Raman Spectroscopic comparative study of Oxytocin and Freeze-dried Extract of *Uvariodendron anisatum Verdeck (Annonaceae)* and their influence on diet induced obesity in Sprague Dawley rats

Zephania Birech[1*], Peter W. Mwangi[2], Prabjot K. Sehmi[2], Nelly M. Nyaga[2]

[1] Department of Physics, University of Nairobi, 30197, Nairobi, Kenya

[2] Department of medical Physiology, University of Nairobi, 30197, Nairobi, Kenya

*Corresponding author: birech@uonbi.ac.ke

Abstract

Obesity is a condition affecting many people in the world. Obese people have increased risks of developing chronic metabolic diseases such as type II diabetes, hypertension, cancer among others. Early and rapid diagnosis of the condition together with effective treatment is therefore necessary. This work investigated, first, Raman spectroscopic similarities between oxytocin and a freeze-dried extract of a local herbal plant exhibiting oxytocin-like properties called *Uvariodendron anisatum Verdeck (Annonaceae)* (UAV). Secondly, whether Raman spectroscopy could be used for comparative studies of the influence of oxytocin and UAV on obese Sprague Dawley (SD) rat models. We also wanted to find a Raman biomarker band for obesity or metabolic syndrome. Both oxytocin and extract samples together with blood extracted from the rats were excited using a 785 nm laser after being placed or applied onto a conductive silver paste smeared glass slides and Raman signals collected, recorded and analyzed.

The Raman spectroscopic spectral profiles of oxytocin and UAV freeze dried extracts were found to be identical showing they were composed of similar Raman active molecules. The prominent peaks were those assigned to disulfide S-S stretching mode at 508 cm^{-1} and to tyrosine at 645 cm^{-1}, 846 cm^{-1} and 1617 cm^{-1}. Raman spectra of blood from rats treated with oxytocin and UAV had indistinguishable profiles thus supporting idea that they were composed of similar active molecules. The spectral profiles were also dissimilar to those from obese and non-obese (normal controls) animals. A prominent peak in spectra of treated rats centered at 401 cm^{-1} could be used as oxytocin biomarker band. Comparison of average intensity trend of fructose bands at around 638 cm^{-1} and 812 cm^{-1} between prepared fructose solution and blood of treated rats, revealed elevated levels of fructose in blood of rats orally administered oxytocin and UAV extracts. The implication was that fructose metabolism in rats administered oxytocin and UAV extracts was upregulated. Principal component analysis (PCA) showed the power of Raman spectroscopy in distinguishing between obese and non-obese SD rats based on spectral profile patterns. It also further revealed that oxytocin and UAV extracts had similar influence on SD rats as their blood's Raman spectral patterns were indistinguishable.

The study revealed that Raman spectroscopy can be a powerful tool for quick obesity (metabolic syndrome) screening with intensity of Raman bands associated with fructose as biomarkers. The same bands can also be used in comparative efficacy studies of anti-obesity drugs. Further studies are needed to validate these Raman spectroscopic results since, to the best of our knowledge, this

was the first such investigation regarding comparison of UAV and conventional oxytocin together with their influence on obese SD rats. Also studies on whether the same results can be seen in human subjects.

Keywords: Oxytocin; Metabolic syndrome; Obesity; *Uvariodendron anisatum Verdeck (Annonaceae);* Fructose

1. Introduction

Obesity, a metabolic condition characterized by abnormal increase in body weight and fat accumulation [1–3], is now a problem globally. According to World Health Organization (WHO), it was estimated that by year 2016 about 650 million people worldwide were obese [2]. The condition is caused by overconsumption of energy dense foods followed by less physical activity. There is a close relationship between being overweight and being obese. The two i.e. obesity and overweight are distinguished by a value known as body mass index (BMI) which basically is a ratio of weight (in kilograms) to the square of height (meters squared). An overweight and an obese human has a BMI value equal or greater than 25 and 30 respectively [2]. An obese person, therefore, is overweight. In rodent models, there is no universally agreed method of determining obese from non-obese rats but often those with fasting blood glucose (FBG) levels above 7 mmol/L [4] and those with increased volumes of subcutaneous and visceral adipose tissues [3] are regarded as obese. An obese individual has risks of developing chronic metabolic diseases such as type II diabetes, hypertension, coronary heart disease, cancer among others[1,3,5]. Management of this metabolic condition involves use of anti-obesity drugs, increase in physical exercise, reduction of high energy diet. These methods are un-popular due to side-effects and high failure rates. New interventions involving natural products with few side effects along with quick diagnostic techniques for monitoring their efficacy and at the same time detecting potential development of the condition are necessary.

