

# Glycoprotein of *Schistosoma mansoni*: isolation, characterization and elucidation of its immunodiagnostic potency

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## ABSTRACT

The diagnosis of *Schistosoma mansoni* in prepatent phase of infection is necessary to prompt liver damage and to prevent late morbidity. This study was designed to purify and characterize a glycoprotein antigen from mature *S. mansoni* fluke using Con-A lectin. In this Study, crude adult worm antigen (AWA) and purified fraction were separated by molecular weight using SDS-PAGE. Chemical structure of purified fraction determined using Mass spectrometry technique. ELISA used to evaluate diagnostic efficacy of both crude and purified antigens. AWA was electrophoresed by SDS-PAGE in to 10 bands with Mwt ranged from 14.2 to 220 kDa while the purified Ag was separated in to two bands with Mwt 74.6 and 20 kDa. The chemical structure of the purified antigen was found to be (Al-Ser-Arg-Ser-Arg-GlcNAc). The purified glycoprotein exhibited 100% positivity with both human sera and mice sera collected at different intervals from infection. In addition, purified Ag detects the infection at early patent period (24h post infection). Results of immune blotting confirmed the immune reactivity of the purified glycoprotein as the human positive sera identified the band at 74.6kDa. Also, ELISA presented 100% sensitivity compared to 84.4% of the available commercial IHA kits. When the purified antigen was tested with serum samples infected with other parasites in ELISA, it showed 100% specificity compared to 96.6% of the commercial IHA kits.

The described antigen is a promising diagnostic antigen for prepatent *S. mansoni* infection, and its immune prophylactic potency will be tested in further study.

**KEY WORDS:** *Schistosoma mansoni*, glycoprotein, Diagnosis, Mass spectrometry, ELISA.

## 1. INTRODUCTION

Schistosomiasis (common name is bilharzia) is a most destructive parasitic disease caused by *schistosoma* species. In tropical region schistosomiasis is the second important disease after malaria (Gryseels, 2006; Peeling, 2006). Worldwide, more than 230 million people at danger of schistosomiasis (Steinmann, 2006). The predestined 166 million cases in Africa represent 90% of the world's cases (King, 2015). In Egypt two species that cause the most morbidity in humans are *Schistosoma haematobium* (urinary schistosomiasis) and *Schistosoma mansoni* (intestinal schistosomiasis). Approximately 60% of people is suffer from intestinal schistosomiasis in Nile Delta zone (Barakat, 2013).

The requirement for accurate diagnosis of disease is critical for epidemiologists and disease control director for all parts of prevention, control and observation to help in assessment of efficacy of anti-schistosomal drug and vaccine to decrease the negative effect of disease on human and animals (Bergquest, 2009; Johanson, 1998; 2010). There are some difficulties in choice between different techniques for diagnosis of schistosomiasis (Doenhoff, 2004). The choice of antigen used in diagnosis is the most important difficult faced. There are several factors that influence this choice which is easily of production, high stability in sample storage, immunogenicity, specificity, sensitivity, and its cost (Hewitson, 2009).

The profuse constituents of the outer surfaces of helminths parasites were glycan (Hokke, 2007). Schistosomes, as other parasitic helminthes, produce many complex glycan structures linked to proteins and lipids as glycoproteins and glycolipids, which are structurally, differ from their host. It is obvious that glycans and glycol conjugates play a vital role in the biology of the parasite, in particular with regard to host-pathogen interactions, but the specific functions them remain obscure (Prasanphanich, 2013; Smit, 2015; Ko, 1990). The expression of glycosyl transferases through different stages of lifecycle was regulated many glycan (Ko, 1990). There is attention in defining these unusual parasite-synthesized glycan antigens and their use in understand immune responses, their function in immunomodulation, and their using as immunodiagnostics and effective vaccine targets. Schistosomes integrate various intriguing glycan structures, a large portion of which are identified with those found in animal cells. These include glycans containing the Lewis<sup>x</sup> antigen (Le<sup>x</sup>), which were the first schistosome glycans to be structurally characterized and shown to be antigenic (Srivastan, 1992; Van Dam, 1994; Nyame, 1998) and specifically synthesized by schistosomes and no other trematodes or nematodes (Khoo, 1995). In addition to Le<sup>x</sup> structures, the adult worms of *S. mansoni* also synthesizes N- linked glycans containing GalNAc $\beta$ 1-4GlcNAc ( LacdiNAc, LDN)

