Fatty Acid-Inducible Part/ANGPTL4 Regulates Lipid Oxidation Response to *Gelsemium Elegans* Alkaloids

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Research

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Abstract

Background The *Gelsemium elegans alkaloids* have been used in animal feed additives and already achieved terrific results in the pig's gut health and weight gain. A series of studies have been conducted to explore the mechanisms by which it improves gut health and promotes growth. However, hardly any research has been done in the metabolic changes *G. elegans alkaloids* elicit in pork muscle. Angiopoietin-like 4 (ANGPTL4), an inhibitor of lipoprotein lipase-mediated plasma triglyceride clearance, is transcribed at a higher rate relative to others in *G. elegans alkaloids* non-feeding muscle than in *G. elegans alkaloids* feeding muscle.

Methods A total of 24 muscle tissues and corresponding blood samples were collected from pigs and Target RNA was extracted using TRIzol reagent according to the manufacturer's instructions. Histological and immunofluorescence analysis combined with Western blot analysis were utilized to further explore the possible mechanism of ANGPTL4 and peroxisome proliferator-activated receptor-δ(PPARD).

Results This phenomenon results from that ANGPTL4 in *G. elegans alkaloids* non-feeding muscle is mediated by elevated plasma free fatty acids via PPARD, presumably leading to reduced local uptake of plasma triglyceride-derived fatty acids and their sparing for use by feeding with *G. elegans alkaloids*. In contrast, the transcription of ANGPTL4 in *G. elegans alkaloids* feeding muscle likely is counteracted via lipoprotein lipase(LPL)-mediated negative-regulation, promoting the use of plasma triglycerides as fuel for active muscles.

Conclusion The experimental result indicates that the muscle and the local regulation of ANGPTL4 via PPARD and LPL have critical roles in governing lipid homeostasis during the feeding process of pigs and will shed light on the molecular mechanism discovered by *G. elegans alkaloids*.

Introduction

Gelsemium elegans (Gou Wen, Da Cha Yao or Duan Chang Cao) has been used as traditional Chinese medicine (TCM) for the treatment of rheumatoid arthritis, neuropathic pain, spasticity, skin ulcers and cancer for many years [1, 2]. The current studies suggest that *G. elegans alkaloids* promote animal growth in pigs and sheep. Thus, the whole plant of *G. elegans* has been widely added to animal feed for livestock. The crude and purified alkaloids of *G. elegans* have demonstrated that this species possesses anti-inflammatory[2], immunomodulating[3], analgesic[2, 4],, anxiolytic, anti-tumour[5, 6], and neuropathic pain-relieving properties[7]. The anti-inflammatory and neuropathic pain-relieving properties were associated with the modulation of neurosteroids in the spinal cord through the activation of spinal a3 glycine receptors[2]. The mechanisms of this anti-tumour reaction may be related to the apoptosis-inducing activities and the inhibition of tumour cell proliferation[5, 6]. A possible explanation for the anxiolytic and analgesic effects is that gelsemine antagonizes glycine receptors stimulates the allopregnanolone biosynthesis. Recent articles propose that *Gelsemium alkaloids* produce antinociception by activating the spinal glycine receptor/allopregnanolone analgesic pathway[7].

Currently, more than 200 compounds, including alkaloids, iridoids, and steroids, have been reviewed[8]; among these compounds, alkaloids and iridoids, which are almost exclusively derived from the genus *Gelsemium* spp., considered as the bioactive components that are responsible for the observed pharmacological effects. *Gelsemine* and koumine are the principal alkaloids in *G. elegans*. Still, their toxicity is relatively weak[9] compared to Gelsenicine, where Gelsenicine uses a smaller dosage to become lethal; thus, the most toxic alkaloid in *G. elegans*[10]. Notably, the dried roots, stems and leaves of *G. elegans* are almost equally toxic as the *Gelsemium semperviresn*[11]. Typical symptoms of intoxication include chest distress, asphyxia, dizziness, tonic convulsions, limb paralysis, and difficulty breathing. Severe poisoning can cause multiple organ failure, which eventually leads to death[2, 12]. Therefore, the essential bioactive components of *G. elegans* have attracted chemists, pharmacologists and toxicologists due to their complex structural features and multiple biological effects. Based on the previous experiments in pigs, we performed a transcriptome analysis of its impact on the liver and intestine(PRJNA526943, SRR8728049- SRR8728060,12 objects), in which we screened a series of expressed genes. Angiopoietin-like protein 4 (ANGPTL4), a secretory glycoprotein, is a member of the angiopoietin family [13]. Previous studies have Suggested thatANGPTL4 is a multifunctional factor in regulating lipid metabolism, wound healing and muscle generation [14, 15]. Furthermore, current research reports that activation of PPARD activates the expression and secretion of ANGPTL4 [16, 17]. However, the expression and secretion of ANGPTL4 in the pork production process remain unknown. In addition, it is unclear that whether a regulatory interaction between ANGPTL4 and PPARD exists in the effects of animal food additives on muscle development in pigs.

