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A NEW LECTIN EXTRACTED FROM *MONIEZIA EXPANSA* HELMINTH CAUSES HUMAN SPERM AGGLUTINATION INCREASED DURING *IN VITRO* ACTIVATION

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ABSTRACT : The present study aims to isolating, partial purifying and characterizing the lectin isolated from *Moniezia expansa* in the sheep's intestines and testing the effect of this lectinin human sperm activity. The effect of partially purified lectin on the sperm studied in males suffers from asthenozoospermia divided into two categories depending on total sperm motility percentage: sever and moderate asthenozoospermia. The measurement of agglutination for two groups asthenozoospermia before and after activation with Ham's F-12 and compare the results with the control sample, the results revealed a significant decrease (P 0.027) in the sperm agglutination of sever and moderate asthenozoospermia compared to normozoospermia. However, it considered as a significant increase (P 0.01) in sperm agglutination in moderate asthenozoospermiaafter activation by Ham's F-12 with lectin compared with lectin only. While significantdifferences (P 0.827) between two parts of the sample after activation by Ham's F-12 with lectin and lectinonly in severe asthenozoospermia. The results showed a positive correlation between total motility percentage and agglutination in moderate asthenozoospermia before and after activation with Ham's F-12 and in severe asthenozoospermia after activation. While a negative correlation was in sever asthenozoospermia before activation.

Key words : *Moniezia expansa*, lectin, spermatozoa, motility, agglutination.

INTRODUCTION

Moniezia expansa is one of the most common intestinal Cestoda also known as sheep tapeworm or double-pored ruminant tapeworm, causing monieziasis that constitutes a problem in sheep breeding, *M. expansa* parasite in the intestine of sheep, cattle and goats as a definitive host (Shalaby and Amer, 2012). The lectins provided an excellent tool for studying glycoprotein redistributions and allowed us to observe variations in the sperm plasma membrane components (Baker *et al*, 2004). Lectins are sugar-binding proteins widely distributed in nature in organisms such as viruses, bacteria, fungi, plants and animals (Sharon and Lis, 2003). Infertility refers to the failure of a couple to conceive after 12 months of unprotected regular intercourse and male factor infertility is partially or fully responsible for about 30–55% of cases of infertility (Hamada *et al*, 2013). Of the primary parameters of semen analysis, motility has a much stronger relationship to both percentage of pregnancy and conception rate when compared to sperm concentration.(Ayala *et al*, 1996). Low motility numbers are referring to as asthenospermia. Asthenozoospermia can range from zero motile sperm to low numbers. The WHO defines asthenozoospermia as falling below 40% motility, but some prefer 20 million total motile counts as a measure instead (WHO, 2010; Hamiton *et al*, 2015). Asthenozoospermia has been established, including disturbances of mitochondrial sheath and axonemal complex formation during spermiogenesis, impaired function of accessory sex glands providing compounds required for movements or epididymis responsible for maturation of spermatozoa, genetic defects and hormonal disturbances. All of these could induce the occurrence of asthenozoospermia (Gonzales *et al*, 2001) and (Piasecka and Kawiak, 2003). The membrane surface of sperm coated with a thick layer of glycan's, i.e., sperm glycocalyx plays an important role in sperm motility, maturation and fertilization (Tollner *et al*, 2012). The carbohydrate composition of the entire surface of spermatozoa undergo striking changes during capacitation, and a close relationship may exist between the sperm surface and the metabolic changes occurring within capacitating spermatozoa (Ahuja, 1984). Our study aimed to characterize the lectin pattern on the sperm plasma membrane of unselected and selected human sperm of normozoospermia donors.

MATERIALS AND METHODS

Ethics

This study done according to ethics criteria of the biological ethics committee. All patients told about the importance and details of research. Moreover, fully explain the aims of this study.

Extraction and characterization of crude lectin

The helminths obtained from infected and slaughtered sheep in the massacre of AL-Hilla city, Babylon province. The tapeworms washed several times with normal saline then crushed manually with a small amount of PBS until the worms completely turned to a thick liquid which then centrifuged 10000 rpm for 30 minutes in a cooling centrifuge. The deposit is neglected and the supernatant was stored in cooling condition until used (Casaravilla, 2003). Estimation of protein concentration used Bradford (1976) after drawing a standard curve by using Bovine serum albumin (mg/ml) as a stock solution.

