Study on reproductive hormone disorder causing N-glycan structure change by swainsonine poisoning

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Abstract

Background: Glycosylation and glycan composition play an important role in the biological properties of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Swainsonine (SW) causes oligosaccharide accumulation and incomplete glycoprotein processing. Previous research has shown that SW reduces serum progesterone concentrations in pregnant livestock. However, we do not know how SW affects the secretion of reproductive hormones. Materials: We used primary culture of mouse endometrial epithelial cells, Real-time polymerase chain reaction, Western blot, Periodic acid-Schiff (PAS) staining, Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF-MS), ELLSA, etc. method. Results: In this study, we observed a significant decrease in the number of surviving fetal mice in the SW treatment group mice and a high level of carbohydrate deposition in PAS stained uterine tissue in the SW treatment group mice. Extensive vacuole degeneration can also be observed in mouse primary endometrial epithelial cell cultures in vitro after SW poisoning. MALDI-TOF-MS shows that the time of exposure to SW is prolonged, the number of di-, tri-, and tetra-complex glycosylated gradually decreases, and the number of tri- hybrid type glycosylated increases, while significantly reduced activity of glycosyltransferases and glycosidases are observed in endometrial epithelial cells. FSH and LH were significantly decreased at 7 and 15 days after pregnancy and 7 days after childbirth, the content of E2 and P4 decreased significantly at 7 days after childbirth, and the expression levels of mRNA and protein of 3β-hydroxysteroid dehydrogenase (HSD-3β) and cytochrome P450 family 19 subfamily A member 1 (CYP19A1) also showed a significant decrease at 15 days after pregnancy and 7 days after childbirth. Conclusions: In summary, The glycan chain structure of FSH and LH glycoprotein hormones were changed by swainsonine, which they are decreased on the expression of the limiting enzyme of steroid hormones indirectly, and ultimately lead to a decrease in the content of E2 and P4. The rate of female mice were decreased by dysfunction of the reproductive hormones.

Background

As a kind of trihydroxy indolizidine alkaloid,swainsonine (SW) is the major toxic constituent in locoweed. SW cation and mannose are similar in structure ,but SW cation has a higher affinity for mannosidase than mannose. SW is well-known to inhibit the activity of lysosomal a-mannosidase I and Golgi mannosidase II [1,2]. On account of the enzymatic dysfunction and the accumulation of complex oligosaccharides in lysosomes caused by swainsonine poisoning,we can infer that SW has an obvious inhibitory effect on a-mannosidase in lysosomes,futhermore, it inhibits glycoprotein synthesis as well as the production of a mixture of mannose and asparagine polysaccharide.These factors lead to vacuolar degeneration in different cells [3].Pregnant and nonpregnant animals (such as sheep,goats and cattle) were studied in vivo,then the experimental result revealed that ingestion of SW-containing plants can reduse serum progesterone concentration and subsequently destroy ovarian function, accompanied by deferred estrus, extended estrous cycle, delayed conception, and abortion [4]. However, we do not know how SW leads to decreased progesterone content. Progesterone, the primary steroid synthesized by luteal cells, is essential for early embryo development, implantation, maintenance of pregnancy, and controlling

the length of reproductive cycles in mammals [5,6]. Therefore, a specific progesterone concentration is required for maintenance of the estrous cycle and a successful pregnancy [7,8]. The hypothalamus-pituitary axis secretes follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which together promote mature follicles, ovulation, production of corpus luteum, secretion of progesterone and estrogen [9-11]. Therefore, this study aimed to determine the effect of SW on disorders of reproductive hormone secretion through histomorphology of mouse uteruses, analysis of cell vacuoles degeneration, level of reproduction hormone, and steroid hormone enzyme expression at the pregnancy period of mice.

Methods

2.1 Experiment animals

Female mice (Source: Fourth Military Medical University) (n=60, 6 weeks old, 30 animals per group, consisting of SW 0.04 mg/kg BW (SW group) and physiological saline 0.04 mg/kg BW (normal group)) were treated 21 days before mating and throughout the mating period. After 21 days of treatment, 60 female mice were observed by vaginal smear method. The day of successful mating was designated as day 0 of pregnancy. We recorded the number of implantations, number of fetuses, and number of absorbed embryos of the female mice at day 15 of pregnancy. A confirmed pregnant mouse continued to receive SW throughout the parturition and lactation periods. The female mice were sacrificed 150 ~ 200mg / kg pentobarbital sodium intraperitoneal injection, if necessary, check whether the animal's heart beats, and the blood, uterus, and ovaries were collected at day 7 or 15 of pregnancy and day 7 of childbirth. These uteruses were stained with Periodic acid-Schiff stain (PAS) at day 15 of pregnancy.

This study was performed with the approval of the local ethics committee, and all the experiments were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All experimental procedures were conducted in conformity with institutional guidelines for the care and use of laboratory animals in Northwest A&F University, China, and conformed to the National Institutes of Health Guide for Care and Use of Laboratory Animals.

Fetuses and the baby mice were euthanized after the study, and the method of euthanasia was consistent with the method of collecting samples to kill the mice150 ~ 200mg / kg pentobarbital sodium intraperitoneal injection, if necessary, check whether the animal's heart beats.

2.2 A mouse primary culture of endometrial epithelial cells

6-to 8-week-old proestrus female mice (22-30 g body weight) were selected for the experiment. Each mouse injected 5IU PMSG (Pregnant mare serum gonadotropin) and human chorionic gonadotrophin (hCG) 5 IU after 48 h. With sterile harvest bilateral uterine surgery and first minced with a scalpel into 1-mm3 pieces in phosphate-buffered saline (PBS) solution and then digested in Hanks Buffered Salt Solution (HBBS) without Ca^{2+} $Mg^{2+}$and containing 6.4 mg/mL collagenase type I, 125 U/mL hyaluronidase, and 0.1 nmol/L gentamycin suspensions containing single cells and luminal epithelial sheets and glandular epithelial fragments. Purified endometrial epithelial cells: Using the attached

purification technology (stromal cells and epithelial cells of different adherent time: 30 min stromal cells, epithelial cells 2-24 h) cells further purification[12].