Here we investigate the outcome of the muscle of pigs fed with *G. elegans* alkaloids and secretion of ANGPTL4 and the molecular mechanisms underlying these effects in their muscle cells. Moreover, we explored the expression of ANGPTL4 and PPARD in muscle tissue and serums alongside their potential correlation in pigs' weight gaining and healthy subjects. To further identify the latent roles of ANGPTL4 and PPARD in pig development, a variety of functional studies were performed using ELISA and WB. The results indicate that transcription f ANGPTL4 in *Gelsemium elegans alkaloids* non-feeding muscle is mediated by elevated plasma free fatty acids via peroxisome proliferator-activated receptor-δ(PPARD), presumably leading to reduced local uptake of plasma triglyceride-derived fatty acids and their sparing for use by feeding with *G. elegans alkaloids*. In contrast, the transcription of ANGPTL4 in *G. elegans alkaloids* feeding muscle is counteracted via lipoprotein lipase (LPL)-mediated down-regulation, which promotes plasma triglycerides as fuel for active muscles. Thus, our data suggest that muscle and the local regulation of ANGPTL4 via PPARD and LPL have vital roles in regulating lipid homeostasis during the feeding process of pigs and will shed light on the molecular mechanism discover of *G. elegans alkaloids*.

Materials And Methods

Porcine CYP7A1 ELISA kit, porcine 3-hydroxymethyl glutaratemonoacyl CoA(HMGCoA) reductase ELISA kit and porcine lipoprotein lipase (LPL) ELISA kit was purchased from Wuhan Mershack biotechnology Co., LTD. PDK4 antibody (bs-0682r), PPAR-δ antibody (BSM-33263m), KLF10 antibody (bs-1838r),

slc22a5 antibody (bs-8149r) and GAPDH antibody (BSM-33033m) were purchased from Beijing Bosenbiotechnology Co., LTD.The other reagents are all domestic analytical pure level.

Collection of muscle tissues and blood samples

A total of 24 muscle tissues and corresponding blood samples were collected from pigs at Hunan New Wellful Co., LTD. (Liuyang, Hunan, China). All tissues and blood samples were collected according to protocols approved by the Research ethics committee of the School of Veterinary Medicine, Hunan Agricultural University(433320027). All experiments were performed according to the institutional guidelines for the care and use of laboratory animals(200705041)

Animals were randomly divided into four subgroups (n = 15) after feeding with the regular diet for eight weeks: Group 1 was continuously fed with the regular diet for seven weeks (Control group); Group 2 was fed with *G. elegans alkaloids* diet for seven weeks (Low-dose group); Group 3 was fed with *G. elegans alkaloids* diet for seven weeks (Middle-dose group); Group 4 was fed with *G. elegans alkaloids* diet for seven weeks (High-dose group). After the treatment mentioned above, pigs were fasted for 12 h before being sacrificed. Bodyweight and liver weight were recorded. The serum was separated from the blood collected and the blood biochemical indexes were determined（Table 1）.

RNA extraction and qRT-PCR

Target RNA was extracted using TRIzol reagent according to the manufacturer's instructions. Primers for ANGPTL4、PPARD、PDK4、SLC22A5, GAPDH and KLF10, were obtained from Invitrogen Bioengineering Corporation (Shanghai, China). The sequences of the primers being used for PCR reactions were listed in Table 2 RT-PCR was performed for 40 cycles at the following conditions: pre-denaturation at 95 °C for 5 min, denaturation at 95 °C for 30 s, annealing at 58.3 °C for 30 s, extended at 72 °C for 30 s. In addition, the expression of target genes was normalized to that of GAPDH, and the relative quantification of mRNA levels was performed using the $2^{-\Delta\Delta Ct}$ method.

Detection of LPL, CYP7A1 and HMG-CoA cytokines in tissue

Dissect the muscle tissue with clean tools, on ice preferably and as quickly as possible to prevent degradation by proteases. Place the tissue in round bottom microfuge tubes immersed in liquid nitrogen, and then keep on ice for immediate homogenization. Add 400 µL complete extraction buffer to the tube and homogenize with an electric homogenizer. Rinse the blade twice using 300 µL complete extraction buffer for each rinse, then maintain constant agitation for two hours at 4 °C. Centrifuge for 20 min at 13,000 rpm at 4 °C. Place on ice, aliquot supernatant to a fresh, chilled tube and store samples at -80 °C. ELISA test is based on the kits according to the manufacturer’s instructions.

