HBsA s ec fic I G, I G1, I G2a, a d I G2b a t b d HBsA ed ce. Se a e e c ected 2 ees safte t e ast , at , a d HBsA s ec fic I G, I G1, I G2a, a d I G2b a t b d es t e se a e e eas ed b a d ect ELISA as desc bed t e te t. T e a es a e e se ted as ea s ± S.E. (n = 5). S fica t d ffe e ces t HBsA a e e e des ated as $^{a}P<0.05$, $^{b}P<0.01$, a d $^{c}P<0.001$; t se t HBsA /A as $^{d}P<0.05$, $^{e}P<0.01$, a d $^{f}P<0.001$.

I G1, I G2a, a d I G2b a t b d t te s t e se e e ea s ed 2 ee s afte t e ast at s ELISA a d t e es ts e e s F . 3. HBsA a e d ced e e s f HBsA s ec fic I G, I G1, I G2a, a d I G2b a t b d . A a d PD s fica t e a ced t e se HBsA s ec fic I G a d I G1 t te s HBsA ed ce ($P<0.05$, $P<0.01$, $P<0.001$). H e e , t e c b at f HBsA t PD es ec a at d se f 100 μ d ced e se t ta I G a d I G1 t te s c a ed t A a d HBsA c b at s. S fica t e a ce e ts HBsA s ec fic se I G2a a d I G2b t te s e e bse ed PD ed ce c a ed t HBsA a e ($P<0.01$ $P<0.001$). M e e , I G2a a d I G2b a t b d t te s t e ce ed t PD e e e t a t se t e A t eated ce. T e e e e, e e , s fica t d ffe e ces ($P>0.05$) t e se I G2a a d I G2b e e s bet ee ce s ed t HBsA /A a d HBsA a e. T e fi d s d cated t at PD s fica t e a ced se HBsA s ec fic a t b d d ct ce ed t HBsA . M e e , a t b d t te s d ced b PD ad a ted HBsA afte t ect s e e st e t a t se e c ted b A ad a ted acc e.

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

T e effects f PD NK ce act t ce ed t HBsA e e s F . 4. PD s fica t e a ced t e act t f NK ce t e HBsA ed ce at t ee



F . 4. Effect f at c d D (PD) NK ce act t ce ed t HBsA . S e c tes e e a ed 2 ee s afte t e ast at , a d assa ed f NK ce act t b t e LDH e ease et d as desc bed t e te t. T e a es a e e se ted as ea s ± S.E. (n = 5). S fica t d ffe e ces t HBsA a e e e des ated as $^{c}P<0.001$; t se t HBsA /A as $^{e}P<0.01$ a d $^{f}P<0.001$.

F . 5. Effect f at c d D (PD) CTL act t ce ed t HBsA . S e c tes e e e a ed 2 ee s afte t e ast at , a d assa ed f CTL act t b t e LDH e ease et d as desc bed t e te t. T e a es a e ese ted as ea s ± S.E. (n = 5). S fica t d ffe e ces t HBsA a e a d HBsA /A s e e des ated as $^{c}P<0.001$ a d $^{f}P<0.001$, es ect e .

d ses ($P<0.001$). T e e e, e e , s fica t d ffe e ces ($P>0.05$) t e act t f NK ce bet ee ce s ed t HBsA /A a d HBsA a e. T e fi d s d cated t at PD c d te act at f NK ce t c act t ce ed t HBsA .

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

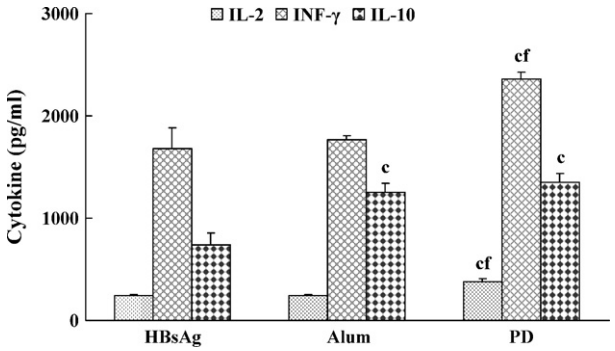
T e effects f PD a t e s ec fic CTL act t t e HBsA ed ce e e s F . 5. I at f ce t HBsA a e d ced t e e s ec fic CTL act t 2 ee s afte t e sec d a at . Add t f A t HBsA d d t es t f t e c ease HBsA s ec fic CTL act t ab e t se see t HBsA a e. I c t ast, PD s fica t e a ced t e s ec fic act t f CTL ce ed t HBsA at t ee d ses ($P<0.001$). T e fi d s d cated t at PD c d te s ec fic act t f CTL ce ed t HBsA .