and GalNAc $\beta$ 1-4(Fuc $\alpha$ 1-3)GlcNAc(LDN-F), the LacdiNAc analog of the Le<sup>x</sup> antigen. Multifucosylated structures have been found on O-glycans of the cercarial glycocalyx with the following terminal structure: (Fuc $\alpha$ 1 $\rightarrow$ 2) $\pm$ Fuc $\alpha$ 1 $\rightarrow$ 2 Fuc $\alpha$ 1 $\rightarrow$ 3GalNAc $\beta$ 1 $\rightarrow$ 4(Fuc $\alpha$ 1 $\rightarrow$ 2) $\pm$  Fuc $\alpha$ 1 $\rightarrow$ 2 Fuc $\alpha$ 1 $\rightarrow$ 3)-GlcNAc $\beta$ 1 $\rightarrow$ 3Gal $\alpha$ 1 $\rightarrow$  (Khoo, 1997) and on glycolipids of *S. mansoni* eggs that consist of a backbone of repeating  $\beta$ 1 $\rightarrow$ 4 linked GlcNAc residues replace with Fuc $\alpha$ 1-2 Fuc $\alpha$ 1-3 side chains (Nash, 1981). The choice of glycans in diagnosis and control of parasitic diseases is the best select because different reasons include that humoral immune response in schistosomiasis was mainly directed towards glycol conjugates (Aronstein, 1983; Ali, 1988; Nyame, 1996), also, Infection with *S. mansoni* was generate IgM and IgG antibodies against the Le<sup>x</sup> structure (Van Dam, 1996; Nyame, 2004). Moreover, parasite derived glycans have structural features differ from mammalian glycans and now it is easy to synthesize complex glycans using combinations of chemical and enzymatic techniques and proved that it is highly antigenic and well recognized by antibodies in sera of infected human and or animals (Parasuraman, 2010). Consequently, the aim of the current research is to isolate and characterize *S. mansoni* glycoprotein antigen and prove its diagnostic potency in detection of *S. mansoni* infection.

## 2. MATERIALS AND METHODS

**Experimental animals: Mice and snails:** Swiss albino mice CD-1 strain and infected *Biomphalaria alexandrina* snails were originally purchased from Schistosome Biological Supply unit (SBSU), Theodor Bilharz Research Institute (TBRI), Giza, Egypt. Then kept under laboratory conditions for life cycle maintenance.

**Rabbits:** Three rabbits of Californ strain were maintained under laboratory conditions were used for preparation of hyper immune sera against *S. mansoni* crude antigen.

Experiments were performed according to the Guide for the Care and Use of Laboratory Animals and Approval of animal rights according to Committee, National Research Centre, Egypt under registration number 1-2 /0-2-1.2012.

**Experimental sera and worm collection:** Blood samples were collected from infected mice at intervals from infection beginning from the first 24 hrs then weekly till the eighth week post infection from the orbital sinus (Duvall, 1967). Blood samples were centrifuged at 500 rpm for 5 minutes to obtain sera. After the 8<sup>th</sup> week, mice were sacrificed by cervical decapitation and portally perfused to collect adult worms from the portal vein and mesenteric venous plexus (Cheever, 1970; Guo, 2011).

**Human sera:** A total of 236 serum samples were given by Dr. Sahar Salim, Medical Parasitology Department, National Liver Institute, Shebin El-Kom, Menoufia University, Egypt. These samples were categorized into: 132 random samples positive serologically by indirect heamagglutination test for schistosome antibodies. 68 sample positive for intestinal schistosomiasis by finding ova in stool samples. 21 samples positive for other parasitic infection (Ascariasis, Echinococcosis, Fascioliasis, Entrobiasis, Trichostrongliasis, Toxoplasmosis and Amoebiasis), and 15 negative samples for schistosomiasis parasitologically.