2.3 Protein extraction

Cells were treated with SW and 0.01M PBS for 12 h and 36 h, washed three times with ice-cold PBS, the cells were transferred to sterile EP tubes and centrifuged at 4℃ at1000 rpm/min for 5 min. The following operations are performed on the ice, and 10^7 cells treated with ice-cold radio-immunoprecipitation assay lysis buffer with 1 mM phenylmethyl sulfonyl fluoride. Repeatedly beat to protein precipitation and centrifuged at 4℃ at14,000 rpm/min for 15 min. The supernatant, which contained total protein extracts of cells, was aliquoted and stored at -80℃. Protein concentration was determined using the BCA Protein Assay Kit [13].

2.4 N-glycopeptide enrichment by hydrophilic interaction chromatography (HILIC)

8g protein sample added UA buffer(8M urea,0.1 M Tris-HCl，pH=8.0), and 4μL DTT to 300ul. Mixed sample centrifuged at 4℃ at14,000 rpm/min for 10 min. And then 180μL UA buffer and 4μL DTT(20mM) added to Ultrafiltration tube at 37℃ oven for 4h. The mixture were centrifuged at 4℃ at14,000 rpm/min for 15 min; To join the 180 uL UA buffe and 10 uL IAA (50 mm), mix vortex, and reacted 30 min in the dark room. After it were high-speed centrifuged at 4℃ at14,000 rpm/min for 15min and added 200ul UA buffer by high-speed centrifugal x 15 min (14000 g),and repeat this step once; Continue to join 200 u L to sialic acid reaction buffer,high-speed centrifugal x 15 min (14000 g), and repeat twice. After the effluent was discarded, 180 uL of desialic acid Buffer was added to the ultrafiltration tube, desialidase was added, and the enzyme was digested overnight. Centrifugation was performed at a high speed (14,000g×15 min). After the effluent was discarded, 200uL 50mM NH4HCO3 was added for centrifugation at a high speed (14,000g×15 min) and repeated twice.After the effluent was discarded, a new receiving tube was replaced, and 180uL 50mM NH_4HCO_3 was added to the ultrafiltration tube, and according to the protein: PNG-F enzyme (100:1 quality ratio,) PNG-F enzyme and 1% Rapigest were added, enzyme digestion at 37℃for 16 h. Elution and collection of carbohydrate chain : The ultrafiltration tube after 16h of enzymatic hydrolysis reaction was placed in a high-speed centrifuge for high-speed centrifugation (14000g×10 min). It was eluted twice with 50mM NH_4HCO_3 (prepared when using), 150uL each time, centrifuged at high speed (14,000g×10 min), and eluted with 150uL double distilled water, centrifuged at high speed (14,000g×10 min).The above solution was recovered, combined, acidified with 0.1%TFA, and freeze-dried for later use [14].

2.5 N-glycan enzyme activity analysis

The protein was extracted from a mouse primary culture of endometrial epithelial cells. The α-mannosidase-I, α-mannosidase-II, N-acetylglucosaminyltransferase-I and N-acetylglucosaminyltransferase-II concentrations were measured using a mouse enzyme-linked immunosorbent assay (ELISA) kit (Good ELISA Kit Producers) according to the manufacturer's instructions. Endometrial epithelial cell culture supernates for 20 minutes at 1000×g. Particulates were

removed and assayed immediately or samples were stored in aliquot at -20℃ or -80℃ for later use, avoiding repeated freeze/thaw cycles.

2.6 Serum reproductive hormone assay

Mouse serum was collected at days 7 and 15 after pregnancy and day 7 after childbirth. Serum follicle-stimulating hormone (FSH), luteotropin (LH), 17β-estradiol (E_2), and progesterone (P_4) were measured using a mouse ELISA kit (Biostest, EM2088, EM2089, EM2093, HM2061) according to the manufacturer's instructions.

2.7 Real-time polymerase chain reaction (PCR)

Total RNA was extracted using Trizol reagent from mouse ovaries at days 7 and 15 of pregnancy and day 7 after childbirth. For the removal of residual genomic DNA, these samples were treated with DNaseI. The first-strand cDNA was synthesized using a first strand cDNA synthesis kit, and quantitative real-time PCR was carried out using SYBR Green (SYBR Green Real-time PCR Master Mix QPK-201; Toyobo Co., Japan.). Specific PCR settings were used in a Bio-Rad iQ5 Real-time PCR system. To verify PCR product purity, samples were subjected to melting curve analyses after real-time PCR reactions. The threshold cycle (CT) numbers were calculated for the amplified cDNA for each investigated mRNA and for the housekeeping gene (β-actin) in each sample. The forward and reverse primers for P450scc(cholesterol side-chain cleavage enzyme) were as follows: (forward: 5'- GAGAGAGAGAAGGATCCAAG AGCT-3'; reverse: 5'- GAGAGAG AGATT CTCGAGTT-3' product size 165 bp), StAR (steroidogenic acute regulatory protein) (forward: 5'-AGAAGTTCAAGTT GT-3'; reverse: 5'-CTTGCTCTCCTCGA-3' product size 202 bp), HSD-3β (3beta-hydroxy steroid dehydrogenase) (forward: 5'-GGCTTCCAACCATCTAAC A-3'; reverse: 5'-CTCCTTCTCCTCCACCAA-3' product size 179 bp), CYP19A1 (cholesterol side-chain lyase) (forward: 5'-GACTC CGGTACGGACG-3'; reverse: 50-TCTTAAGTACGCTC-30 product size 207 bp) [13].