50 µl of RBCs suspension solution 2% from each blood group as prepared in paragraph 3-3-3-1 added to 50 µl of a series of dilute solutions of lectin in a V shape well plates, incubated for 30 minutes at 37°C (Al-Mawla, 2013).

Purification of crude lectin

The lectin purified by using DEAE – cellulose. The later prepared by dissolving the DEAE – cellulose with 0.15 M PBS and degassing the air by the vacuum pump. Then the resin loaded in glass column in dimensions (1.25 × 50 cm). The lectineluted with 0.15 M PBS pH= 7.3 and each 5ml of elution collected in test tubes. Each 5ml fromthe eluted solution monitored using UV to measure the absorbance at 280 nm by UV spectrophotometer (Whitaker, 1972). Partially purified lectin then was loaded slowly over the Sephacryl S-200 in the column; 5ml for each fraction collectedand then examined by absorbance at 280 nm of each fraction.

Molecular weight estimation of purified lectin

The molecular weight of lectin estimated by gel filtration method using Sepharose- 6B peaked according to the recommended of the manufacturing company (Pharmacia catalog). Then standardization did, by plotting the elution volume of each standard protein to the void volume of the blue dextran 2000 versus the log of each standard protein molecular weight (Stellwagen, 1990) and thus the molecular weight of GTFs was accordingly calculated.

Specificity of purified lectin to sugars

Seven different types of sugars (Maltose, Xylose, Galactose, Sucrose, Glucose, Fructose and Lactose) used with a concentration of 100 molars, which mixed separately with 25 microliters with the same volume of purified lectin. Incubated at 37°C for half an hour, 50 microliters of red blood cells suspension 2% were added, distributed on seven wells in the dilution plate separately for each sugar type and then incubated at 37°C for half an hour and the agglutination titer was measured (Al-Mawla, 2013).

The effect of pH on purified lectin

The solution was prepared with different ranging from 3 to 10 (as described by Kuku and Oladiran (2004) by the transfer of the purified lectin (25ìl) and pH solution (25ìl) in titration plate then mixed with 50ìl on RBCs 2% suspension, incubated 37°C (Kuku and Oladiran, 2004).

The effect of purified lectin on male sperms

Semen samples

Semen samples obtained by masturbation from forty-five consenting donors after 3 to 7 continuous days of sexual abstinence. The samples received at the laboratory and a basal semen analysis conducted within one hour. At this point, the semen sample split into two aliquots, oneused as an entire sperm sample, and the other one conducted by the washout and centrifugation procedure. All semen samples classified as normozoospermic and asthenozoospermia according to the World Health Organization (2010).

Preparation of test solutions

To quantify the sperm agglutinating immobilizing activities and titer of lectins. Dilutions of test solutions of different lectin concentrations were prepared by dissolving lectin with PBS.PH 7.2 by the dilution factor. The lectin adjusted to 40µg, 20µg, 10µg, 5µg and 2.5µg, respectively in per ml of PBS for the experiment and kept at fourº before the experiment.

Washout and centrifugation procedure

A highly motile sperm population selected with the washout and centrifugation procedure using Ham'sF-12 medium (AL Basheer scientific bureau). 0.5 ml of the semen sample was diluted 0.5:0.5 in the medium. After 10 minutes of centrifugation at 250 g, the supernatant discarded. Then 50ì l of the pre-warmed medium was carefully added to the pellet without mixing and incubated for 30min at 37ºC to allow motile sperm to migrate from the pellet to the medium. A motile spermpopulation selected recovering the supernatant fresh and selected semen samples and addition 40µg/ml of lectin and estimation of sperm agglutination (Al-Harrby, 2002). A

motile sperm population selected recovering the supernatant fresh and selected semen samples and addition 40µg/ml of lectin and estimation of sperm agglutination (Rose *et al*, 1976).

Agglutination specifically refers to motile spermatozoa sticking to each other, head-to-head, tail-to-tail or in a mixed way. The motility is often vigorous with a frantic shaking motion, but sometimes the spermatozoa agglutinated that their motion is limited. The major type of agglutination (reflecting the degree (grades 1–4) and thesite of attachment (grades A–E) should be recorded (Rose *et al*, 1976).

Grade 1 : isolated <10 spermatozoa per agglutinate, many free spermatozoa.