**Preparation of adult worm crude antigen:** The collected worms were homogenized in 0.01M phosphate buffer saline (PBS) pH 7. 2. The suspension was then centrifuged at 14,000 rpm for 30 minutes at 4°C, then supernatant "adult worm antigen (AWA)" was collected and assayed for protein content (Lowery, 1951). The supernatant was aliquoted into 1.5 ml aliquots in plastic eppendorf tubes then stored at -20°C until used.

**Hyper immune sera preparation:** About 100 mg (Gubadia and Fabgemi, 1997) of *S. mansoni* adult worm antigen was mixed with an equal volume of Freund's complete adjuvant and injected subcutaneously into each rabbit (Fabgemi, 1995). The first booster dose was administered 14 days post the initial dose after emulsification in Freund's incomplete adjuvant. Second and third booster doses were given at 21 and 28 days respectively (Csizmas, 1960). Sera were collected 3 days post last injection from ear vein, and then serum samples were stored at -20°C until used.

**Indirect haemagglutination inhibition assay (IHIA):** Identification of sugar structures associated with adult worm antigen was performed using indirect haemagglutination inhibition assay. Sheep erythrocytes "SRBCs" were collected from Shebin Ekoum abattoir. Aliquotes of SRBCs were formalized, tanned and coated with adult *S. mansoni* crude antigen (Laemml, 1970). Different sugars were tested for their competitive inhibition of the binding activities between *S. mansoni* antigen and rabbit hyper immune sera rose against *S. mansoni* crude antigen.

**Lectin affinity Chromatography:** Prepacked *Concanavalin ensiformis* Con a column obtained from Sigma Chem. Co. St. Louis, MO, was utilized for isolation of glycoprotein fraction of *S. mansoni*. The fraction was assayed for protein content (Lowery, 1951).

### Characterization of the isolated glycoprotein:

**SDS-PAGE:** The technique was performed according to the procedures described by Laemml (Wray, 1981) for characterization of adult *S. mansoni* crude antigen and the purified glycoprotein fraction. The antigens were mixed separately with reducing sample buffer containing 5% 2-mercaptoethanol and electrophoresed on SDS-PAGE 12% slab gel. After separation, slab gel was silver stained (Towbin, 1979). The gel was then destained with a destaining solution till the bands become clear.

**Immuno blotting:** The immunogenicity of the isolated fraction was tested by immune blotting assay. The protocol described the immune blotting of the purified glycoprotein fraction onto nitrocellulose membrane (Towbin, 1979). Subsequent detection of protein was performed using human antisera positive for *Schistosoma* antibodies, Horse-raddish peroxidase conjugated goat-anti-human IgG was added. ECL western blotting detection reagent (Amersham, UK) was utilized for visualization of the immune reactive bands.

**Chemical characterization:** The analysed *S. mansoni* purified glycoprotein fraction was homogenous by thin-layer chromatography (TLC), which was performed on EM silica gel 60 F sheet (0.2 mm) with  $\text{CHCl}_3/\text{CH}_3\text{OH}$  (9:1 v/v) as the developing eluent and rate of flow (Rf)=0.2. The spot was detected with U.V model UVGL-58 as a violet spot. EI Mass spectra recorded on a Varian MAT 311 a spectrometer.

**ELISA:** The assay was utilized to evaluate the diagnostic potentials of the crude and isolated fraction in experimental *S. mansoni* infection in mice, potency of the fraction in the diagnosis of schistosome natural infection in human. In addition, the sensitivity specificity of the isolated fraction with human samples infected with other parasites. The test was carried out as described by Oldham (1983) and the optimal concentration of the antigen and dilution of serum were determined by checkerboard titration. Anti-mouse and anti-human IgG horse radish peroxides were used and cut off point of point of optical density values was determined according to Hillyer (1992).