2.8 Western blot

Total protein was extracted using RIPA reagent from mouse ovaries at days 7 and 15 of pregnancy and day 7 after childbirth. Firstly,rinsed the ovaries three times with ice-cold PBS and lysed in radioimmunoprecipitation assay (RIPA) buffer with 1% phenyl methylsulfonyl fluoride (PMSF) for 5 minutes. Secondly, centrifuged the lysates at 15,000 g at 4℃ for 10 minutes and collected the supernatants. Thirdly,determined the protein concentrations by the BCA protein assay kit (ThermoFisher Scientific, Waltham, MA, USA). Fourthly,electrophoresed ovary lysates(30 mg/lane) with 10% SDS-PAGE and then transferred them onto polyvinylidene fluoride (PVDF) membranes. Fifthly, blocked the membranes with 5% skim milk at room temperature for 2 hours. Sixthly,incubated the membranes with specific primary antibodies (1:1000 in PBS buffer) recognizing P450scc, StAR, HSD-3β, CYP19A1, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH, Cell Signaling Technology, Danvers, MA, USA)at 4℃ overnight . Seventhly,after washing with Tris-buffered saline (TBS) (pH8.0) containing 0.1% Tween-20, horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G (IgG, Bioworld Technology Co., St Louis Park, MN, USA) in washing solution was added and incubated for 1 hour at room

temperature.Finally, immunoreactive proteins were detected using SuperSignal chemiluminescence and protein bands were digitally imaged for densitometric quantification using a software program (Eastman Kodak Company, Rochester, NY, USA).

2.9 Statistics

For each experiment, 4 to 5 female mice per group were used. Data were reported as mean±SD. Statistical analysis was performed by SPSS software and included Student's t-test or one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. A *P* value < 0.05 was considered significant. **P*<0.05 each group versus the control group at the same time.

Results

3.1 Effect of SW on mouse embryo toxicity

The female mice were injected with SW by intraperitoneal injection and died at 15 days after pregnancy. We observed the number of implantations, embryo number, and number of embryos absorbed. The results showed that embryo number of the SW treatment group was significantly decreased in normal groups (*P*<0.05). The embryo absorption number is significantly higher than that of mice in the normal group (*P*<0.05). However, there was no significant difference between the SW group and normal group in number of implantations (*P*>0.05) (Table-1).

Table 1. Effect of swainsonine on mouse embryo toxicity

	Number of implantations	Embryo number	Embryo absorption number
SW groups	13±2	6±1	7±1
Normal groups	15±2	13±2*	2±1*

3.2 Histological changes in the uteruses of female mice after SW treatment

The female mice were intraperitoneally injected with SW and died at 15 days after pregnancy. We collected uterine tissue from both groups of mice mouse. Extensive carbohydrate accumulation was observed on the endometrium lamina propria using PAS staining (Figure 1 B). Subsequently, we cultured the primary endometrial epithelial cells of mice in vitro after SW poisoning and extensive vacuole degeneration was found in the endometrial epithelial cells (Figure 1 D).

3.3 Change of N-glycan structure after SW treatment

The results showed that the compound type glycan peak (tetra-glycosylated 2540 ($HexNAc_6Hex_7Fuc$), tri-glycosylated 2337 ($HexNAc_5Hex_7Fuc$)), disappeared in the SW-I group (SW, 12h) compared with the control group. Meanwhile, the new structure of hybrid glycan type (2133 ($HexNAc_4Hex_7Fuc$), 1768 ($HexNAc_3Hex_6Fuc$), and 1622 ($HexNAc_3Hex_6$) appeared in the SW-I group (SW, 12h) compared with the control group. The results showed that the compound glycan type peak (tetra-glycosylated 2540 ($HexNAc_6Hex_7Fuc$), tri-glycosylated 2337($HexNAc_5$ Hex_7Fuc), tri-glycosylated 2174 ($HexNAc_5Hex_6Fuc$), di-glycosylated 1809 (HexN Ac_4Hex_5Fuc), and di-glycosylated 1663 ($HexNAc_4Hex_5$)) disappeared in the SW-II group (SW, 36 h) compared with the control group. Meanwhile, the new glycan structure of hybrid type (2133 ($HexNAc_4Hex_7Fuc$), 1768 ($HexNAc_3Hex_6Fuc$), and 1622 ($HexNAc_3Hex_6$) peak of spectra mass) appeared in the SW-II group (SW, 12 h) compared with the control group (Figure 2).

3.4 Effect SW on N-glycan enzyme activity in mouse endometrial epithelial cells

The total proteins were extracted from the primary endometrial epithelial cells of mice in vitro and the activity of glycosyl transferase and glycosidase was analyzed by ELISA methods. The results found that activity of a-mannosidase-II and N-acetyl glucosaminyltransferase-II in SW group cells were significantly decreased compared with normal cells after culturing for 12, 24 and 36 h (P<0.05); The activity of a-mannosidase-I and N-acetylglucosaminyltransferase-I in SW group cells were significantly decreased compared with normal cells after culturing for 24 and 36 h (P<0.05) (Figure 3).

3.5 Effect SW on reproductive hormone levels in mouse blood

The serum sample were collected from two groups of mice on days 7 and 15 of pregnancy and day 7 after childbirth.The level of P4, E2, FSH and LH were measured by ELISA methods.The results found that the FSH and LH level of SW group mice were significantly decreased compare to the normal group mice at days 7 and 15 after pregnancy and days 7 after childbirth (P<0.05). The E2 and P4 content of SW group mice were significantly decreased compare to the normal group mice at days 7 after childbirth (P<0.05) (Figure 4).