One of the non-conventional potential alternative obesity treatments gaining a lot of attention lately involves use of oxytocin[6–8]. Oxytocin (OT) is a hormone associated with labor, lactation [9–11] and regulation of social behavior in mammals [10,12]. It has chemical formula $C_{43}H_{66}N_{12}S_2$ and is locally produced in the brain and released to the circulatory system[12,13]. The compound consists of nine amino acids in the sequence: cysteine - tyrosine - isoleucine - glutamine - asparagine - cysteine - proline - leucine - glycine (CYIQNCPLG)[14]. The role of OT in weight reduction in obese rhesus monkeys[6] and in rats[7,15] has been reported. In mice[16] and male humans[16], OT caused a decrease in calorie intake. It has also been found that the hormone suppresses eating behaviour resulting in reduction of blood glucose levels, increase in insulin levels [10,17,18], reduction of glucose intolerance and insulin resistance[7] and a shift in diet preference from carbohydrates to fats[16]. The hormone is also reported to make cells resistant to diabetic conditions [13] and improve their insulin sensitivity [18]. In a study on African American

males, the levels of oxytocin in blood of type II diabetic subjects were found to be lower than in healthy ones[19]. In the same study, subjects with higher levels of OT had lower body weights. Administration of OT is through intranasal [12,16], intraperitoneal [20] and subcutaneous[16] routes. Oral administration is rare due to its impaired and unpredictable absorption rates in the gastric system [21] though a review of it is being suggested elsewhere[22].

All these findings indicate a special role OT plays in the treatment and prevention of obesity and diabetes. Extended studies are, therefore, needed to investigate the influence of OT on metabolic diseases and other potential uses in non-conventional treatments.

In many parts of remote rural Kenya where hospital facilities are distant, traditional herbalists and birth attendants use herbal extracts for labor induction just as oxytocin. One of the herbs commonly used, and which is the subject of our study is *Uvariodendron anisatum Verdeck (Annonaceae)* (UAV)[23,24]. This work sought to investigate first, similarities of UAV freeze dried extracts and oxytocin using Raman spectroscopy and secondly their influence on diet induced obesity in Sprague Dawley (SD) rats. This study, to the best of our knowledge, was the first of its kind. It was found that little Raman spectral differences exist between oxytocin and UAV extracts and no distinguishable differences were observed on their influence on obese SD rats. Their administration resulted in elevated levels of fructose in blood as revealed by intensity analysis of assigned Raman bands.

Experimental

Plant collection and extract preparation

Fresh whole plants of *Uvariodendron anisatum Verdeck (Annonaceae)* were collected from their natural habitat in Meru county, Kenya. The plant was confirmed at the University of Nairobi herbarium and a voucher specimen deposited. The plant materials were air dried for a week before being milled and grounded into powder. The powder (1 kg) was macerated in distilled water in a weight to volume ratio of 1:8 for twenty (20) minutes and 8 litres of solution made. The resulting suspension was then filtered using cotton wool and followed by Whatman's filter paper. The resulting filtrate was frozen and lyophilization done to obtain freeze-dried extract. The freeze-dried extract was weighed, placed in amber colored sample bottles and stored in a deep freezer.

Animal experiments

Twenty freshly weaned Sprague Dawley (SD) rats weighing around 95 g were used in the experiment. They were housed, 5 members each, in metallic cages (dimensions 109 cm by 69 cm

by 77.5 cm) with floor covered with wood shaving. The shavings were replaced thrice every week. Lighting of the cages was maintained at a 12-hour day and night cycle. For the first 8 weeks, all the animals were fed on a high fat (15%) and high fructose (20%) diet *ad libitum.* Weight and Fasting (5 hour fast) blood glucose levels including oral glucose tolerance tests were measured on both day 0 and day 56 (last day of 8th week). On day 1, the animals were confirmed to be non-diabetic as the FBG levels were on average 4.38 +/- 0.33 mmol/l which was less than 7.5 mmol/L, a limit suggested by Wang *et al*[4]. The blood drawn was hence labelled as non-obese (Nob) and stored. The weights and blood glucose level values (averaged 325 g and 6 mmol/L respectively) obtained on the 56th day were used to designate the rats as obese. The rats were then regarded as obese (with metabolic syndrome). These animals were thereafter randomly grouped, with 5 members each, into: Obese (Ob; fed on high fat 15%, high fructose 20% diet *ad libitum* as before), Oxytocin treated (Oxy; same feeding as obese and administered oxytocin 1 mg/kg), *Uvariodendron anisatum Verdeck (Annonaceae)* (UAV) extract treated at low dose (LDOx; same feeding as obese and administered a dose of 100 mg/kg) and high dose (HDOx; same feeding as obese and administered a dose of 200 mg/kg). The oxytocin and UAV treatment was carried out for 7 days. Blood glucose testing was done using a commercial glucometer (StatStrip Xpress Nova Biomedical, Waltham MA, USA) and weight measurement using an electronic beam balance. The solvent used in dissolving both the oxytocin powder (Sigma-Aldrich, USA) and the freeze-dried UAV extracts was normal saline (0.9% NaCl in water). All the prepared solutions of oxytocin and the extracts were administered daily by oral gavage. The blood samples (~50 µL) were drawn from each rat via lateral vein sampling after local anesthesia of the tail by topical application of lidocaine. All the rats were then euthanized following an overnight fast using 20% Phentobarbital (1ml/kg of body weight) injected intraperitoneally at the end of the experiment. Confirmation of death was via loss of the pupillary light reflex. The drawn blood from each rat was stored in sodium citrate vacutainers to prevent clotting and refrigerated at 4˚C.