Grade 2 : moderate 10–50 spermatozoa per agglutinate, free spermatozoa.

Grade 3 : large agglutinates of >50 spermatozoa, some spermatozoa still free.

Grade 4 : gross all spermatozoa agglutinated and agglutinates interconnected.

RESULTS AND DISCUSSION

Characterization of lectin

Specificity of the crude lectin to blood groups (ABO) : The crude of lectin extracted from *M. expansa* shows its ability to agglutinate the erythrocyte of thehuman; the blood group O positive shows the highest agglutination titer with the crude lectin (1: 5120). Hou *et al* (2010) extracted lectin from red kidney bean and the result of haemagglutination test with chicken red blood cells of degreased and ungreased red kidney beanpowder with reversed micelles was 283 and 164 titer/mg, respectively. The specific haemagglutination activity expressed as unit mg–one protein (Nagre *et al*, 2010). AL-Morshidy *et al* (2016) shows that the lectin isolated from adult worm *Fasciola gigantica* agglutinate human RBCs (1:5120 and 1:1280 before and after purification, respectively).

Purification of lectin

Precipitation with ammonium sulfate : The considered the most important step in the purification process. For that purpose, the sulfate or chloride of sodium or ammonium or ethanol, the previous study depends on the precipitation of lectin with different saturation percentages of ammonium sulfate (AL-Mawla, 2013). Bhowal *et al* (2005) used 80% ammonium sulfate to precipitate lectin from fungus *Macrophomina phaseolina*.

Ion exchange chromatography : After ammonium sulfate precipitated, lectin was passed through DEAE-cellulose column that already equilibrated with acetate buffer (0.1M, pH 5.6), and the absorbance was read at 280nm for wash fraction. The elution step for binding protein (which carry a negative charge). In this step solution of lectin eluted with a gradient of 0.1-1 M of NaCl solutions, the peaks of lectin explained in Fig. 1.

Pan *et al* (2010) used DEAE-Se AL-Mawla (2013) used DEAE-Cellulose in ion-exchange chromatography to purified lectin isolated from *Phaseolus vulgaris*, the agglutination activity in this step was 45.5 agglutination unit/mg.

Bhowal *et al* (2005) used DEAE-Sephadexa 50 in the purification of asialic acid-specific lectin from the phytopathogenic fungus *Macrophomina phaseolina*, the specific activity of the lectin increased nearly to 2000 with a 62-fold purification compared to the culture filtrate. The protein recovered was 3.25%.

Gel filtration chromatography : Lectinsolution obtained from ion-exchange chromatography was concentrated then passed through a Sephacryl S-200 column (1.5×70 cm) that equilibrated with PBS (0.1M, pH 7.3). The fractions collected and measured at 280nm absorbency, the peaks oflectin explained in Fig. 2.

Belew *et al* (1978) showed the results show that Sephacryl S-200 superfine has good resolving power for proteins having molecular weights below 30 000. Pan *et al* (2010) used gel filtration chromatography on Sephacryl S-200 HR and Superdex 200 10/300 GL columns to purify lectin isolated from the gill of bighead carp (*Aristichthys nobilis*).

Lectin molecular weight estimation : The molecular weight of lectin estimated by using a gel filtration method. Sepharose 6 B gel used in dimension (1.5×70 cm) to detect the elute volume by Blue dextran-2000. It is represented the Vo.Yufang (2010) mentioned that lectin isolated from beans plant seeds was monomer contains from one band when electrophoresed and its molecular weight 30000 D. AL-Mawla (2013) mentioned that lectin isolated from beans plant seeds was monomer contains from one band when electrophoresed and its molecular weight 35500 Dalton.

Stability of partially purified lectin with different temperature degrees : The results showed that the effectiveness of lectin increased by increasing the temperature and reached a maximum of 40°C and gave a high indication of the direction of the temperature. Bala *et al* (2010) found that the isolated lectin from the seeds of the plant has thermal stability ranging from 10 to 70 degrees Celsius and optimum heat of 37^0 Celsius.