3.6 The expression analysis of speed limit of steroid hormone enzyme

The ovary samples were collected from the two groups of mice, and the total RNA and protein were extracted at days 7 and 15 of pregnancy and day 7 after childbirth. The P450scc, StAR, HSD-3β, and CYP19A1 mRNA and protein expression were measured by real-time PCR and western blot. There were no significant differences between SW and normal group mice in P450scc, StAR mRNA, and protein expression at days 7 and 15 of pregnancy and day 7 after childbirth (P>0.05). There were no significant differences between SW and normal group mice in HSD-3β and CYP19A1 mRNA and protein expression at day 7 after pregnancy (P>0.05). The HSD-3β and CYP19A1 mRNA and protein expression in SW group mice were significantly lower than those of normal group mice at day 15 of pregnancy and day 7 after childbirth (P<0.05) (Figure-5).

Discussion

Our current understanding of SW is that it causes changes to N-glycan chain structures and glycoform that lead to disorders of reproduction hormones. According to the indicators of SW, it is locoweed's (*Astragalus* spp. and *Oxytropis* spp.) main toxic substance. The female mice were intraperitoneally injected with SW, and the number of live fetuses and fertilized implantations were observed at day 15 after pregnancy. We found that the number of live fetuses in SW group mice were lower than those of normal group mice. Therefore, we collected mouse uteruses for PAS staining and found extensive accumulation of red material (related carbohydrate) in the endometrium lamina propria. Subsequently, we cultured the primary mouse endometrial epithelial cells, which displayed vacuolar degeneration after SW treatment [15-17]. We extracted total protein from two group cells and analyzed it by MALDI-TOF-MS. The results showed that the number of hybrid type N-glycan were higher, and the number of compound type glycan were decreased compared with normal cells. It was caused by the half-chair structure of the SW cation, and the mannose cation formed in the process of mannose's hydrolysis is very similar [18]. SW is a well-known inhibitor of lysosomal a-mannosidase-I and Golgi a-mannosidase II. Poisoning induces enzymatic dysfunction and the accumulation of oligosaccharides in lysosomes [19,20]. Treatment of cells with the Golgi mannosidase II inhibitor, SW, results in the accumulation of hybrid type structures with a Man5GlcNAc2 core and one complex branch on the a1,3-core mannose [21]. In the present study, a-mannosidase-I,II activity declined, and N-acetylglucosamine transferase-I,II activity was decreased in endometrial epithelial cells after SW treatment. $Man_5GLcNAc_2$ is modified by N-acetyl glucosaminyl transferase, which links with an N-acetylglucosaminyl transferase to form $GLcMan_5\ GlcNAc_2$. Then, it is transported to a Golgi apparatus cavosurface by II-type a-mannose glycosidase shear, losing two mannose forms of GLcMan3GlcNAc2 [22]. Combined, these results support an essential role for the activity of glycosyl transferase, and glycosidase was significantly decreased as an extension of time for SW in the N-glycan process. Meanwhile, the number of compound-type glycans were gradually reduced, and the number of hybrid-type glycan were increased. Therefore, the conclusion was extensive vacuolar degeneration of the cells, causing accumulation of hybrid type N-glycan.

FSH and LH are glycoprotein hormones and they are similar in chemical structure [23]. They are made up of two different subunits, called alpha (α) and beta (β), which are connected by non-covalent bond to form two diverse heterodimers [24]. These 2 hormones are identical in the protein portion of the α-subunit, while they are various in the protein portion of the β-subunits. Through an N-glycosidic bond, N-glycans (oligosaccharides) can be covalently linked to the proteins of asparagine (Asn) residues [25]. A research group in USA first reported that FSH exists in human pituitaries and urinary preparations as 2 main glycoforms, respectively are tetra-glycosylated (FSH tetra) and di-glycosylated FSH (FSHdi) [26,27].Follow-up studies from the same research group illustrated that most low-glycosylated FSH was tri- but not di-glycosylated [28,29]. These glycoprotein hormones' α-subunit seems always to be modified by two glycans at positions 52 and 78 [28]. Each one of the four pituitary gonadotroph in glycoforms, designated FSHdi, FSHtetra, LHdi, and LHtri, is heterogeneous and present in blood as spectra of isoforms varying in their glycan compositions [30]. In the present study, tri-glycosylated were increased,

while di-glycosylated and tetra-glycosylated disappeared with the extension of SW poison attack time. We speculated that the N-glycan structure of the peptide chains of FSH and LH were also changed after SW treatment. Glycosylation and glycan composition are of fundamental importance for the biological properties of FSH and LH [31,32]. Above all, SW can change N-glycan chain structure and glycoform and increase the number of hybrid-type glycan, although the levels of FSH and LH trend significantly downward.