Raman spectroscopy

Raman spectroscopy was carried out using confocal Raman system (STR, Seki Technotron Corp) equipped with a 785 nm laser and a spectrometer (Princeton Instruments). The conductive silver paste smeared microscope glass slides used as Raman sample substrates were prepared as described in [25]. Spectral callibration of the Raman spectroscopic device was also done as

described in reference [25]. The experimental parameters were: grating, 600 groves/mm; Centre wavelength, 850.97 nm (980 cm^{-1}); exposure time, 10 sec; spectral accumulation, 5 sec; microscope objective, X10 Max Plan. A small amount of blood (~ 10 µL) was pipetted onto the silver smeared glass slide. Ten spectra per rat's blood sample were recorded making a total of 250 (50 data sets for non-diabetic samples included) spectral data sets with each group having 50 data sets. Pre-processing of the data was done as described by Birech *et al*[25], analysis and plotting of the spectral data were achieved using MATLAB 2017a and ORIGIN (Originpro 9.1) software.

2.4 Ethical approval

Ethical approval for the study was granted by the Biosafety, Animal Care and Use Committee, Faculty of Veterinary Physiology, University of Nairobi.

2. Results and Discussion

2.1 Raman spectra of oxytocin and freeze-dried extracts of Uvariodendron anisatum Verdeck (Annonaceae) (UAV)

The Raman spectra from UAV freeze dried extracts and those from commercially available oxytocin displayed identical profiles and so indicating similar molecular composition (see Fig. 1). The discrepancy in the spectral profiles was only observed from the different forms of the sample (i.e. solid or solution). The spectra of oxytocin solution and UAV extract's dry powder (oxytocin powder and UAV extract's solution) displayed identical spectral profiles as seen in Figure 1a (1b). The exact reason why the extracts solids and oxytocin solution (also extract's solution and oxytocin powder) had similar Raman spectral profiles is still unclear. It was thought that the interaction between the silver smear and the samples are responsible for the observed variations. The interactions must have influenced conformations of the various bonds in the oxytocin hormone (which is composed of nine amino acids i.e. it is a nanopeptide) [14]. The most affected was the disulfide S-S stretching mode at 508 cm^{-1} in the oxytocin powder resulting in red-shifting to 401 cm^{-1} in the extract's solution [14,26]. These same signals were observed to be broad in Figure 1a. The conformational angle must have been less than 60^{o} about the C-S bond as was argued earlier by Maxfield and Scheraga [14]. The other prominent bands observed were those centered at wavenumbers 645 cm^{-1}, 846 cm^{-1} and 1617 cm^{-1} ascribed to tyrosine with the commonly known

830/850 cm^{-1} doublet seen in oxytocin powder and solution [26,27]; 1240 cm^{-1} assigned to amide III with anti-parallel β-sheet structure [26]; 1450 cm^{-1} assigned to C-H deformation in isoleucine and 1658 cm^{-1} attributed to amide I vibrations with anti-parallel β-sheet conformation [26].

Figure 1. Figure displaying Raman spectra obtained from (a) UAV extract's dry powder and oxytocin solution and (b) UAV extract's solution and oxytocin powder. All the samples were placed on conductive silver paste smeared glass slides.

2.2 *Raman spectra of blood from SD rats*

The Raman spectra of blood obtained from SD rats that were obese (Ob), non-obese (NOb), obese and administered oxytocin (Oxy), obese and administered UAV's extract at low dose (LDOx) and high dose (HDOx) are displayed in Figure 2a. The intense peak at 401 cm^{-1} also seen in extract's solution (see Fig. 1b) was present in all blood from rats administered oxytocin and UAV extracts but less significant in obese and non-obese rats. This band may be used as oxytocin biomarker band in blood and reflects elevated levels of the hormone in the treated animals. In other murine studies, subjects administered oxytocin exhibited increased levels of the hormone (i.e. oxytocin) in serum [10] and in plasma [7] thus supporting our observation through the assigned Raman peak. Elsewhere, it was reported that in human subjects that were obese and with type II diabetes mellitus, levels of oxytocin were significantly lower compared to healthy subjects [8,19]. The band centered at 478 cm^{-1} was associated with both fructose and glucose's skeletal vibrations with tentative assignments; C-C-C , C-C-O, C-O deformations and C-C torsional vibrations [28]. The bands centered at 638 and 812 cm^{-1} were attributed to fructose and tentatively assigned to ring deformation and C-C stretching vibrations respectively [28]. Interestingly, these two latter bands (fructose bands) exhibited a decrease in intensity upon administration of both oxytocin and UAV extracts to the diabetic rats as seen in Figures 2b and 2c. In order to interpret this trend, solutions of fructose in normal saline were prepared with concentrations ranging from 0.005 – 0.015 mMol/L and Raman spectra obtained after pipetting onto the conductive silver coated glass slides. The trend of the average intensity of peak centered around 812 cm^{-1} as a function of fructose concentration (see Figure 2d) was identical to that from blood of SD rats (Figure 2b and 2c). The implication of this was that fructose levels in blood of obese rats are lower than in non-obese and treated rats (both oxytocin and UAV extract treated). At the same time, oral administration of oxytocin and UAV extracts causes elevated levels of circulating fructose in SD rats.

