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DATA NOTE

Mass spectrometry data of diabetic rat sperm proteome treated with *Gynura procumbens* aqueous extract [version 1; peer review: 1 approved, 1 approved with reservations]

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Abstract

Diabetes mellitus has a deleterious effect on the male reproductive system, especially on sperm quality and spermatogenesis. *Gynura procumbens* (*G. procumbens*) is a traditional herb known for its ability to improve the fertility of diabetes-induced male rats. This study was designed to identify the differential expression of sperm proteins after treatment with *G. procumbens* aqueous extract on diabetes-induced male rats. The sperm proteome was profiled using label-free shotgun proteomics analysis. Sprague Dawley rats used in this study were divided randomly into four groups. One group was a normal control group (healthy rats), while the three other groups were induced with 50 mg/kg bodyweight (BW) of streptozotocin (STZ) to emulate the diabetic condition. The diabetic rats were divided into negative control (non-treated diabetic), metformin-treated (positive control) and *G. procumbens* aqueous extract-treated (450 mg/kg BW) groups. Oral treatments were administered for 14 consecutive days before the rats were euthanized. Total sperm protein samples were extracted from the caudal epididymis and run through SDS-PAGE. Later, samples were digested using trypsin before liquid chromatography-tandem mass spectrometry (Thermo Orbitrap Fusion) analysis. The acquired data were processed using MaxQuant and Perseus software. The mass spectrometry proteomics data is available through ProteomeXchange Consortium via the PRIDE partner repository, with the dataset identifier PXD011373.

Keywords

Gynura procumbens, diabetes mellitus, fertility, proteomic, Sambung nyawa

Open Peer Review

Approval Status

	1	2
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11 Jun 2019	view	view

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2. **María Eugenia Cabrillana** , Histology and Embryology Institute of Mendoza, Mendoza, Argentina
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Any reports and responses or comments on the article can be found at the end of the article.

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Introduction

Diabetes mellitus is a metabolic disorder that is often associated with male infertility, causing disruption to spermatogenesis, testicular impairment, and erectile and ejaculation dysfunction¹. Disruption to spermatogenesis and testicular impairment in diabetic rats has been reported to cause low sperm quality². Current medicine, such as metformin and other oral anti-hyperglycaemic drugs, claimed to significantly reduced blood glucose level; however, they could not improve the fertility problems associated with diabetes³. Medicinal plants or herbs have been long used in traditional practice and can be considered as alternative treatments for some diseases. *Gynura procumbens* is one of the herbs that has shown potential for treating various diseases such as diabetes mellitus, cancer and, recently, fertility. A previous study demonstrated that *G. procumbens* had potential as anti-hyperglycaemic agent by lowering the blood glucose level of diabetes-induced male rats⁴. It is claimed that *G. procumbens* imitates the mechanism of action of insulin, increasing the glucose uptake into the skeletal muscle⁵. Additionally, *G. procumbens* extract has the ability to improve the fertility of diabetes-induced male rats^{3,6}. To further elucidate the effect of *G. procumbens* aqueous extract (GPAE) on sperm quality, a proteomic analysis was performed to identify and quantify the differential expression of total sperm proteins after treatment with *G. procumbens* extract.

Materials and methods

Ethical statement

This research has obtained ethical approval from the Animal Ethics Committee of Faculty of Medicine, Universiti Kebangsaan Malaysia (FST/2013/MAHANEM/31-JAN./492-FEB.-2013-FEB.-2015). All efforts were made to ameliorate harm to animals, achieved by inducing to imitate diabetes conditions with streptozotocin (STZ) intravenously at a dose of 50 mg/kg bodyweight (BW) in all groups except the control normal group, which was left uninduced.

Source and husbandry of animals

A total of 28 male Sprague Dawley rats aged eight weeks (120–200 g) were used in this study. All rats were proven fertile and provided by the Animal House of Universiti Kebangsaan Malaysia. All animals were acclimatized for seven days, wherein their food pellet (Rat Chow, Barastoc, Ridley, Australia) and drink intakes were given *ad libitum*. The rats were kept in PVC cages at controlled room temperature with a 12-hour light/12-hour dark cycle.

Experimental design

G. procumbens were harvested from Universiti Kebangsaan Malaysia glass house (2.9300°N, 101.7774°E). The *G. procumbens* aqueous extract was prepared as described previously³. Briefly, the leaves part of *G. procumbens* were harvested and dried in the oven for 72 hours at 48°C. The dried leaves then were ground to dust and extracted for three hours at 60°C. The extract produced was in liquid form, later sent for freeze-drying and kept in 4°C for freshness.