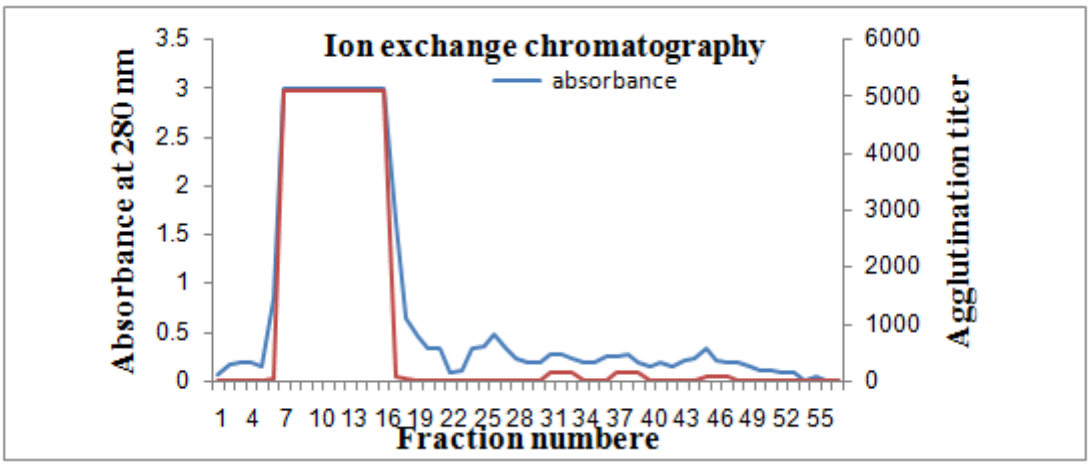

Fig. 1 : Ion exchange chromatography by using DEAE-Cellulose, (1.5 × 17) cm in dimensions, to purified lectin extracted *M.expansa*, the column washed with PBS, elution with the linear-gradient (0.7M NaCl), flow velocity 30 ml/hr. 5 ml/fraction.

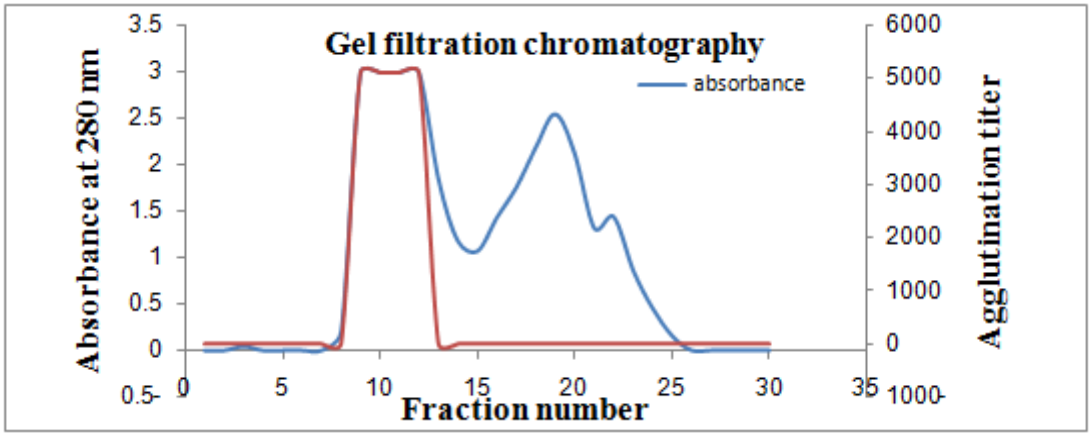

Fig. 2 : Gel filtration chromatography by using Sephacryl-S 200 (1.5×70 cm) in dimensions, to purified lectin extracted from *M. expansa*, the column washed with PBS, flow velocity 30 ml/hr. 5 ml for each fraction.