Figure 2: Figure showing (a) Average Raman spectra from blood of obese(Ob), non-obese (NOb),

oxytocin treated obeserats (Oxy) and UAV extracts treated obese rats at low dose and high dose (LDOx and HDOx) SD rats (b) and (c) Average Raman intensity of peak centered at 812 cm^{-1} and 638 cm^{-1} respectively and (d) Average Raman intensity of peak centered around 812 cm^{-1} from fructose solution in normal saline.

Here, Raman spectroscopic study indicates that fructose metabolism in the liver [29–31] is upregulated by oral administration of oxytocin and UA extracts hence the increased concentration in blood. It should also be noted that during treatment, the animals were still on a high fat and high fructose diet. The high levels of fructose in blood are usually filtered out through the kidneys and it is expected that their levels in urine are high as reported elsewhere in diabetic humans[32]. In other studies, intraperitoneally injected oxytocin on mice resulted in reduced fructose concentration in seminal vesicles and coagulating glands [20]. It was not clear presently whether the method of administration of the extract and oxytocin brings about fructose level variations in different body organs. Oral administration of oxytocin is unpopular due to impaired or unpredictable absorption rates in the gastrointestinal tract [21,33]. The work here, therefore, suggests that intensity of Raman spectral bands at 638 and 812 cm^{-1} assigned to fructose could be used in quick indication of fructose level variation in oxytocin treated subjects and in obesity (or metabolic syndrome) screening. The band can be used also in comparing anti-obesity influence of conventional oxytocin and similarly used traditional plant extracts. The other bands centered at 1033, 1130, 1318 and 1443 cm^{-1} are associated with the branched chain amino acids (BCAAs). The peaks centered at 1033 and 1130 cm^{-1} are ascribed to C-N stretch, NH_3 rocking, HCCH torsional vibrations in leucine [34]; CO stretch, OH bending vibrations in both valine and isoleucine [34].

2.3 *Principal component analysis (PCA)*

When Raman spectroscopy is to be used to make quick examination of influence of oxytocin and UA extracts on obesity, a method to distinguish between spectral profiles from the different blood samples is needed. In this work, principal component analysis (PCA) was used. The method utilizes spectral patterns in segregating between spectral data. The spectral pattern variations are expressed in terms of percentage variance and ranking done[35,36]. The results are represented on a set of orthogonal axes referred as principal components (PCs). The PC with the highest variance is called PC1, flowed by PC2 and so on [35]. Each of the spectral data set is displayed as a point (score) on a PC plane. For our work it was found that Raman spectral data from blood of obese,

non-obese were clearly differentiated from each other and from the rats administered oxytocin and UAV extracts (Oxytocin, low dose and high dose) as displayed in Figure 3. The low and high doses of the UAV extract did not show distinguishable differences in the score plot.

Figure 3: Figure displaying PCA score plot from Raman spectral data of SD rat's blood. PC1 and PC2 had explained variance of 74.7% and 9.5% respectively. The spectra from obese rats were clearly distinguished from non-obese and the treated rats.

The results of the study indicate that Raman spectroscopy can be used as a label free obesity detector or screener with bands associated with fructose as biomarkers. At the same time, the results show that Raman profiles from blood of oxytocin treated rats and those treated with UAV extracts contained similar Raman active molecules. The two compounds (i.e. oxytocin and UAV extracts) influenced obesity in the rats since the spectral profiles were modified. This was also supported by the fact that the average weights and FBG values of the treated animals decreased (325 g to 260 g and 6.3 +/- 0.3 mmol/L to 4.7 +/- 0.4 mmol/L respectively) in the first 7 days after commencing treatment. The low and high doses of the UAV extract did not exhibit discernible differences on the rats as the blood had identical profiles. Herbalists and traditional birth attendants in parts of rural Kenya use UAV to induce labor [23,24]. The Raman study results reveals that the herb has identical effects in obese SD rats as the conventional oxytocin. This implies that the herb is composed of similar Raman active molecules. Further studies need to be done to validate the Raman spectroscopic results reported here as this, to the best of our knowledge, is the first such investigation as regards comparison of UAV and conventional oxytocin and on their influence on obesity in rats.

3. Conclusion

The study revealed that Raman spectroscopy can be a powerful tool for comparative study of anti-obesity drugs as spectral profiles from obese, non-obese and treated rats were distinguishable. The peaks associated with fructose could be used as biomarker bands for the distinction. The method further showed that oxytocin and UAV are composed of identical Raman active molecules and possesses similar anti-obesity effects. They both also cause elevated levels of fructose in blood of rats.

References

1. Chien M-Y, Ku Y-H, Chang J-M, Yang C-M, Chen C-H. Effects of herbal mixture extracts on obesity in rats fed a high-fat diet. Journal of Food and Drug Analysis. 2016;24: 594–601. doi:10.1016/j.jfda.2016.01.012

2. WHO. Obesity and overweight [Internet]. World Health Organization; 2018. Available: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight

3. Kn BP, Gopalan V, Lee SS, Velan SS. Quantification of Abdominal Fat Depots in Rats and Mice during Obesity and Weight Loss Interventions. Paulmurugan R, editor. PLoS ONE. 2014;9: e108979. doi:10.1371/journal.pone.0108979

4. Wang Z, Yang Y, Xiang X, Zhu Y, Men J, He M. [Estimation of the normal range of blood glucose in rats]. Wei Sheng Yan Jiu. 2010;39: 133–137, 142.