Experimental methods and procedures were detailed as previously⁷. Briefly, male Sprague-Dawley rats were used in

this study, which were randomly hand-picked and divided into four groups, with seven rats in each group. Randomization was performed in choosing the rats to avoid bias in the experiment. One group served as a normal untreated group (N), while the three other groups were induced with 50 mg/kg BW STZ intravenously for type 1 diabetes induction. The blood glucose level of each rats was measured after 72 hours of STZ induction. Rats with blood glucose level at 13 mmol/L and more were considered as diabetic and used in this study. These diabetic rats served as (i) diabetic-untreated (negative control), (ii) diabetic-metformin treated (positive control) (500 mg/kg BW) and (iii) diabetic-GPAE treated (450 mg/kg BW) groups. Treatment was given via oral gavage for 14 consecutive days after seven days of STZ induction at the Animal House, Universiti Kebangsaan Malaysia. On the 15th day, all rats were euthanized by inhalation of diethyl ether for sperm protein analysis.

Sample collection and preparation

Sperm protein extraction was performed as described previously⁸. Briefly, sperm samples from each rat were collected separately from the caudal epididymis and minced in Biggers-Whitten-Whittingham medium (91.06 mM Sodium chloride, 4.78 mM Potassium chloride, 2.0 mM Calcium chloride, 1.17 mM potassium phosphate, 2.44 mM Magnesium sulphate, Sodium hydrogen carbonate, 0.25 mM Sodium pyruvate, 21.55 mM Sodium lactate, 5.55 mM glucose, and bovine serum albumin)⁹. Briefly, the samples were incubated in a 5% CO₂ incubator for 30 minutes at 37°C, to allow the sperm to swim up. Sperm samples were then centrifuged at 4000 rpm for 15 minutes and the supernatant was removed. The pellet was mixed with lysis buffer (7 M Urea, 2 M Thiourea, 4% 3-[(3-Cholamidopropyl)-dimethyl-ammonio]-1-propanesulfonate (CHAPS), 0.8% Immobilized pH Gradient (IPG) buffer, 1 mM phenylmethane sulfonyl fluoride (PMSF)). The samples were then centrifuged at 15,000 rpm for 20 minutes at 4°C. The supernatant was collected, added to 60 mM dithiothreitol (DTT) and kept at 80°C until used. The concentration of sperm proteins was determined using the Bradford assay¹⁰.

SDS-PAGE

A total of 100 µg of the sperm protein samples from each group were used for one-dimensional SDS-PAGE. Protein samples were loaded onto 12.5% SDS-PAGE gels and run for eight minutes or until all the proteins stacked up in the resolving gel. The gel was then cut for trypsin digestion using the in-gel trypsin digestion method¹¹. For this purpose, excised protein bands were treated with DTT for one hour at 57 °C to reduce the disulphide bridges, followed by Iodoacetamide for another hour at room temperature in the dark for alkylation purposes, as previously described¹¹. Gels were rehydrated with 50 mM ammonium bicarbonate and dehydrated with acetonitrile for three times, respectively. Enzymatic digestion was performed by incubating samples with trypsin for 30 minutes at 4 °C in a 1: 50 (w/w) ratio. The digestion products were then incubated with 50 mM ammonium bicarbonate for overnight at 37 °C. Digested peptides were sent for liquid chromatography with tandem mass spectrometry (LC-MS/MS) for protein identification and quantification.

LC-MS/MS analysis

LC-MS/MS analysis was performed as described in Kamaruzaman *et al.*⁷. Briefly, the peptide samples were analysed using a liquid chromatography (LC) system (UltiMate 3000 RSLCnano, Dionex) coupled to a Linear Trap Quadrupole (LTQ) Orbitrap Fusion mass spectrometer (Thermo Fisher, Bremen, Germany). A total of 1.0 μ L of digested samples from each group were injected into a reverse phase column (15 cm $\times$ 75 μ m internal diameter, particle size of 2 μ m, 100 Å, C18 PepMap column) and eluted at a flow rate of 300 nL/min. The elution mobile phase composition was 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). The eluted peptides were separated in 5–40% solvent B for 91 minutes, 95% solvent B for 2 minutes, 95% solvent B for 6 minutes and back to 5% solvent B for 2 minutes. Data were acquired in data dependent mode. Full scan spectra in the range of 310–1800 m/z were acquired. The automatic gain control (AGC) was targeted at 4.0×10^5 with a maximum injection time of 50 ms and 3 seconds in top speed mode, where precursors were selected with a maximum cycle time of 3 seconds. Precursors with an assigned monoisotopic m/z and a charge between 2 and 7 were further analysed. All precursors were filtered using a 20 second dynamic exclusion window with an intensity threshold of 5000. The MS/MS spectra analyses were performed using rapid scan rate and collision-induced dissociation (CID), with normalized collision energy (NCE) set to 30% and high energy collision-induced dissociation (HCD) at 28%, a 1.6 m/z isolation window, AGC targeted at 1.0×10^2 and a maximum injection time of 250 ms.