Histological and immunofluorescence analysis

The muscle tissues were assessed by hematoxylin-eosin(H&E) staining as described in Liu et al[18]. Frozen muscle sections (5 µm thick) were treated with 0.1% Triton X-100 in PBS and were incubated for

45 min at room temperature with the primary antibody mix and diluted in 0.05% Tween20 in PBS. After three washing steps with PBS, sections were incubated for 45 min at room temperature with the appropriate fluorescent-labelled secondary antibodies.

Western blot

Total protein lysates of muscle tissues were obtained by incubation on ice with RIPA containing 1% PMSF, cocktail and phosphatase inhibitors. The supernatant was collected after centrifugation at 12,000×*g* for 15 min at 4 °C. Protein quantification was performed with a BCA kit. Aliquots containing 30 μg of protein were separated by SDS-PAGE (5% concentrated gel and 10% separated gel) and transferred to PVDF membranes. The membrane was incubated with 5% nonfat dried milk at room temperature for two hours, followed by specific primary antibodies at 4 ° C overnight and the corresponding secondary antibodies at room temperature for one hour. Immunoreactive bands were visualized by ECL and quantified by software Image J (NIH, USA).

Statistical analysis

All data are shown as mean± S.E.M. All statistical analyses, including one-way ANOVA, t-test and Pearson's correlation, were executed using SPSS 20.0. All experiments were independently repeated at least replicates, where the alpha level is set to 0.05 for significance.

Result

The most frequently expressed gene in the *G. elegans* alkaloids groups was ANGPTL4, a sensitive target of the PPAR transcription factors that encodes a secreted inhibitor of the enzyme LPL[19,20,21].. LPL catalyzes the hydrolysis of circulating triglycerides (TG), which plays a crucial role in the uptake of fatty acid in skeletal muscle[22].

G. elegans alkaloids activate the mRNA expression of the PPAR signalling pathway

Relative quantitative PCR results showed that the expression of the ANGPTL4 gene in the muscle of the drug group was significantly increased (Fig. 1a), which was significantly different from that of the control group ($P < 0.05$). Meanwhile, KLF10, PDK4 and SLC22A5 gene expression was significantly increased in muscles of the drug group (Fig. 1b,c,e). However, the results of relative quantitative PCR indicated that PPARD gene expression in muscles of the drug group was significantly reduced (Fig. 1d).

ELISA was also used to detect the concentration of LPL in muscle tissue. As shown in Figure 2a, the concentration of LPL in the experimental group was lower than that in the control group, with statistical significance ($P < 0.05$). Figure 2b showed that the concentration of CYP7A1 in the experimental group was lower than that in the control group, with statistical significance ($P < 0.05$). As shown in Figure.2c, the concentration of HMG-CoA in the experimental group was higher than that in the control group, with statistical significance ($P < 0.05$).

H&E staining and immunohistochemical analysis:

Hematoxylin-Eosin (H&E) staining is one of the most commonly used techniques, a widely used stain in medical diagnosis. The H&E staining of muscle from different experimental group(ctrl, low, mid, high)showed in Fig S1. Tissue stained with hematoxylin and eosin shows cytoplasm stained pink-orange and nuclei stained darkly, either blue or purple. We can see the difference in the range of cytoplasm and nuclei in the groups. However, the target changes still need more information.

Immunohistochemical (IHC) results showed that the expression of (a)Angptl4, (b)KLF10, (b)PDK4, (d)SLC22A5 protein in the experimental group was significantly higher than that in the control group (Fig S2a, b, c, d). IHC results also showed that PPARD protein expression in the experimental group was significantly lower than in the control group (Fig. 4e).

Discussion

Effects of *G. elegans alkaloids* on the protein expression of ANGPTL4 pathway-related genes in different groups of pigs are shown in Fig3. The SLC22A5, KLF10, PDK4, and ANGPTL4 protein abundance slightly up-regulated through the change of the *G. elegans alkaloids* from low to high. On the contrary, the PPARD protein abundance significantly down-regulated through the concentration. Finally, we summarize the result of different visions of the PPARD related pathway in Table 3.

Previous studies have shown that PPARD is mainly involved in regulating fat metabolism, which is closely related to obesity, diabetes and other diseases[23]. It plays an essential regulatory role in fatty acid oxidation in muscle, fat and other tissues and is also related to insulin sensitivity since it regulates the expression of enzymes needed for fat metabolism.

According to our former transcriptome, the RT-PCR analysis presents the best results, and a significant difference in each gene is observed. ELISA analysis shows the LPL and CYP7A1 down-regulated. The HMG-CoA, however, positively regulated significantly in the ELISA test. The Western-blot analysis and Immunohistochemistry analysis demonstrate the same results in the changing style according to the *G. elegans alkaloids.*

PPARD regulates lipase oxidation and energy decoupling and its primary regulatory mechanism. Firstly PPARD is a regulatory factor for the expression of enzymes needed in fat metabolism, which enhances the activity of PPARD to increase the expression of various enzymes, thus increasing fat metabolism and energy consumption[24]. Secondly, PPARD regulates the body's fat metabolism and is a sensor of plasma-free fatty acids in the liver[25]. Thirdly, PPARD is closely related to fat metabolism and cell proliferation, cell differentiation, migration, apoptosis, and inflammation. For macrophages, artificial PPARD ligands were used to treat them, and the inflammatory molecular products were reduced, suggesting that PPARD has anti-inflammatory effects[26]. Moreover, PPARD p 53 gene in mice, macrophages of mononuclear cells in protein chemistry agent and the expression of matrix metalloproteinases nine less than the control group, PPARD can slow down the role of inflammation[27].