5. Vadivelu B, Arumugam VA, Subbarayan S, Alshatwi AA, Krishnamoorthy R. Effect of Macrotyloma uniflorum on antiobesity in rats fed with a high fat diet. Saudi Journal of Biological Sciences. 2018; doi:10.1016/j.sjbs.2018.05.003

6. Blevins JE, Graham JL, Morton GJ, Bales KL, Schwartz MW, Baskin DG, et al. Chronic oxytocin administration inhibits food intake, increases energy expenditure, and produces weight loss in fructose-fed obese rhesus monkeys. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2015;308: R431–R438. doi:10.1152/ajpregu.00441.2014

7. Deblon N, Veyrat-Durebex C, Bourgoin L, Caillon A, Bussier A-L, Petrosino S, et al. Mechanisms of the Anti-Obesity Effects of Oxytocin in Diet-Induced Obese Rats. Lobaccaro J-MA, editor. PLoS ONE. 2011;6: e25565. doi:10.1371/journal.pone.0025565

8. Ding C, Leow MK -S., Magkos F. Oxytocin in metabolic homeostasis: implications for obesity and diabetes management. Obesity Reviews. 2019;20: 22–40. doi:10.1111/obr.12757

9. Gimpl G, Fahrenholz F. The Oxytocin Receptor System: Structure, Function, and Regulation. Physiological Reviews. 2001;81: 629–683. doi:10.1152/physrev.2001.81.2.629

10. Scerbo MJ, Gerdes JM. Bonding With β-Cells—A Role for Oxytocin in Glucose Handling. Diabetes. 2017;66: 256–257. doi:10.2337/dbi16-0053

11. Ivell R, Kimura T, Müller D, Augustin K, Abend N, Bathgate R, et al. The Structure and Regulation of the Oxytocin Receptor. Experimental Physiology. 2001;86: 289–296. doi:10.1113/eph8602185

12. Parker KJ, Oztan O, Libove RA, Sumiyoshi RD, Jackson LP, Karhson DS, et al. Intranasal oxytocin treatment for social deficits and biomarkers of response in children with autism. Proceedings of the National Academy of Sciences. 2017;114: 8119–8124. doi:10.1073/pnas.1705521114

13. Jankowski M, Broderick TL, Gutkowska J. Oxytocin and cardioprotection in diabetes and obesity. BMC Endocrine Disorders. 2016;16. doi:10.1186/s12902-016-0110-1

14. Maxfield FR, Scheraga HA. A Raman spectroscopic investigation of the disulfide conformation in oxytocin and lysine vasopressin. Biochemistry. 1977;16: 4443–4449. doi:10.1021/bi00639a018

15. Blevins JE, Baskin DG. Translational and therapeutic potential of oxytocin as an anti-obesity strategy: Insights from rodents, nonhuman primates and humans. Physiology & Behavior. 2015;152: 438–449.

doi:10.1016/j.physbeh.2015.05.023

16. Lawson EA, Marengi DA, DeSanti RL, Holmes TM, Schoenfeld DA, Tolley CJ. Oxytocin reduces caloric intake in men: Oxytocin Reduces Caloric Intake. Obesity. 2015;23: 950–956. doi:10.1002/oby.21069

17. Klement J, Ott V, Rapp K, Brede S, Piccinini F, Cobelli C, et al. Oxytocin Improves β-Cell Responsivity and Glucose Tolerance in Healthy Men. Diabetes. 2017;66: 264–271. doi:10.2337/db16-0569

18. Elabd S, Sabry I. Two Birds with One Stone: Possible Dual-Role of Oxytocin in the Treatment of Diabetes and Osteoporosis. Frontiers in Endocrinology. 2015;6. doi:10.3389/fendo.2015.00121

19. Eisenberg Y, Dugas LR, Akbar A, Reddivari B, Layden BT, Barengolts E. Oxytocin is lower in African American men with diabetes and associates with psycho-social and metabolic health factors. Rosenfeld CS, editor. PLOS ONE. 2018;13: e0190301. doi:10.1371/journal.pone.0190301

20. Kumar A, Farooq A. Effect of oxytocin on the concentration of fructose in the accessory glands of mouse. Life Sciences. 1994;55: PL19–PL24. doi:10.1016/0024-3205(94)90086-8

21. Noriega-Guerra L, Aznar R, Arevalo N, Martinez-Zalce G, Martinez del Campo E, Lepe CM. Disadvantages of administration of oxytocin by oral absorption. American Journal of Obstetrics and Gynecology. 1966;96: 849–856. doi:10.1016/0002-9378(66)90681-8

22. McQuaid-Hanson E, Leffert LR, Bateman BT. Oral intake during labor: an alternative interpretation of recent data. American Journal of Obstetrics and Gynecology. 2016;215: 672. doi:10.1016/j.ajog.2016.06.030

23. Misonge J, Karimi E, Ombega J, Sani D, Ogeto J, Ogeto G, et al. PHYTOCHEMICAL SCREENING AND EVALUATION OF OXYTOCIC LIKE ACTIVITY OF UVARIODENDRON ANISATUM VERDEC (ANNONACEAE). Published 2015. 2015; 301.