Protein identification and quantification

Data processing was conducted according to previous studies^{12,13}. Raw files for each biological replicate were analysed together using the MaxQuant software (version 1.5.3.30)¹⁴. The derived peak list was searched using the Andromeda search algorithm embedded in the MaxQuant workflow. The protein sequence database used for protein identification analysis was the *Rattus norvegicus* database, obtained from Uniprot database (proteome ID: UP000002494, accessed on February 2016). The MaxQuant parameters were set as enzyme trypsin and allowed up to two miss cleavages. The peptide length was set minimally up to seven amino acids. The spectra search included carbamidomethylation of cysteine as a fixed modification and oxidation of methionine was set as a variable modification. Since no labelling was performed, multiplicity was set to one. Peptide spectrum match (PSM) and protein identification were filtered using a target-decoy approach with a false discovery rate (FDR) of 1 %. The second peptide feature was enabled. The label-free quantification (LFQ) of protein was done using the MaxLFQ algorithm integrated in MaxQuant. Other MaxQuant settings were set as default. All resulting information was reported in the “proteinGroups” output file (proteinGroups.txt in the combined file), containing the full list of identified and quantified proteins. This file has been deposited to the ProteomeXchange Consortium¹⁵ via the PRIDE partner repository with the dataset identifier PXD011373.

The data were further analysed for protein quantification using Perseus software (version 1.5.4.1)¹⁶. The hits to the reverse database, contaminants and proteins identified with modified peptides were eliminated. Then, the LFQ intensity ratios were transformed by $\log_2$. Missing values were imputed by drawing random numbers from a normal distribution to stimulate signals from low abundance proteins, using the default parameters described previously¹². The value of $\log_2$ (LFQ ratios) for each sample were averaged and statistically analysed as per Kamaruzaman *et al.*⁷. In short, data were displayed as mean $\pm$ standard error of mean. The statistical analysis for protein LFQ intensities were compared using Perseus software (version 1.5.4.1) (one-way ANOVA, $p < 0.05$).

Dataset

The dataset is composed of raw and processed LC-MS/MS data for the proteome profiling of rat sperm. Samples were collected from the caudal epididymis of four different groups of rats; normal-untreated, diabetic-untreated (negative control), diabetic-metformin treated (positive control) and lastly, diabetic-GPAE treated. LC-MS/MS, followed by MaxQuant and Perseus analyses, were carried out to identify and quantify the total sperm proteins (Table 1).

Protein identification revealed 473 proteins, and using a stringent search in MaxQuant software (including filtering contaminants and hits to decoy database), a total of 88 proteins were found in all groups. Identified proteins were later quantified using MaxQuant and Perseus software. This information revealed the effect of *G. procumbens* on the male diabetic rat reproductive system, specifically on the sperm proteins that are involved in fertilization.

Summary

The elucidation of sperm proteins using high-throughput proteomics techniques is still rather limited, especially in complementary medicine studies. Most of the previous studies^{17,18}, especially those involving herbal extract treatments on murine model organisms, mainly utilized a 2D gel-based approach, which is far less sensitive than shotgun proteomics¹⁹. Most of these studies identified only around 200–300 proteins, which may not profile the entirety of the sperm proteome. This shortcoming may be due to the low throughput of the gel technique, as well as the inability to visualize low-abundance proteins¹⁶.

On the other hand, shotgun proteomics mainly relies on the acquisition of peptide identities from digested total protein samples using an LC-MS/MS approach. In this study, 2411 peptides were found, which corresponds to 473 proteins. The high number of identified proteins suggests the applicability and sensitivity of shotgun proteomics, as compared to the more conventional 2D gel approach. Hence, shotgun proteomics, particularly using LC-MS/MS Orbitrap, can be considered as an effective strategy for producing a comprehensive proteome profiling of rat sperm, especially regarding GPAE treatment. Additionally, this dataset can be used as a primary guide for the characterisation of protein biomarkers for sperm quality and fertility in male rats.

Table 1. Data summary.