**Indirect haemagglutination assay (IHA):** IHA assay was utilized for the detection of *S. mansoni* antibodies using erythrocytes coated with specific adult worm antigen (Van Gool, 2002). The IHA test kit (Fumouze Diagnostics Company, Paris, France) was prepared following the manufacturer's instructions. 50 $\mu\text{l}$  of PBS was added to each well, in tubes 10 $\mu\text{L}$  of stock sera added to 390  $\mu\text{l}$  of PBS, 50  $\mu\text{l}$  of sera stock dilution were delivered to the wells of the micro plate and mixed well with PBS, then 50  $\mu\text{L}$  of the mixed serum stock dilution was added to the first well, mixing it well with the buffer solution and then discard 50  $\mu\text{L}$  to obtain dilution 1:80. Then carefully, one drop of the sensitized red blood cells was delivered into each well. One drop of un-sensitized red blood cells was added to one well serving as serum control. Very carefully, the wells content was homogenized and allowed to remain motionless protected from vibration, for 2 h. The plate was examined for any agglutination. Each sample yielded agglutination reaction equal to/or more than 1:80 corresponds with a past or an already treated infection.

**Statistical analysis:** Data of ELISA were analyzed for the means and standard deviation according to Senedecor and Cochran 1982. Significance of the results was evaluated using one way ANOVA using statically package for social science version 15 (SPSS) computer programs (2002).

### 3. RESULTS

**Indirect haemagglutination inhibition assay (IHIA):** Identification of sugar structures associated with adult *S. mansoni* was investigated by testing some sugars for their ability to competitively inhibit the binding activities in the rabbit hyper immune sera towards *S. mansoni* crude antigen in haemagglutination inhibition assay (HIA). The results clarify that glucose achieved the highest inhibition percentage of the binding activities at both low (83.3% at 25mM) and high (58.3% at 200mM) concentrations of sugar as demonstrate in figure.1. While mannose did not inhibit the binding activities at low concentration (25mM) and represent moderate inhibition percentage (41.7%) at high concentration (200mM). Fucose showed weak inhibition percentage (16.7%) at high concentration (200mM).

**Isolation of *S. mansoni* glycoprotein:** Con a lectin affinity chromatography was utilized for purification of corresponding glycoprotein. The protein content of isolated fraction was observed to be 49.43 $\mu\text{g}/\text{ml}$  which performs only 10.9% from the total loaded protein which was 452.4 $\mu\text{g}/\text{ml}$ .

**SDS-PAGE:** The electrophoretic profiles of crude antigen display multiple bands with molecular weights ranged from 14.2-220 kDa (Fig. 2). However, in the isolated glycoprotein fraction it was detected two bands at molecular weights 74.6 kDa and 20 kDa.

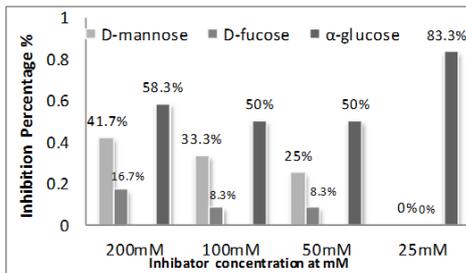
**Immuno blotting:** To assess the immunogenicity of the isolated bands of the purified glycoprotein fraction using *S. mansoni* positive human serum samples immune blot analysis was conveyed. The serum recognized single band at molecular weight 74.6 kDa (Fig.3).

**Chemical characterization by Mass spectrometry:** The Thin Layer Chromatography (TLC) gave one spot in  $\text{MeOH}/\text{CHCl}_3$  (80:20 v/v) as an eluent. By immersing the TLC sheet in 5%  $\text{H}_2\text{SO}_4/\text{Me}-\text{OH}$  solution and heating it gave burning red color for GlucNAc sugar moiety. The mass spectrum of the monomer  $m/z = 968$  [M + 3H]. Al-Ser-Arg-Ser-Arg-GlucNAc. The fragmentation or peak at  $m/z = 91$  is an indication for the Alanine (Al) residue [M + 3H]. The fragmentation or peak at  $m/z = 218$  is an indication for the GlucNAc residue [M - 3H]. The fragmentation or peak at  $m/z = 295$  is an indication for the Alanine + Serine (Al + Ser) residue [M + 1H]. The fragmentation or peak at  $m/z = 468$  is an indication for the Alanine + Serine + Arginine (Al + Ser + Arg) residue [M]. The fragmentation or peak at  $m/z = 579$  is an indication for the Alanine + Serine + Arginine + Serine (Al + Ser + Arg + Ser) residue [M + 4H]. The fragmentation or peak at  $m/z = 745$  is an indication for the Alanine + Serine + Arginine + Serine + Arginine (Al + Ser + Arg + Ser + Arg) residue [M - 2H]. The electrophoresis gave 2 bands at molecular weights 74.6 kDa and 20 kDa which means that the glucopeptide polymer is: O-glycan Al-(Ser-Arg-Ser-Arg-GlucNAc)<sub>94</sub>; EI-Mass: [(m/z, %) = 968 (M+ +3H, 80)]<sub>95</sub>.