24. Misonge J, Ogeto J, Sengera G, Mwalukumbi J, Mwaura A, Juma S. Evaluation of phytochemistry and uterotonic activity of root aqueous extract of Uvariodendron anisatum verdec. Used in childbirth in Eastern/Central Kenya. 2014;

25. Birech Z, Mwangi PW, Bukachi F, Mandela KM. Application of Raman spectroscopy in type 2 diabetes screening in blood using leucine and isoleucine amino-acids as biomarkers and in comparative anti-diabetic drugs efficacy studies. Multhoff G, editor. PLOS ONE. 2017;12: e0185130. doi:10.1371/journal.pone.0185130

26. Tu AT, Bjarnason JB, Hruby VJ. Conformation of oxytocin studied by laser Raman spectroscopy. Biochim Biophys Acta. 1978;533: 530–533.

27. Hernández B, Coïc Y-M, Pflüger F, Kruglik SG, Ghomi M. All characteristic Raman markers of tyrosine and tyrosinate originate from phenol ring fundamental vibrations: Characteristic Raman markers of tyrosine and tyrosinate. Journal of Raman Spectroscopy. 2016;47: 210–220. doi:10.1002/jrs.4776

28. Ilaslan K, Boyaci IH, Topcu A. Rapid analysis of glucose, fructose and sucrose contents of commercial soft drinks using Raman spectroscopy. Food Control. 2015;48: 56–61. doi:10.1016/j.foodcont.2014.01.001

29. Sun SZ, Empie MW. Fructose metabolism in humans – what isotopic tracer studies tell us. Nutrition & Metabolism. 2012;9: 89. doi:10.1186/1743-7075-9-89

30. Kolderup A, Svihus B. Fructose Metabolism and Relation to Atherosclerosis, Type 2 Diabetes, and Obesity. Journal of Nutrition and Metabolism. 2015;2015: 1–12. doi:10.1155/2015/823081

31. Elliott SS, Keim NL, Stern JS, Teff K, Havel PJ. Fructose, weight gain, and the insulin resistance syndrome. The American Journal of Clinical Nutrition. 2002;76: 911–922. doi:10.1093/ajcn/76.5.911

32. Kawasaki T, Akanuma H, Yamanouchi T. Increased Fructose Concentrations in Blood and Urine in Patients With Diabetes. Diabetes Care. 2002;25: 353–357. doi:10.2337/diacare.25.2.353

33. Fjellestad-Paulsen A, Söderberg-Ahlm C, Lundin S. Metabolism of vasopressin, oxytocin, and their analogues in the human gastrointestinal tract. Peptides. 1995;16: 1141–1147. doi:10.1016/0196-9781(95)00088-2

34. Ganesan A, Brunger MJ, Wang F. A study of aliphatic amino acids using simulated vibrational circular dichroism and Raman optical activity spectra. The European Physical Journal D. 2013;67. doi:10.1140/epjd/e2013-40376-x

35. Jolliffe I. Principal Component Analysis. In: Lovric M, editor. International Encyclopedia of Statistical Science. Berlin, Heidelberg: Springer Berlin Heidelberg; 2011. pp. 1094–1096. doi:10.1007/978-3-642-04898-2_455

36. Lever J, Krzywinski M, Altman N. Points of Significance: Principal component analysis. Nature Methods. 2017;14: 641–642. doi:10.1038/nmeth.4346