Current research suggests that PPARD quantity is low when expressed in the fat cells and white adipose tissue(WAT); however, raising brown adipose tissue (BAT) and metabolism of fatty acid oxidation and oxidative phosphoric acid dissolves the decoupling of related gene expression. Ultimately leads to less diet-induced obesity in mice, reduce fat cell triglycerides (TG) levels and the concentration of plasma-free fatty acids[28].PPARD may also be associated with the differentiation of preadipocytes, and PPARD gene knockout mice with BAT and WAT are associated with weight loss[29]. In addition, mice with the PPARD gene knocked out were fed on a high-fat diet, and it was found that plasma TG and very-low-density lipoprotein(VLDL) levels were significantly increased, VLDL content in the liver was increased. Furthermore, lipoprotein esterase activity was decreased, which further indicated that PPARD could affect the lipid metabolism function of the liver by regulating blood lipid levels[30].

This part is also basically consistent with our previous studies on physiological and biochemical indicators(Table 4), so our *G. elegans alkaloids* regulation is likely to be regulated through the PPAR pathway.

Conclusion

A critical finding in this paper is the regulation of *G. elegans alkaloids* on the PPAR pathway. *G. elegans alkaloids* can prevent the expression of PPARD and induce the ANGPTL expression, thereby regulating lipid metabolism and oxidative stress of muscle. The other important finding in this paper is the regulation of *G. elegans alkaloids* on PPAR-related pathways. *G. elegans alkaloids* can also up-regulate the expression of PDK4, KLF1, and CYP7A1 and thereby modulate the antioxidative and pro-oxidative enzymes to activities. The proposed regulatory mechanism of *G. elegans alkaloids* against lipid was shown in Fig. 4.

Abbreviations

T3	triiodothyronine
T4	thyroxine
INS	insulin
TP	total protein
ALB	albumin
GLOB	globulin
A/G	albumin/globulin
AKP	alkaline phosphatase
ALT	alanine transaminase
AST	aspartate transaminase
AST/ALT	aspartate transaminase/alanine transaminase
BUN	blood urea nitrogen
TCHO	cholesterol
LDH	lactate dehydrogenase
C3	complement 3
C4	complement 4
IgA	immunoglobulin a
IgG	immunoglobulin g
IgM	immunoglobulin m
ANGPTL4	angiopoietin-like 4
PPARD	peroxisome proliferator-activated receptor-δ
LPL	Lipoprotein lipase
TCM	traditional Chinese medicine
HMG-CoA	3-hydroxymethyl glutaratemonoacyl CoA
H&E	hematoxylin-eosin
IHC	Immunohistochemical

Declarations

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Author contributions

WY, HXP and SZL designed research; WY, SSY and WLL performed research; LYS, FYJ and ZNJ analyzed data, and LYS, GJP and WY wrote the paper. All authors have read and approved the manuscript.

Competing Interests

The authors declare that they have no competing interests.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All tissues and blood samples were collected according to protocols approved by the Research Ethic Committee of the School of Veterinary Medicine, Hunan Agricultural University(433320027). Furthermore, all experiments were performed under the institutional guidelines for the care and use of laboratory animals(200705041).

Consent for publication

All authors agreed to have the findings of this research published.

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Tables

Table 1 Statistical significance between groups

1. Thyroxine(T4)			
	Low dose	Medium dose	High dose
Control	Significance	Insignificance	Insignificance
2. Insulin level(INS)			
	Low dose	Medium dose	High dose
Control	N/A	N/A	Significance
Low dose	N/A	Significance	N/A
3. Total protein(TP)			
	Low dose	Medium dose	High dose
Control	Insignificance	Insignificant	Significance
Low dose	N/A	N/A	Significance
4. Globulin assay(GLOB)			
	Low dose	Medium dose	High dose
Control	Significance	Insignificance	Insignificance
5. White ball ratio			
	Low dose	Medium dose	High dose
Control	Significance	Insignificance	Significance
6. Alkaline phosphate(AKP)			
	Low dose	Medium dose	High dose
Control	Insignificance	Significance	Insignificance
Low dose	N/A	Insignificance	Insignificance
Medium dose	Insignificance	N/A	Significance
High dose	Insignificance	Significance	N/A
7. Alanine aminotransferase(ALT)			