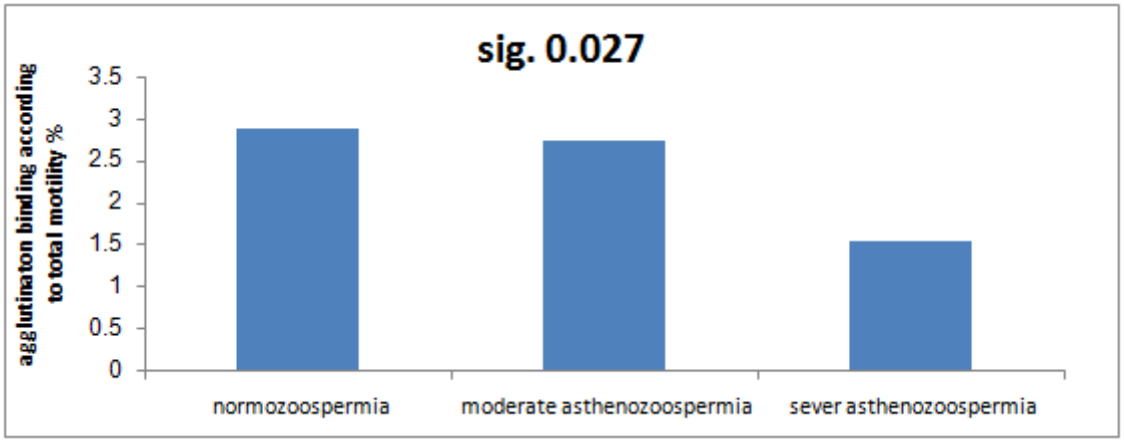

Fig. 3 : Lectin binding according to total sperm motility percentage, entire sperm sample (Normozoospermia, Severe and Moderate asthenozoospermia). P-value (0.027).

The effect of different pH on partially purified lectin : The effect of pH is its effect on the protein ion state and the range of amino acid amino acids that are necessary to maintain the stability of the protein, as the results show, the optimal pH for partially purified lectin efficacy is eight. This shown in the pH numbers 5 - 11 and the lectin loses its full efficacy at 4 and 11, these results also mentioned by Moreira and Perrone (1977), AL-Mawla (2013).

The specificity of the partially purified lectin to carbohydrates : The current study showed that the partially purified lectincould precipitate sugars maltose, xylose, glucose, galactose, sucrose, fructose and lactose. This indicates the difference of specialization of lectin

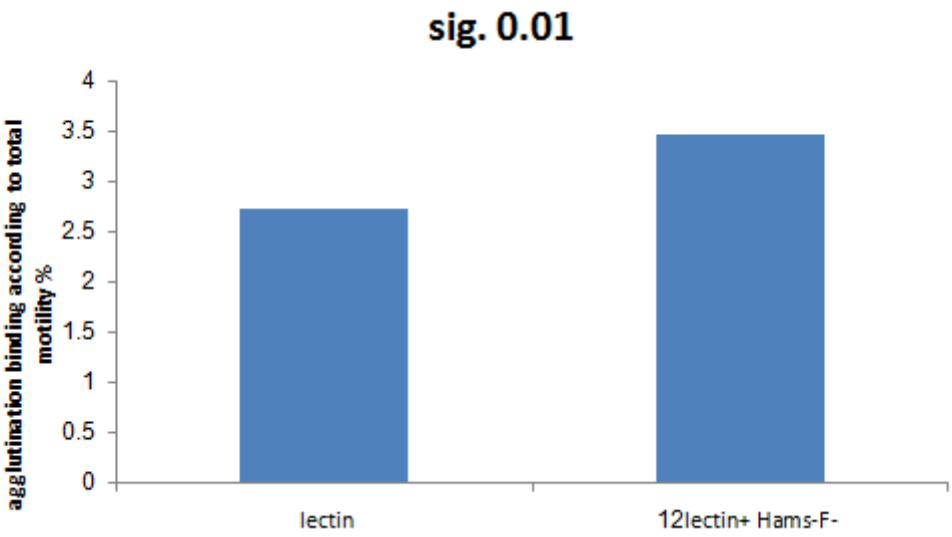

Fig. 4 : Lectin binding according to total motility percentage and sperm population recovered in Ham´sF-12 after centrifugation technique, P-value 0.01

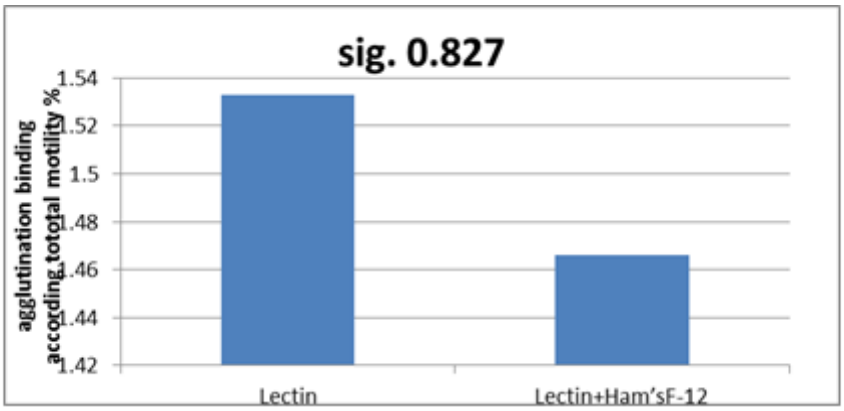

Fig. 5 : Lectin binding according to total motility percentage and sperm population recovered in Ham's F-12 after centrifugation technique, P-value (0.827).