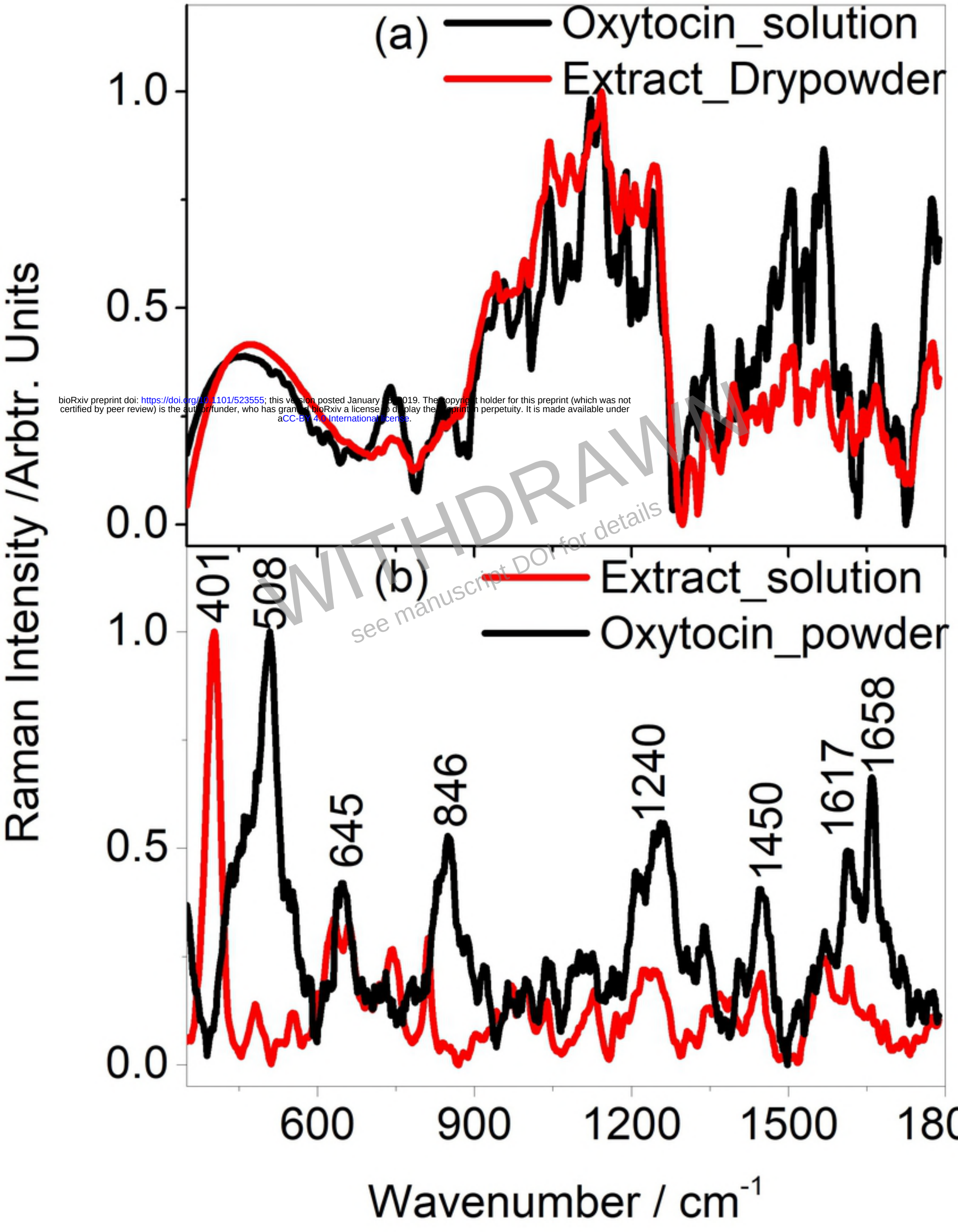

Figure 1

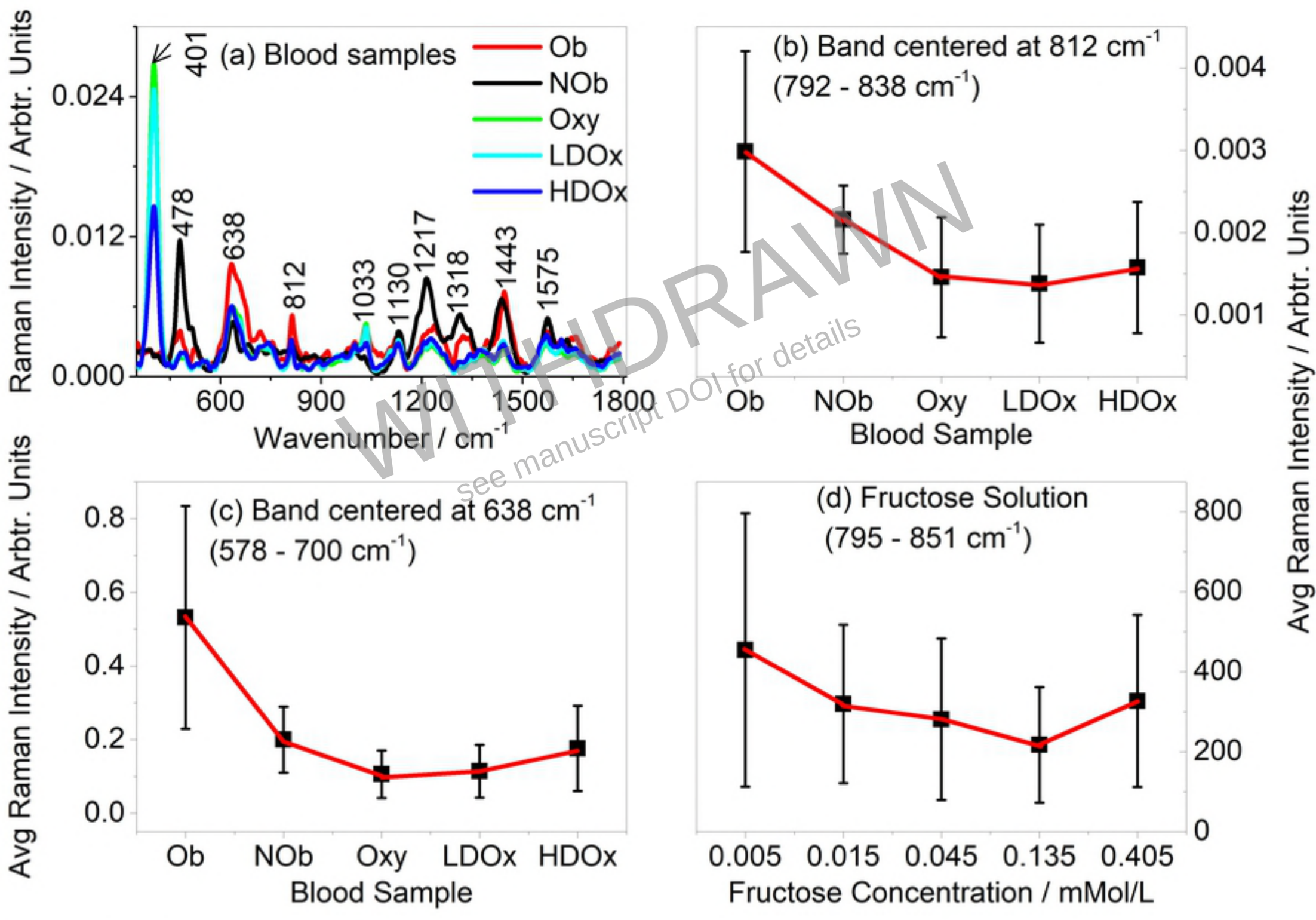