Gonadotropin plays an important role in the formation of ovarian steroid hormones. LH can stimulate the production of androgens, while FSH stimulates the expression of aromatase. This promotes the synthesis of estrogen in granular cells. FSH can increase the expression and activity of P450arom in granular cells, and LH can increase the expression of P450scc in luteal cells. This increases the production of estradiol and progesterone, respectively [33]. LH can combine with LH receptors on the follicle membrane cells and promotes the synthesis of androgens on membrane cells. The generated androgens pass through the basement membrane and into the granular cells [34]. FSH combines with the FSH receptors on the granular cells and activates the expression of aromatase. Under the action of this enzyme, androgens are aromatized into estrogens. LH can also regulate the transcription of the important regulatory enzymes StAR, P450scc, 3β-HSD, and CYP19 in the sex steroidogenesis pathway through the cAMP/PKA signal pathway. In this experiment, the levels of estradiol and progesterone significantly declined in both late pregnancy and the pre-partum period. Steroidogenic enzymes play an important role in the production of steroid hormones [35]. The expression of StAR catalyzes the combination of mitochondrial inner membrane cholesterol and the cytochrome P450 cholesterol-side chain cleavage enzyme P450scc to start the production of steroids. This is the first enzymatic step of steroid hormone production [36]. Under the action of P450scc, cholesterol is converted to pregnanolone. As a precursor, pregnanolone is metabolized into progesterone by the action of 3β-HSD [37]. Under the catalysis of cytochrome P450 17a-hydroxylase, pregnanolone is used as a substrate to produce androgen [38]. Finally, androstenedione or testosterone is used as a substrate for the synthesis of estradiol, catalyzed by the cytochrome P450 aromatase (P450arom, encoded by the CYP19 gene). In the test, there was no obvious change in the content of testosterone (not listed in the text). There was no significant difference in the expression of mRNA and protein between P450scc and StAR, while the 3β-HSD and CYP19A1 were significantly down-regulated. In summary, the experiment results show that SW poisoning only affects the expression of the rate-limiting enzyme in the later generation stage of steroid hormones. The decline of steroid hormone activity is mainly due to the decrease in the content of FSH and LH, which could not activate the steroid hormone rate-limiting enzyme. This can lead to the decline of aromatase activity, causing a decrease in the content of estradiol and progesterone.

Conclusions

SW inhibits the activity of glycosyltransferases and glycosidases in the processing of N-glycans, its N-glycan chain structure changes, and the number of hybrid glycans accumulates, resulting in vacuole degeneration of cells. Because the increased glycans are all tri-hybrid-type glycosylated, it is speculated that the structure of the peptide chains and sugar chains of FSH and LH changes, resulting in a decrease

in activity of gonadotropin, which indirectly leads to a decrease in the rate-limiting enzyme activity of steroids, eventually resulting in a decrease in the content of E2 and P4. This leads to the disorders of reproductive hormone secretion and the decreased rate of conception in mice.

Abbreviations

MALDI-TOF-MS: Matrix-assisted laser desorption ionization-time of flight mass spectrometry

PAS: Periodic acid-Schiff

HILIC: Hydrophilic interaction chromatography

ELISA: Enzyme-linked immunosorbent assay

SW: Swainsonine

FSH: Follicle-stimulating hormone

LH: Luteinizing hormone

hCG: human chorionic gonadotrophin

HSD-3β: 3β-hydroxysteroid dehydrogenase

CYP19A1: Cytochrome P450 family 19 subfamily A member 1

PMSG: Pregnant mare serum gonadotropin

PBS: Phosphate-buffered saline

TBS: Tris-buffered saline

HBBS: Hanks Buffered Salt Solution

DMEM: Dulbecco's Modified Eagle Medium

FBS: Fetal bovine serum

SDS-PAGE: Sodium dodecyl sulfate polyacrylamide gel electrophoresis

CAN: Acetonitrile

FA: Fatty acids

CT: The threshold cycle

PMSF: Phenyl methylsulfonyl fluoride

PVDF: Polyvinylidene fluoride

Declarations

Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request. All data generated or analysed during this study are included in this published article and its supplementary information files.

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Author Contributions

BY Z conceived and designed the study; HC Z contributed to sample collection; RQ T analyzed the data; CC W and Y W wrote the paper. At the same time all authors have read and approved the manuscript.

Competing Interests

The authors declare no competing financial interest.

Ethics approval and consent to participate

In this study, there were permit to experiment with the approval of the Institutional Animal Care and Use Committee of Northwest A&F University, Yangling, China.