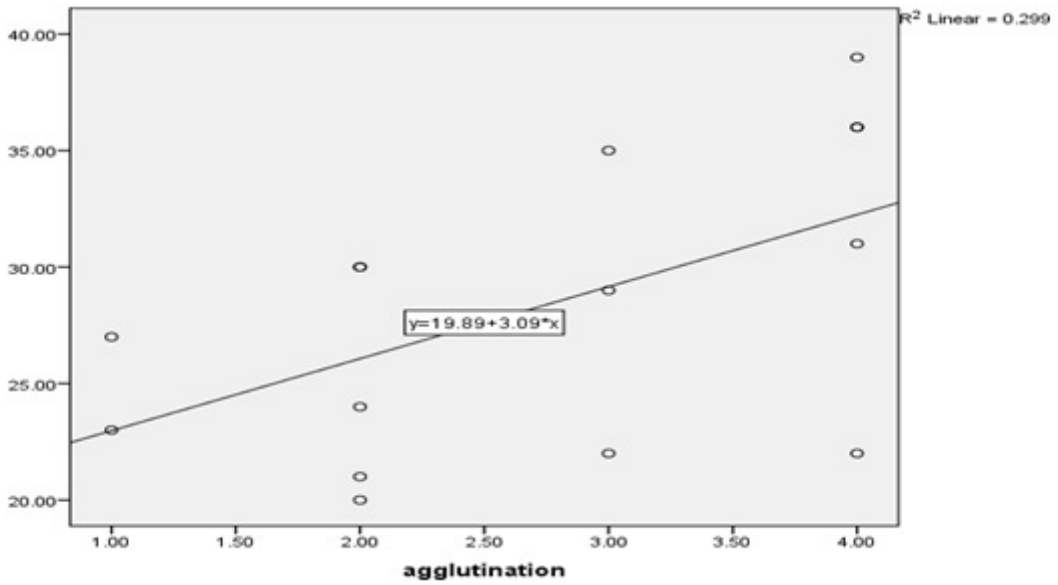

Fig. 6 : Correlation between total motility percentage and agglutination of moderate asthenozoospermia after activation with Ham´s F-12.

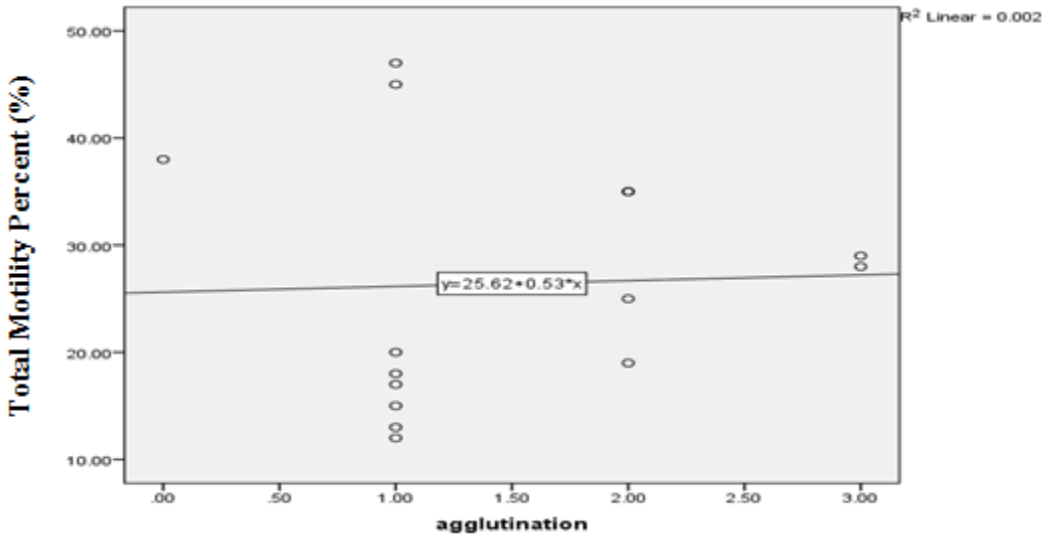

Fig. 7 : Correlation between total motility percentage and agglutination of severing asthenozoospermia after activation with Ham's F-12.

for carbohydrates and these acids have a significant role in maintaining the composition or installation of the site of the link (Sharma *et al*, 2009).