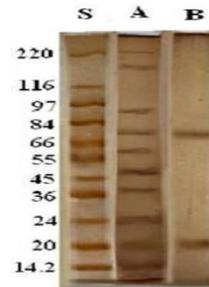
**Diagnostic potency of the isolated glycoprotein fraction:** The best concentration of *S. mansoni* crude antigen was 0.5 µg/ml while that of the glycoprotein fraction was 20 µg/ml and dilution of serum was 1:100. Figure.4, displays the isolated glycoprotein based ELISA had an excellent ability to diagnosis of schistosomiasis at different weeks of infection with significant difference in comparison with crude *S. mansoni* antigen especially at first, six and eight weeks post infection. However, the IHA kits diagnose mice schistosomiasis only at eight weeks post infection.

**Diagnosis of human intestinal schistosomiasis:** Figure.5, indicate the potency of isolated glycoprotein fraction in diagnosis of 200 human serum samples with natural intestinal schistosomiasis infection, whereas, all samples were positive with a percentage of 100 %, while no negative samples were founded.

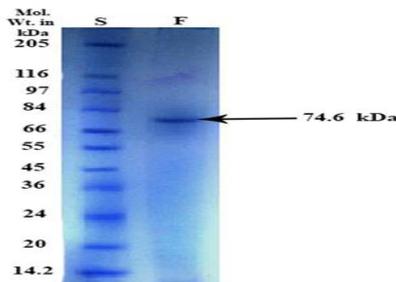
**Sensitivity and Specificity of the isolated glycoprotein fraction:** The purified glycoprotein fraction proved potency against available commercial IHA kits. Where, sensitivity of it was 100% against 84% for available commercial IHA. When both assays tested with human serum samples infected with other parasites the purified fraction achieve 100% specificity in comparison with 96.6% for IHA kits.



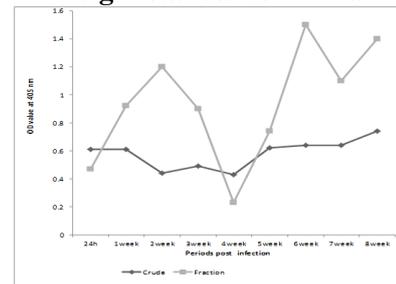
**Figure.1. Carbohydrate inhibition of rabbit hyper immune sera binding activities to *S. mansoni* crude adult extract coat SRBCs**



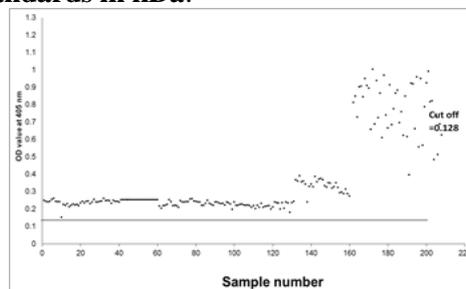
**Figure.2. SDS-polyacrylamide gel electrophoresis silver stained of *S. mansoni* antigens. Lan (A): The crude extract of *S. mansoni* worms. Lan (B): The isolated glycoprotein fraction. Lan (S): molecular weight standards in kDa**