Consent for publication

Not applicable

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Figures

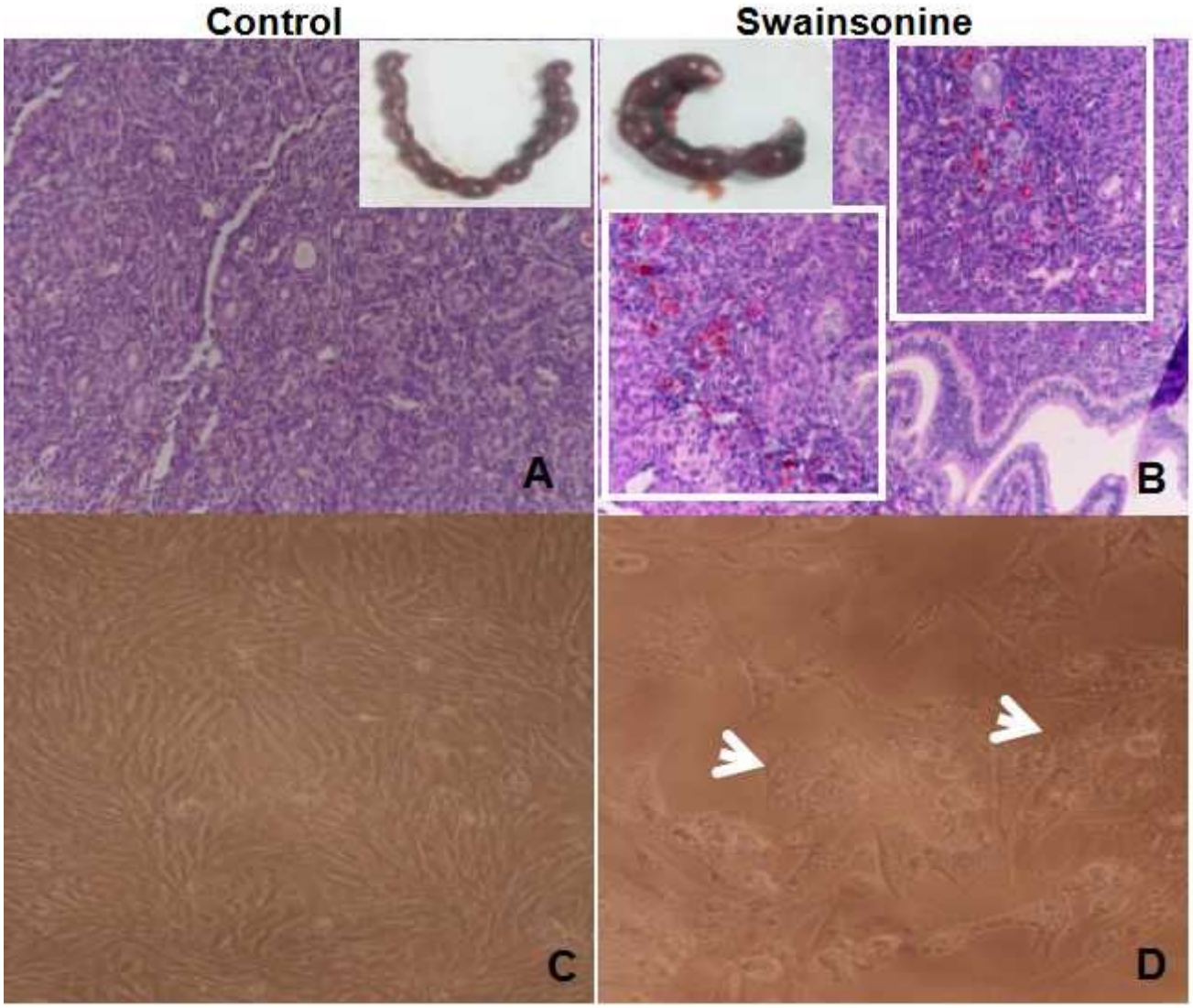

Figure 1

The uterus tissue stained by Periodic acid-Schiff (PAS) at day 15 of pregnancy after physiological saline (A) and SW (B) treatment after culturing the endometrial epithelial cells for 36 h with PBS (C) and SW concentrations 1 mg/mL (D).

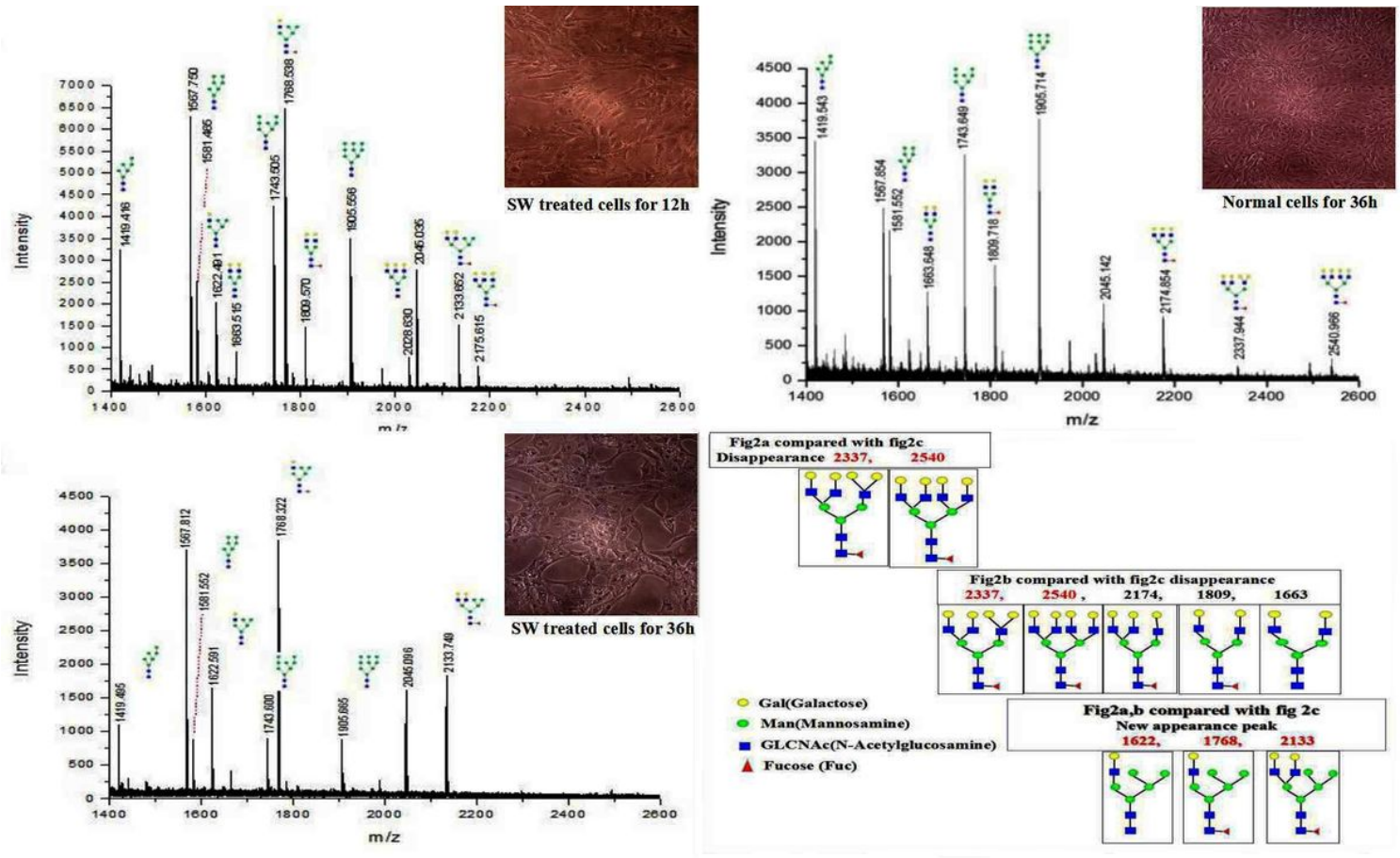

Figure 2

Quantitative MALDI-TOF MS spectra of glycan profile in SW and control groups. (a) The mouse endometrial epithelial cells were treated by SW for 12 h (the upper left); (b) The mouse endometrial epithelial cells were treated by SW for 36 h (the down left); (c) The mouse endometrial epithelial cells were not treated by SW for the control group (the upper right); (a and b) disappearance and appearance peak compared with (c) (the down right).