The effect of partially purified lectin on sperm agglutination : Motility of sperm is a serious factor and of significance for transport to the site of fertilization and the events essential to the penetration of the oocyte. Asthenozoospermia, or low sperm motility may be caused by sperm structural or functional insufficiency (Diekman, 2003; Gatti *et al*, 2004; Pang *et al*, 2011), by a harmful effect of seminal plasma (Feugang *et al*, 2016), or by a combination of these. It is a common cause of human male infertility. One of the primary parameters is movement, as the movement has a strong relationship to pregnancy rates and the rate of reproduction compared to the concentration of sperm. Male infertility diagnosed by semen evaluation; however, some damages cannot be found by usual techniques and needs specific procedures to detect the injury, especially in sperm membranes (Souza *et al*, 2005).

To date, the role of animal lectin on spermatozoa not reported, although it has previously shown to the effect of plant lectin on spermatozoa. The membrane surface of mature sperm coated with a thick layer of glycan, i.e., the sperm glycocalyx, including O- and N-glycan, which protects sperm during transit in the female reproductive tract and assists with other key functions (Diekman, 2003; Gatti *et al*, 2004). This study showed no significant differences in sperm agglutination assay between normozoospermia and moderate and severe oligozoospermia (Fig. 3). The mature coating of glycan on the surface of sperm is a prerequisite for them to gain fertilizing capability. Lectins are protein or glycoprotein, usually, without catalytic activity that can bind to specific carbohydrates expressed on different cell surfaces (Marques and Barracco, 2000). The lectins provided an excellent tool for studying glycoprotein redistributions and allowed us to observe variations in the sperm plasma membrane components. It previously observed through this methodology that modification of the lectin-binding pattern detected in mice sperm after capacitation (Baker *et al*, 2004).

The present study showed no significant increase in agglutinationin moderate asthenozoospermia after activation by Ham's F-12 compared to the lectin only (Fig. 4). The lectin used to change in permeability of sperm membrane consequent to lectin binding to the sperm surface or to the attachment of spermatozoa to the zonapellucid surface considered a preliminary step that may or may not lead to acrosomal exocytosis (Schwarn and Kohler, 1979). An agglutination assay may prove useful as a director for the occurrence of capacitation, which used to develop compare surface properties of in capacitated and capacitated mammalian sperm.

One of the major outcomes of this study is that significant increase in agglutination of severe asthenozoospermia after activation by Ham's F-12 compare with used lectin only (Fig. 5). Male infertility diagnosed by semen evaluation; however, some damages cannot be found by usual techniques and needs specific procedures to detect the injury, especially in sperm membranes. Studies have been carried out to develop new techniques for seminal evaluation it is well known that cell surface glycan or glycocalyx play important roles in sperm motility, maturation and the membrane surface of sperm is coated with a thick layer of glycan (Xin *et al*, 2014). Lectins, high molecular weight glycoproteins with different sugar-binding specificity, can agglutinate

different cell types. The recovery of high-quality spermatozoa facilitated by the agglutination induced by the lectin binding (Pérez *et al*, 1999).

Total motility percent (%)

Our results appeared positive correlation between sperm motility percent and agglutination of sperm in moderate asthenozoospermia and after activation in severe asthenozoospermia (Figs. 6 and 7). Lectins, high molecular weight glycoproteins with different sugar-binding specificity, can agglutinate different cell types. The recovery of high-quality spermatozoa facilitated by the agglutination induced by the lectin binding (Pérez *et al*, 1999).

The correlation between total motility percent and agglutination showed negative in sever as then ozoospermia before activation. The glycan moieties covering cell surfaces are involved in many physiological and pathological processes related to the cell. Disturbances in the cell environment related to diseases frequently trigger changes in glycan, such as fucosylation, sialylation, abnormalities in glycan structure and uncommon glycan (Svarovsky and Joshi, 2014).

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