	Low dose	Medium dose	High dose
Control	Insignificance	Significance	Insignificance
Low dose	N/A	Significance	Insignificance
Medium dose	Significance	N/A	Significance
High dose	Insignificance	Significance	N/A
8. Aspartic acid			
	Low dose	Medium dose	High dose
Control	Significance	Significance	Significance
9. AST/ALT measurement			
	Low dose	Medium dose	High dose
Control	Insignificance	N/A	Insignificance
Low dose	N/A	N/A	Insignificance
Medium dose	N/A	N/A	N/A
High dose	Insignificance	N/A	N/A
10. Urea			
	Low dose	Medium dose	High dose
Control	Insignificance	Insignificance	Insignificance
11. lactate dehydrogenase			
	Low dose	Medium dose	High dose
Control	Significance	Significance	Significance
12. Immunoglobulin A assay			
	Low dose	Medium dose	High dose
Control	Insignificance	Significance	Insignificance
Low dose	N/A	Insignificance	Insignificance
Medium dose	Insignificance	N/A	Significance

High dose	Insignificance	Significance	N/A

Table 2 Primer sequences used for real-time quantitative PCR.

Gene	Primer	Sequence（5’-3’）
KLF10	Forward	TGCTCAACTTCGGCGCTTC
	Reverse	AGCTTTGTTCCACGAATACACA
SLC22A5	Forward	GCTACAGTCCTGGTGATGG
	Reverse	AGTAGGGAGACAGGATGCT
PDK4	Forward	AGACACCATAATGTAGTTCCT
	Reverse	GGATTTGCTGTCTGTAAGTC
PPARD	Forward	AACGAGGGGAGTCAGCACA
	Reverse	CGATGTCGTGGATCACAAAG
ANGPTL4	Forward	GGAGAAGCAGCACTTGAGAA
	Reverse	GGGTCATCTTGGGTAGTCTTT

KLF10, Kruppel Like Factor 10; SLC22A5, Solute Carrier Family 22 Member 5; PDK4, Pyruvate Dehydrogenase Kinase 4 ; PPARD, peroxisome proliferator activated receptor δ; ANGPTL4（Angiopoietin Like 4

Table 3 The Gene and related proteins changes through the GE alkaloids treated muscle

	Gene or related proteins	Treated（Low, Mid, High）VS CTRL
RT-PCR analysis	Angptl4	+ +
	KLF10	+ +
	PDK4	+ +
	SLC22A5	+ +
	PPARD	− −
Western-blot analysis	SLC22A5	+
	KLF10	+
	PPARD	− −
	PDK4	+
	ANGPTL4	+
ELISA analysis	LPL	− −
	CYP7A1	− −
	HMG-CoA	+ +
Immunohistochemistry analysis	Angptl4	+ +
	KLF10	+ +
	PKD4	+ +
	SLC22A5	+ +
	PPARD	− −

++（*Up-regulated significantly*）（− −）*Down-regulated significantly*

Table 4 Effect of GE alkaloids on blood biochemical indexes of piglets

	Control group	Low-dose group	Middle-dose group	High-dose group
T3	2.18±0.17	2.07±0.08	1. 91±0.27	1.96±0.21
T4	95.57±12.38	106.14±7.06	97.14±6.04	98.57±7.66
INS	0.80±0.28	0.81±0.25	0.89±0.29	1.10±0.28
TP	62.43±2.88	61.00±1.15	64.14±1.68	66.14±3.39
ALB	35.29±2.56	35.29±1.38	36.00±1.53	36.71±1.60
GLOB	28.14±2.12	24.71±1.80	29.86±1.86	28.86±3.85
A/G	1.22±0.13	1.48±0.16	1.24±0.12	1.41±0.20
AKP	218.14±42.01	195.86±21.15	187.43±14.55	181.71±24.34
ALT	44.00±3.12	46.00±6.43	37.57±1.27	44.86±6.96
AST	42.29±7.32	35.29±2.21	32.57±4.12	37.86±6.57
AST/ALT	0.94±0.21	0.72±0.08	0.87±0.09	0.92±0.16
BUN	2.91±0.24	2.79±0.48	2.93±0.14	2.81±0.39
TCHO	2.23±0.19	2.12±0.17	2.14±0.14	2.37±0.17
LDH	713.43±65.73	601.00±29.66	605.71±28.62	617.86±120.94
C3	0.20	0.20	0.20	0.20
C4	0.01	0.01	0.01	0.01
IgA	0.11±0.01	0.11±0.02	0.13±0.03	0.15±0.02
IgG	5.35±0.38	5.51±0.48	5.04±0.44	5.23±0.39
IgM	0.93±0.11	1.00±0.11	0.95±0.11	0.93±0.14