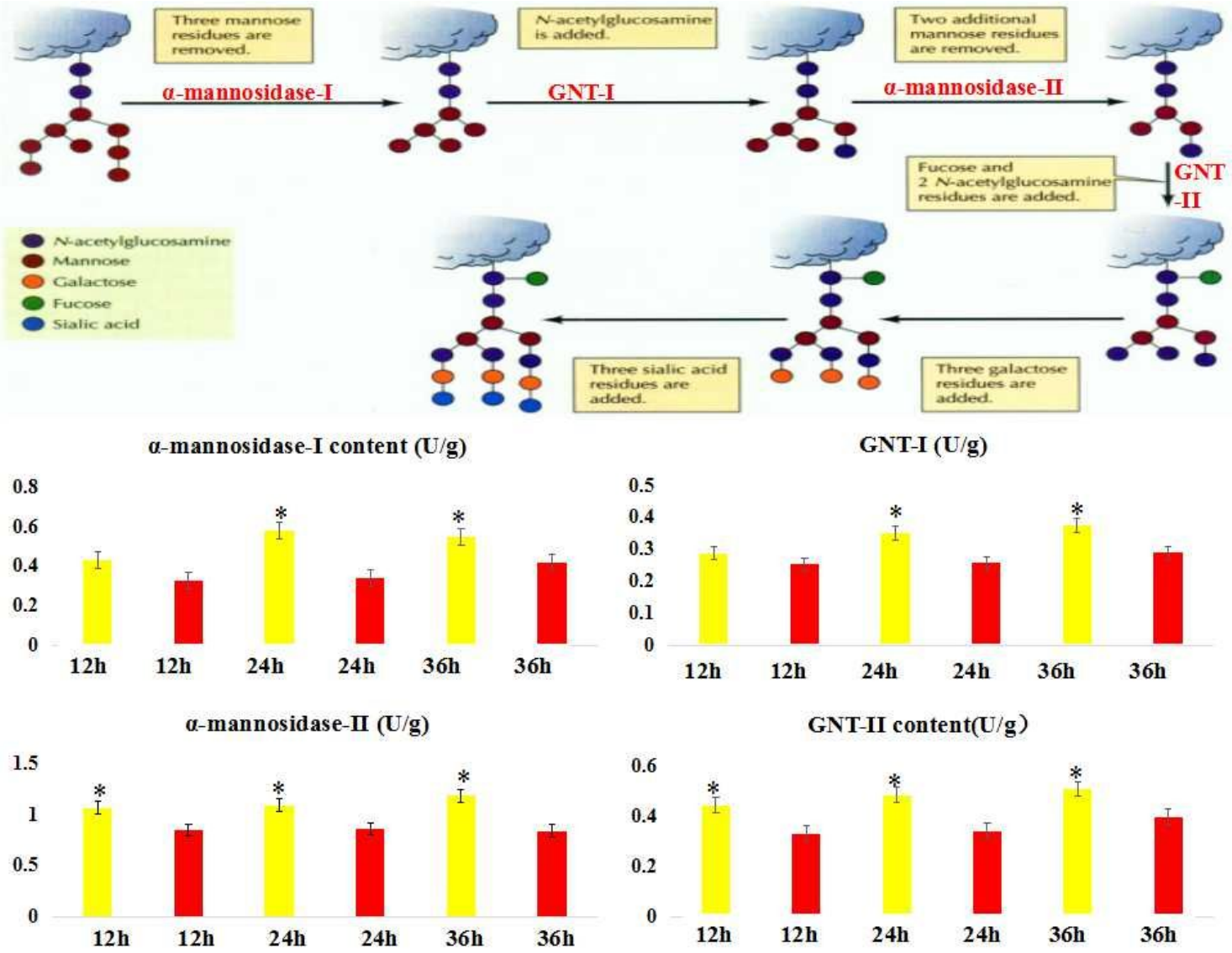

Figure 3

The oligosaccharide in the N-glycan maturation process (up). Effect of swainsonine on N-glycan enzyme activity in mouse endometrial epithelial cells (down).