Figure 2

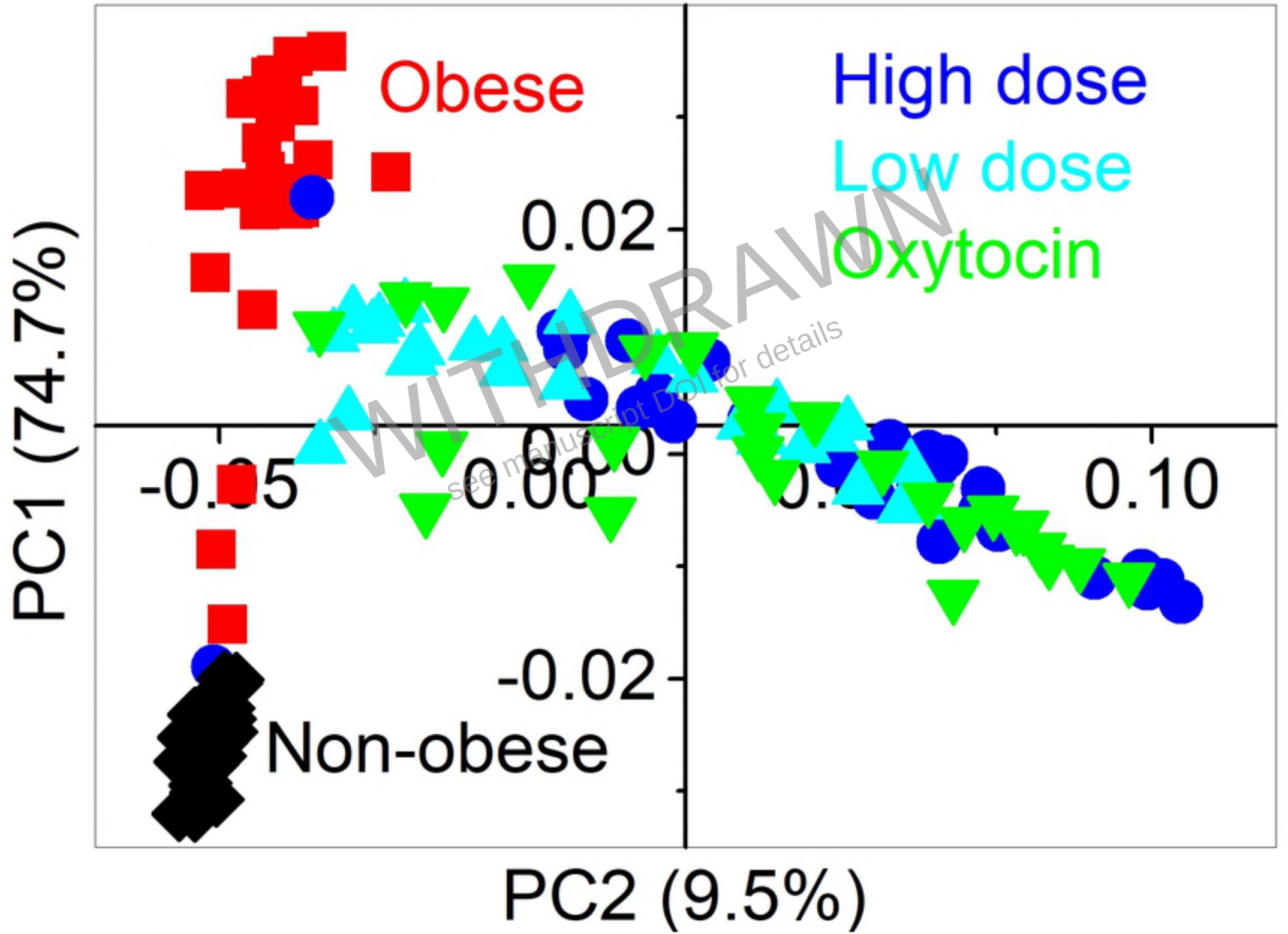

Figure 3