**Figure.3. Immunoreactive band of the isolated glycoprotein fraction identified by *S. mansoni* positive human sera using immunoblot assay. Comassie blue stained. Lan (F): glycoprotein fraction isolated by lectin affinity chromatography. Lan (S): Molecular weight standards in kDa.**



**Figure.4. Comparative diagnostic potentials of crude antigen and the isolated glycoprotein in experimentally infected mice using ELISA**



**Figure.5. Scatter graph representing the potency of the isolated glycoprotein fraction in the diagnosis of human natural schistosomiasis**

**DISCUSSION**

The current results of haemagglutination inhibition assay clearly demonstrate that maximum inhibition was achieved by glucose, followed by mannose while fucose was less inhibitor at all molar concentration. This means that the dominant carbohydrate structures associated with *S. mansoni* adult worm are mainly glucose. The current results agree with Wuhrer (2006), who observed that all *Schistosoma* stages can express glycosphingo lipid GalNac(β1-4)Glc(β1-1) ceramide which has been termed “schisto - core”. Meanwhile, Hayunga (1983), suggested

that antibody binding sites on the glycoprotein tegumental antigen with molecular weight 58 kDa most possible contain moieties which were similar to mannose, fucose, glucosamine, and galactosamine in structure. Comparable results were observed by Hayunga (1983), who identified glucosamine in the isolated antigen. While, the present isolated glycoprotein fraction includes glucose in its acetylated form. Recently, it was obvious that acetylation of sugars prompt their biological activity as proposed by Kuhn (2014), where he prove that N-acetylglucosamin (GlucNac) have role in remodeling the bacterial peptidoglycan where it was play as antibacterial agents and provide a chance for evolution of new antibiotics. The glycoprotein fraction described herein is in acetylated form therefor it is advantage.

Affinity chromatography has been presented as a very effective technique to purify specific diagnostic antigens to avoid cross reactivity between other parasites (Fukuda and Kobata, 1993). According to haemagglutination inhibition results, Con-A lectin affinity chromatography column was applied for isolation of glycoprotein fraction. In accordance, Abdel-Rahman (2014), used Con-A lectin column to isolate glycoprotein fractions from *F. gigantica* mature fluke. This agrees with Simpson and Smithers (1980), who reported the highest specific binding "from six types of lectin" was carried out by con-A and the agglutinin of molecular weight 60,000 Da from *Recinus communis*. Con-A has a broad specificity for mannose, glucose, glucosamine and other related sugars. Moreover, Con-A column was the less costly.

In the current study SDS-PAGE was showed a complex profile of *S. mansoni* worms crude extract revealed 10 bands with molecular weights ranged from 14.2-220 kDa. While, Basyoni and Abd El-Wahab (2013) separated 9 bands with molecular weights ranged from 16-120 kDa. Of them, 7 bands of molecular weights of 92, 70, 67, 54, 44, 30 and 20 kDa were the most distinguished. When adult *S. mansoni* vomit (AWV) prepared by Planchart, (2007) electrophoresed under reducing conditions in 12.5% SDS-PAGE, it displayed at least 20 components with molecular masses between 12 and 116 kDa. Moreover, Boctor and Shaheen (1986), used SDS-PAGE 5-20% gradient gel to characterize crude soluble worm antigen preparation (SWAP) showing that SWAP contained at least 30 bands with both silver stain and Coomassie blue staining methods with molecular weights ranged from  $20 \times 10^3$  to  $200 \times 10^3$ . The distinction between the present data and those reported before may be due to antigen preparations and gel concentrations used (5-20% or 12.5% or 12%).