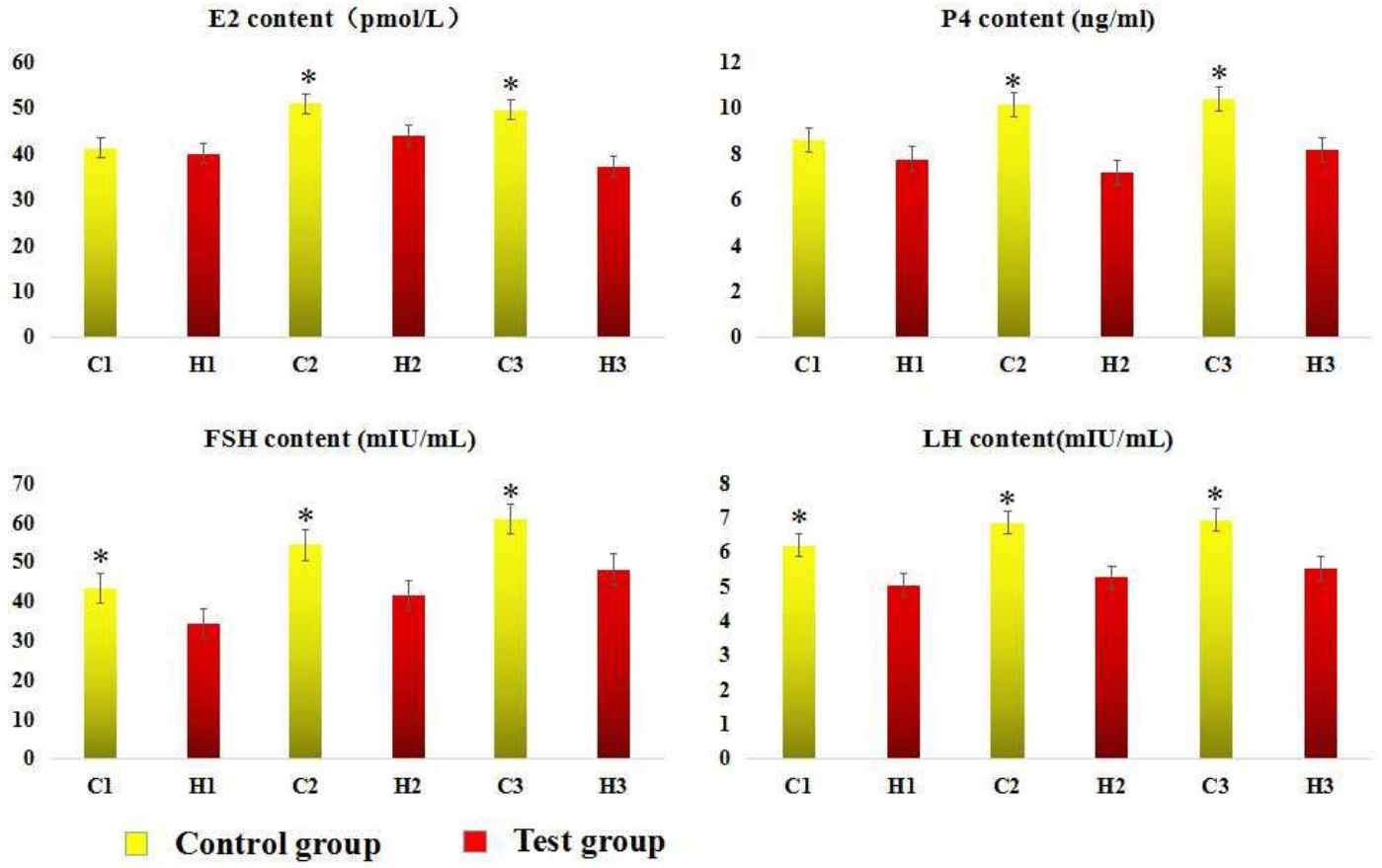

Figure 4

Effect of swainsonine on reproductive hormone levels in mouse blood.

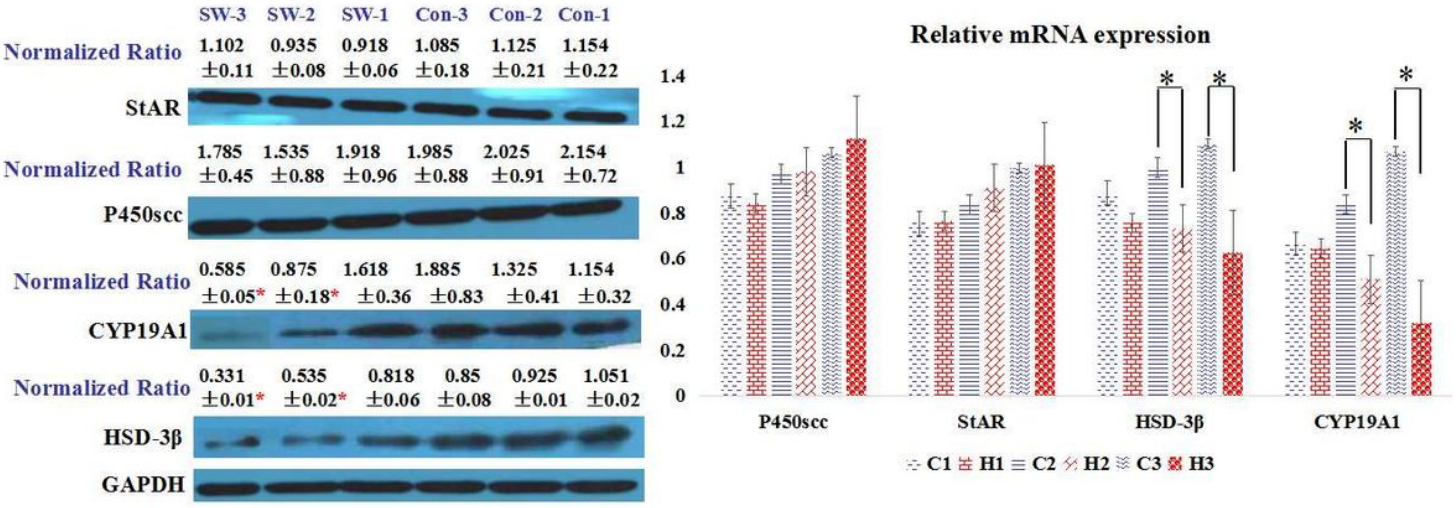

Figure 5

Effect of swainsonine on expression of steroid hormone enzyme mRNA and protein.

Supplementary Files

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