T3:triiodothyronine; T4:thyroxine; INS:insulin; TP:total protein; ALB: albumin; GLOB: globulin; A/G:albumin/globulin; AKP: alkakine phosphatase; ALT: alanine transaminase; AST:aspartate transaminase; AST/ALT: aspartate transaminase/alanine transaminase; BUN:blood urea nitrogen; TCHO:cholesterol; LDH: lactate dehydrogenase;C3:complement 3; C4:complement 4; IgA:immunoglobulin a; IgG:immunoglobulin g; IgM: immunoglobulin m

Figures

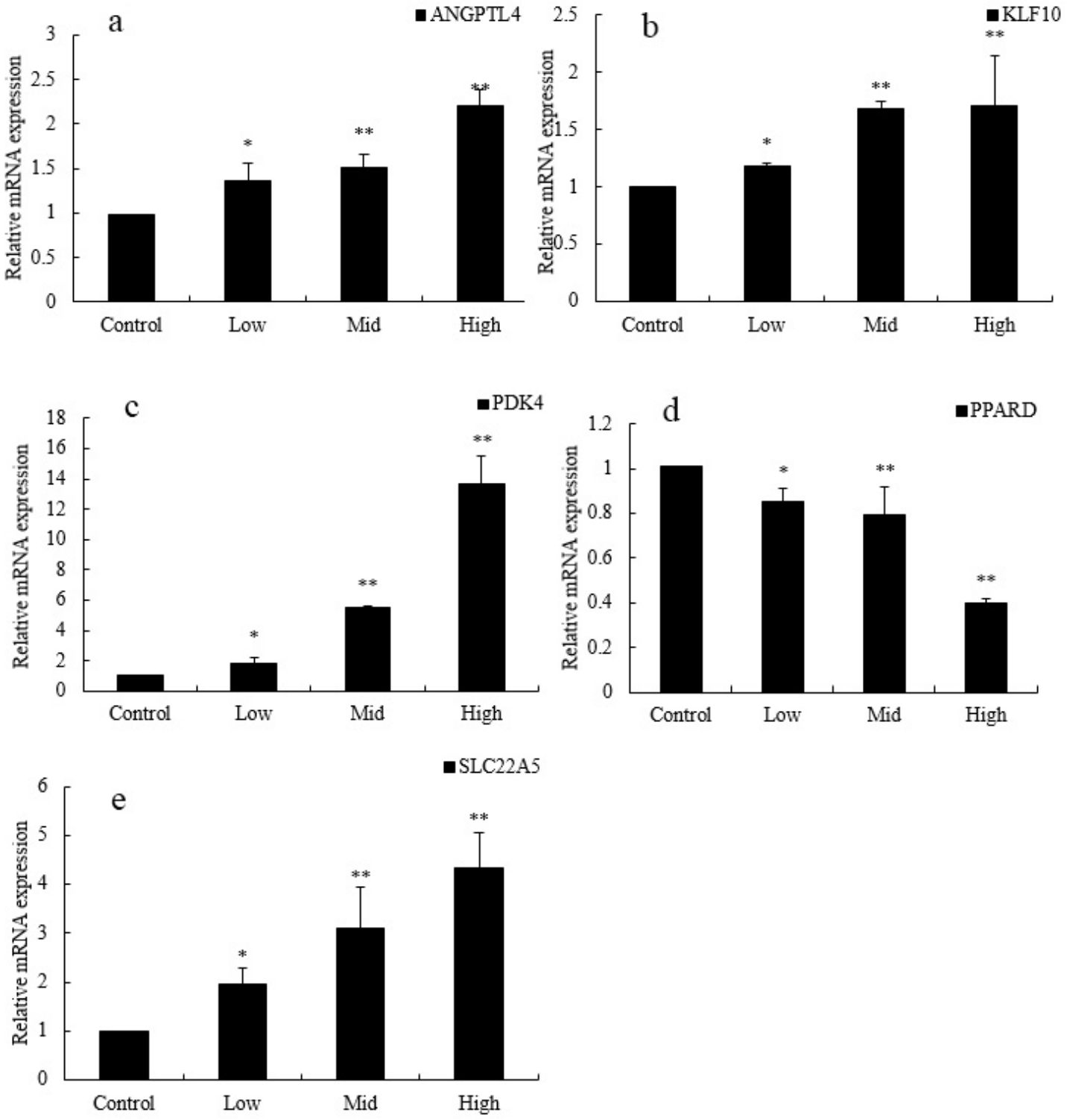

Figure 1

G. elegans alkaloids induces ANGPTL4, KLF10,PDK4 and SLC22A5 gene expression in pig muscle. KLF10, PDK4, and SLC22A5, are PPARD target as well as LPL. On the contrary, the PPARD gene expression is significantly being dissuaded. Error bars represent SEM. *Significantly different according to paired Student t test ($P < 0.01$). ANGPTL4：Angiopoietin Like 4；SLC22A5：Solute Carrier Family 22

Member 5KLF10, Kruppel Like Factor 10; PDK4,Pyruvate Dehydrogenase Kinase 4;PPARD,Peroxisome Proliferator Activated Receptor Delta.