Subject area	Reproductive Biology
More specific area	Proteomics of rat sperm
Type of data	Raw and processed data
How data was acquired	Experiments performed using LC system (UltiMate 3000 RSLCnano, Dionex) coupled to an LTQ Orbitrap Fusion mass spectrometer (Thermo Fisher Scientific, Bremen, Germany)
Data format	Raw, processed
Experimental factors	Male Sprague-Dawley rats aged 8 weeks (120–200 g) were randomly divided into four groups. One group served as normal untreated group while three other groups were induced with STZ (50 mg/kg). These three groups were divided into: (i) diabetic-untreated group (negative control), (ii) diabetic metformin-treated group (positive control) and (iii) diabetic-GPAE-treated group (450 mg/kg).
Experimental features	Rat sperm proteome description and relative quantification. Extracted proteins were run on SDS-PAGE before being reduced, alkylated and tryptic digested. The peptide samples were then subjected to LC-MS/MS for protein profiling and analysed using MaxQuant and Perseus software.
Data source location	UKM, Bangi, Malaysia (2.9300°N, 101.7774°E)
Data accessibility	The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium ¹⁵ via the PRIDE partner repository with the dataset identifier PXD011373 .
Related research article	K.A. Kamaruzaman, W.M. Aizat & M.M. Noor, <i>Gynura procumbens</i> Improved Fertility of Diabetic Rats: Preliminary Study of Sperm Proteomic. Evid. Based Complement Alternat. Med. (2018) 1-13 ⁷ .

Data availability

Underlying data

Dataset on ProteomeXchange, Accession number PXD01133:
<https://identifiers.org/px/PXD011373>

Rattus norvegicus reference proteome was obtained from Uniprot, Proteome ID UP000002494: <https://www.uniprot.org/proteomes/UP000002494>.

Grant information

This research has been funded by Economic Transformation Programme Research Fund (ETP-2013-080) and a Research University Grant (GUP-2016-056).

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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- The article entitled "Mass spectrometry data of diabetic rat sperm proteome treated with *Gynura procumbens* aqueous extract", was clearly written.
- The datasets were not clearly presented and the format was not accessible.
- Perhaps a table showing the difference or not, in the proteins detected by mass spectrometry, between the control and the experimental groups could improve this article. It is not easy for the reader to look for the results in the PRIDE database.
- In the summary, no reference was made about the effect of the *Gynura procumbens* aqueous extract, on the proteins analyzed in all groups. Therefore it is not possible to understand whether this technique is useful for detecting differences between treatments.

Is the rationale for creating the dataset(s) clearly described?

Yes

Are the protocols appropriate and is the work technically sound?

Yes

Are sufficient details of methods and materials provided to allow replication by others?

Yes

Are the datasets clearly presented in a useable and accessible format?

No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Male reproduction.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 21 February 2020

<https://doi.org/10.5256/f1000research.21062.r59564>

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Yun Shin Sew

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This article presented proteomics datasets on the sperm quality of diabetic male rats treated with *Gynura procumbens* extract. The study was well planned, executed and the article is well drafted. Indeed, the authors addressed the effectiveness of this *G. procumbens* medicinal plant in treating diabetes mellitus and infertility in a previously published article by Kamaruzaman *et al.* (2018)¹. While in this article, the authors detailed out the experimental design and methodology for the preparation of proteomic data and how the data were analysed using MaxQuant and Perseus analysis softwares. However, there are a number of issues which need to be clarified and additional information are necessary in order to improve this article:

1. Given that the raw and processed data have been deposited in Proteome Xchange database (PXD011373), however the link for the 'Dataset FTP location' seems to be not working. By trying another link given under 'PRIDE project URI', the file with name 'combine.rar' was downloadable but failed to be opened. I would suggest the authors to check again on the data accessibility issue and fix any possible error in order to share those information with readers.
2. For the section 'Dataset', additional information are needed particularly related to the processed data. For example, the number of animal sample per group used for generating the processed data, how was the raw data of biological replicates in each group combined for analysis? Was there a normalization step across datasets for each group performed? What were the parameters used for stringent search in MaxQuant and Perseus analysis softwares, including thresholds and cut off values set for identifying a total of 88 proteins in all groups?
3. There were three different dosages of *G. procumbens* aqueous extract (150, 300 and 450 mg/kg) used for treatment referring to Kamaruzaman *et al.*(2018)¹, however this article presented and deposited dataset for only one treatment 450 mg/kg. Perhaps the authors can state somewhere in the article (i.e. experimental design section) regarding the rationale of choosing this particular treatment dataset?

4. Under the section 'Sample collection and preparation' there was a typo observed in the second last sentence where the samples kept at 80°C until used, it should be -80°C instead. In short, I would recommend this article to be indexed with minor revisions as the above.

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Is the rationale for creating the dataset(s) clearly described?

Yes

Are the protocols appropriate and is the work technically sound?

Yes

Are sufficient details of methods and materials provided to allow replication by others?

Partly

Are the datasets clearly presented in a useable and accessible format?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Proteomics, Transcriptomics, Metabolomics, Microbiome.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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