In addition, in the current data glycoprotein isolated fraction was separated into two bands with molecular weights 74.6 and 20 kDa as proved by SDS-PAGE. While 74.6 kDa proved its immunogenicity than 20 kDa as demonstrated by western blotting. These results are in accordance with Attallah, (1999) who used BRL4 MAb to identify a 74 kDa antigen in three developmental stages antigenic extract of both *S. mansoni* and *S. haematobium*. While, Ibrahim, (2010) utilized a monoclonal antibody 12D/10F "an IgM that recognized an adult *S. mansoni* tegument antigen" in the molecular weight regions of 50-65 kDa which is glycoprotein in nature like the fraction isolated in the current study. Moreover, Coelho-Castelo (2010), separated a mannose-binding protein from adult *S. mansoni* tegument and cercariae, namely Sm60; by SDS-PAGE it revealed two bands at molecular weights of 60/66 kDa. This Sm60 is similar the present fraction prepared here in being glycoprotein but differ in the bounded glycan which may be due to the type of the used affinity chromatography column, as the author used immobilized mannose for isolation. Hayunga, (1983) described the Con A-binding antigen as having a molecular weight of 58 kDa. However, Carvalho, (2011) identified proteins of adult worm preparation of approximately 200, 100, 43 and 18 kDa to be immunogenic with sera from infected mice. Also, they identified similar reaction with schistosomula antigen preparation as the mice sera recognized 100, 43 and 18 kDa.

Structurally recognition of the isolated glycoprotein fraction was performed utilizing mass spectrometry technique. Mass spectrometry results display the chemical structure of the isolated glycoprotein to be O-glycan Al-(Ser-Arg-Ser-Arg-GlucNac)<sub>94</sub>, coincide with this result that of Wuhner, (2006) who reported that *S. mansoni* adults express dimeric, fucosylated LDN N-glycan {GalNac(β1-4)[±Fuc(α1-3)]GlcNac(β1-3)GalNac(β1-4) }[±Fuc(α1-3)]GlcNac(β1-3)}. Similarly, observed that *S. mansoni* express glycans with LDN and LDNF which are targets to the host humoral immune response. In addition, Nyame (2004), reported the occurrence of LDN (F) repeats parallels the poly (LacNac) and poly (Lewis X) chain found on N-glycans from total *S. mansoni* worm glycoproteins. In accordance, Van Die and Cummings (2010), reported that LDN, LDNF and LewisX were expressed within the different schistosomal stages. Both LDN and LDNF were used as a backbone for further modifications leading to several multifucosylated immunogenic structures that are found in egg and cercarial glycoproteins and glycolipids such as LDN-DF or F-LDN-DF (Khuo, 1997; Wuhner, 2002; Jang-Lee, 2007), or repeats of LDN and LDNF as found on N- glycans of schistosomes (Wuhner, 2006).

In the current research the ELISA technique was used to assess the diagnostic ability of glycoprotein isolated fraction in detection of *S. mansoni* infection. The present results pointed toward the fact that the diagnostic potency of presented fraction was most potent than that of crude *S. mansoni* antigen in detection of schistosomiasis at all phase of infection especially at early stage .in contrast the HIA kit detect the schistosomiasis at eight weeks post infection only.

The present study evaluated both sensitivity and specificity of the purified antigen using ELISA for the detection of *S. mansoni* in comparison with the commercial IHA kits. The purified fraction using ELISA achieved maximal sensitivity (100%) and also specificity (100%) while the available commercial IHA kits exposed lower values which were 84.4% “sensitivity” and 96.6% “specificity. The current results come to agreement with Ibrahim and Ibrahim (2014), who reported higher sensitivity of ELISA 93.3% than that of IHA 84.4% with samples collected from school-children in central Sudan while disagree in evaluating specificity as they reported that ELISA specificity reduced to 53.8 % however it was 95.1 % when detected by IHA . On the other hand, the present results disagree with Attallah (1999), who found the use of ELISA for diagnosis of schistosomiasis represent 56% specificity. However, the fast dot-ELISA was detected circulating antigens in urine of schistosoma infected patients with 89% specific. This difference may be related to the antigen nature utilized because Attallah (1999), tested a 74 kDa antigen which is protein versus to the used antigen herein which is glycoprotein.

#### 4. CONCLUSION

A glucose specific fraction from *S. mansoni* adult worms resolved into two bands with molecular weight 74.6 kDa and 20 kDa. The fraction is consisted of O-glycan Al-(Ser-Arg-Ser-Arg)<sub>94</sub>. It recorded 100% sensitivity & 100% specificity and its diagnostic value is higher than the available commercial IHA kits in addition to its potency in the diagnosis of prepatent and patent infection while IHA failed to diagnose it.

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