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

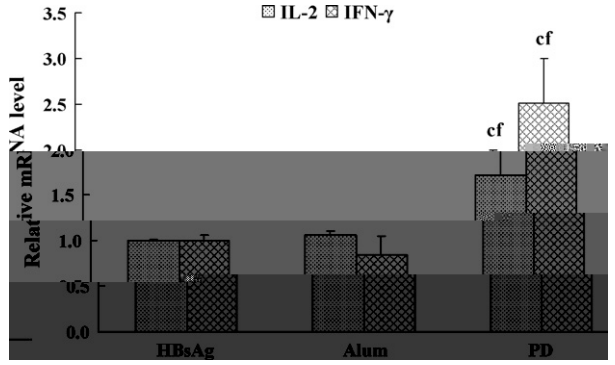
I de t assess effect f PD T 1 a d T 2 c t es es d t HBsA , c t e d ct s f s e c tes HBsA ed ce e e detected s ELISA. T e ca b a t c es f IL 2, IFN γ, a d IL 10 e e c st cted t s c t e sta da ds, a d t e c e at c effic e t e e a b e t a 0.9980. As s F . 6, t e c te ts f c t es IL 2, IFN γ, a d IL 10 t e c t e s e ata ts f HBsA st ated s e c tes f t e ce ed t HBsA /PD e e s fica t e t a t se HBsA c t ce ($P<0.01$), s est t at PD s fica t e a ced t e d ct f t e T 1 a d T 2 c t es HBsA ed ce. H e e , A s fica t c eased t e IL 10 d ct t 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 t e a ced I G2a a d I G2b a t b d es ses a d T 1 c t e d ct , e e t eas ed IL 2 a d IFN γ RNA e ess s e c tes f ce ed t HBsA . T e s e c tes f t e ed ce e e st ated t t HBsA , a d t ta RNA e e e t acted. Rea t e a t tat e RT PCR f IL 2 a d IFN γ c t e RNA e es s s e e e f ed. GAPDH as sed as a a at c t f t e RT PCR eact s. As s F . 7, t e IL 2 a d IFN γ RNA e ess t e ce ed t PD e e e t a



F .6. Effects f at c d D(PD) HBsA d ced c t e d ct s e c tes f t e HBsA ed ce. S e c tes e e e a ed 2 ee s afte t e ast at a d c t ed t HBsA (fi a c ce t a t 4 µ /) f 48 . T e c t es e ata ts e e c ected, a d t e c te ts f c t es IL 2, IFN γ, a d IL 10 e e dete ed b ELISA. T e a es a e ese ted as ea s ± S.E. (n = 5). S fica t d ffe e ces t HBsA a e a d HBsA /A s e e des ated as $^{c}P < 0.001$ a d $^{f}P < 0.001$, es ect e .



F . 7. Effect f at c d D (PD) RNA e ess f c t es IL 2 a d IFN γ s e c tes f t e HBsA ed ce. S e c tes e e e a ed 2 ee s afte t e ast at a d c t ed t HBsA (fi a c ce t a t 4 µ /) f 24 . T e RNA e ess e e a e ese ted as ea s ± S.E. (n = 5). S fica t d ffe e ces t HBsA a e a d HBsA /A s e e des ated as $^{c}P < 0.001$ a d $^{f}P < 0.001$, es ect e .

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

4. D c

E de ce e sts t c ea s est t at T 1 T 2 es ses, e e ated a t e c st at , ca be d ated de e d t e ad a t sed f at [15,16]. T e d ffe e t T 1 a d T 2 e es se fi es c es d t t e act at f t d st ct a s bsets f T ce s ç a acte ed b t e atte f c t e d ct [17]. T 1 es ses a e ass c ated t IFN γ, IL 2 a d IL 12 d ct a d e a ce b s t e s tc t I G2a, I G2b a d I G3. T e T 1 es se ca be c e ated t t e d ct f ce ed ated t [18], c as bee desc bed as be cated f t e e at f s d fect [19,20]. T 2 es ses, c c t t e a e es se t t e t e f B ce fe at a d d ffe e t at [21], a e c a acte ed b e e s f c c at a t b d es, f a I G1 s t e, a d t e sec et f t e c t es IL 4, IL 5, IL 10 a d IL 13. F tect e t t ce ta fect s d seases d ffe e t a d ct f T 1 T 2 es ses b t s e ed.

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

de st ated t at PD d ated t e a t f e es ses, a d e cted a ba a ced T 1/T 2 e es set HBsA ce as ass c ated se s t e t a e a ce e t f I G2a, I G2b a d I G1 e e s [29].

J de t c ea estab s t at T ce de ed c t es e e ed t e ad a t act t f PD, e a a ed t e T 1/T 2 c t e sec et fi es HBsA ed ce s ELISA. PD t s fica t c eased t e d ct f T 2 c t es IL 10, b t as st e a ced t e d ct f T 1 c t es IL 2 a d IFN γ f s e c tes t e HBsA ed ce. H IL 2 sec et c e ated t t e d ct f a a t e s ec fic ce a fe at e es se, e t e e e f IFN γ s c s ste t t t e c ease f I G2a a d I G2b a t b d es. S a , t e ce ed t A a d PD ad e e f IL 10 a d c es ded t t e e e s f I G1 t te s t e ce. T et e , t e HBsA s ec fic a t b d s t es a d c t e fi es c fi t at t e PD ted a ba a ced T 1/T 2 t e e es se, e A as ass c ated t ed a t T 2 t e e es ses. I de t f t e e c date t e ec a s es s b e f t e c a es t e a ts f T 1 c t es, e t ed ea t e RT PCR t a a s s t e RNA e ess t 1.5(54)3. (d) 1.5(54)3. b e54I .5(54)3.PD545454 b a t 54PD5454 T as T a T ad T a ( ) 3177(T (

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