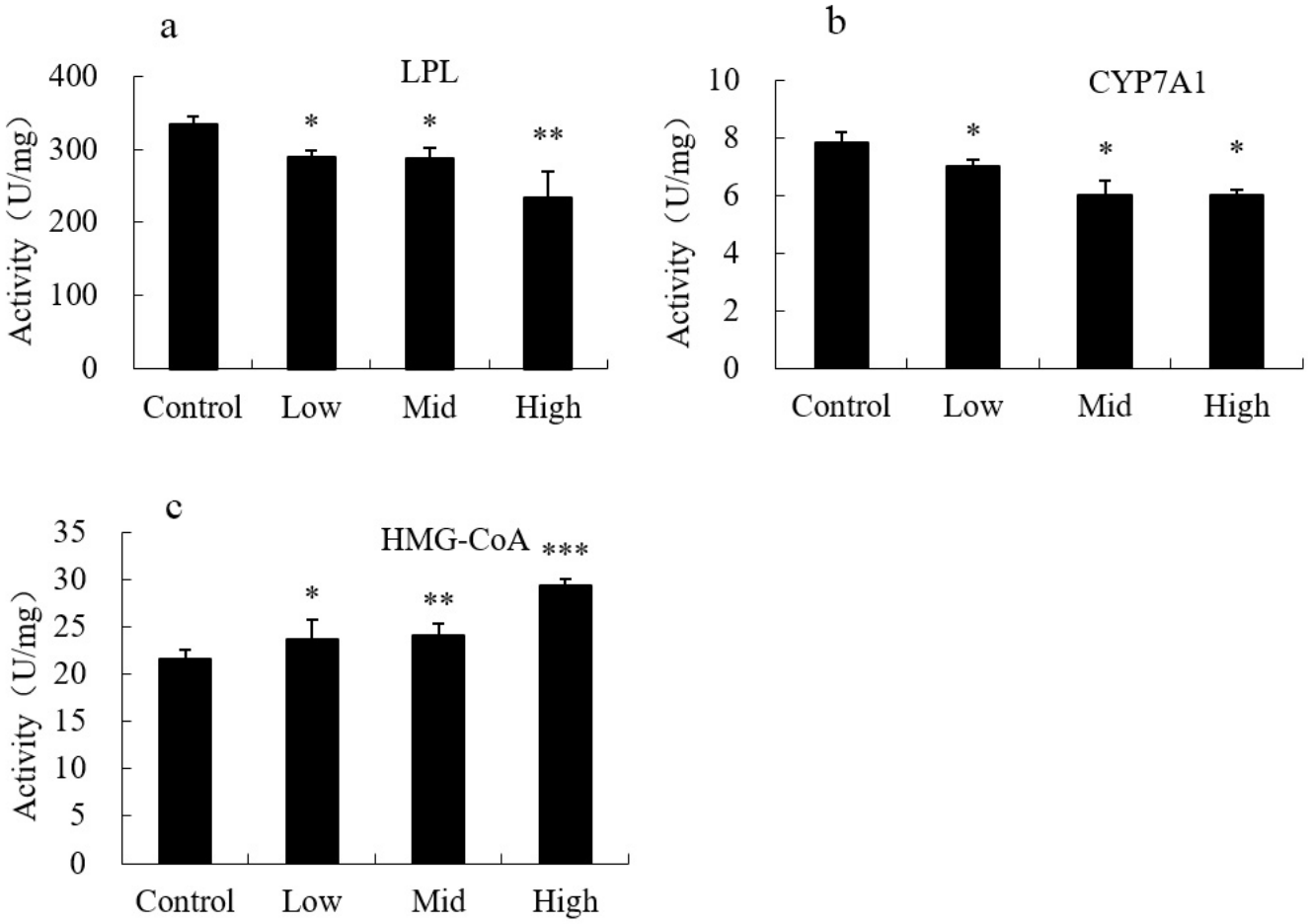

Figure 2

LPL, CYP7A1 and HMG-CoA protein levels in post-treated muscle tissue as determined by ELISA. Error bars represent SEM. *Significantly different according to paired Student t test ($P < 0.01$).

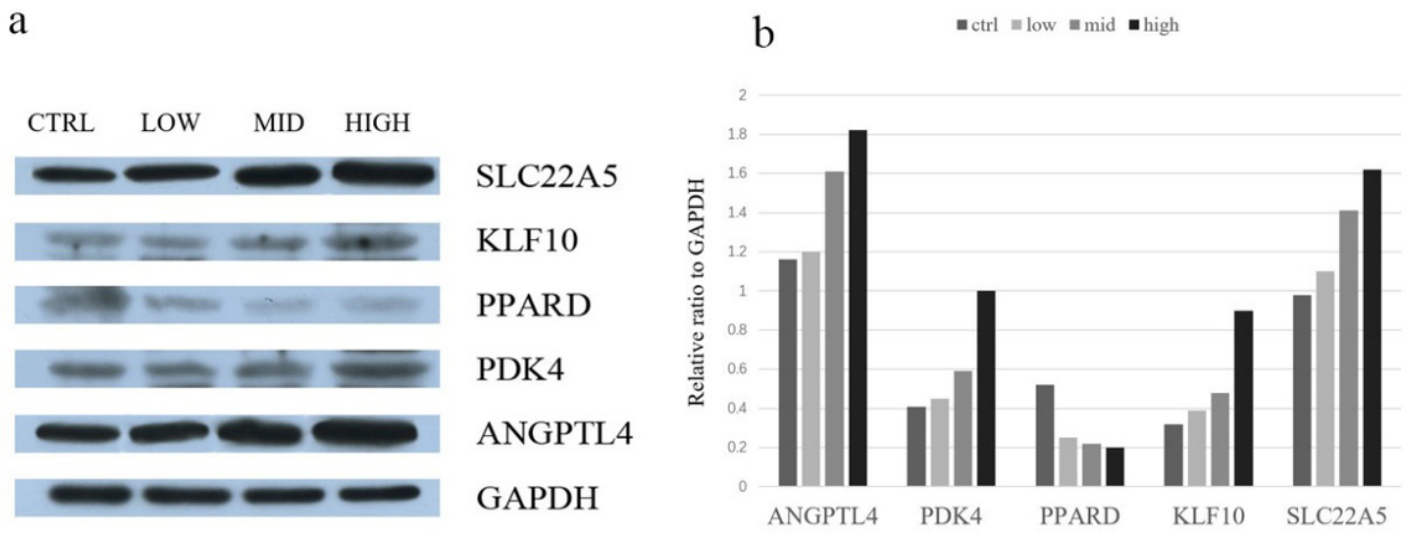

Figure 3

Effects of G. elegans alkaloids on the protein expression of ANGPTL4 pathway related genes in different groups of pigs. a) SLC22A5, KLF10, PPARD,PDK4,ANGPTL4 protein abundance in pig skeletal muscle. b) The relative ratio of GAPDH show in the histogram. ANGPTL4：Angiopoietin Like 4；SLC22A5：Solute Carrier Family 22 Member 5；KLF10,Kruppel Like Factor 10;PDK4,Pyruvate Dehydrogenase Kinase 4;PPARD,Peroxisome Proliferator Activated Receptor δ； GAPDH：Glyceraldehyde-3-Phosphate Dehydrogenase.

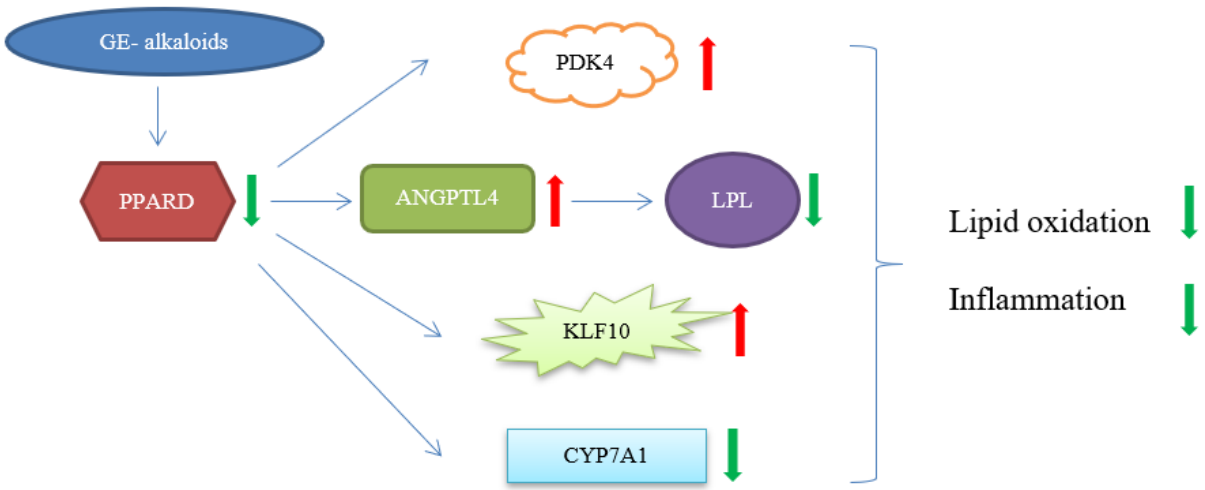

Figure 4

Proposed mechanism of G. elegans alkaloids on pig muscle Red arrow means up-regulated, and the green arrow means down-regulated

Supplementary Files

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- Supplement.docx