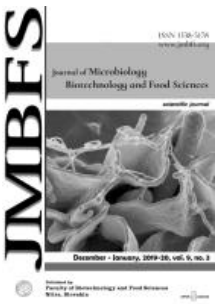

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# THE HEXOKINASE 1 GENE OF BLOODSTREAM FORM *Trypanosoma brucei brucei* (FEDERE ISOLATE) CONSERVES AMINO ACIDS IN DOMAINS AND MOTIFS PECULIAR TO THE HEXOKINASE 2 SUPERFAMILY

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**ABSTRACT**

*Trypanosoma brucei brucei* hexokinase is the first key regulatory enzyme of the glycolytic pathway which serves as the only source of ATP and nucleic acid precursors for the parasite in the infective stage. This enzyme is attracting much interest because during the infective stage of the parasites, the bloodstream form (BSF) of this parasite depends solely on glycolysis because of its poorly developed mitochondrion. This research, therefore, attempted to characterize the hexokinase 1 gene of the *T. b. brucei* (Federe isolate) which is prevalent in Nigeria. The parasites were grown in rats and purified by diethyl aminoethyl (DEAE) cellulose chromatography. The genomic DNA was isolated, and the parasites hexokinase 1 gene amplified using consensus primers. The amplicon was purified and sequenced. The sequences were studied using software from the National Centre for Biotechnology Information (NCBI) and CLAWSTAL W server. The nucleotide sequence (Accession number MH198230) and the translated amino acids revealed high similarity with sequences of *Trypanosoma brucei brucei TREU 927, T. brucei gambiense and Trypanosoma cruzi* but low similarity with Human Glucokinase (hexokinase IV). The translated peptide sequence contains the N-terminal peroxisome-targeting signal (PTS-2) that is peculiar to glycosome containing organisms; ATP-binding and the hexokinase binding sites, and other amino acids in the domain peculiar to hexokinase 2 super-family were also conserved. These showed that *T. brucei brucei* hexokinase 1 conserves the unique features peculiar to the hexokinase 2 super-family and its uniquely from its mammalian forms make it a potential target for drug or vaccine against trypanosomiasis.

**Keywords:** *Trypanosoma brucei brucei*, hexokinase 2 superfamily, hexokinase 1, glycolytic pathway, African trypanosomiasis

## INTRODUCTION

African trypanosomiasis is a deadly neglected tropical disease that affects both humans and animals. It is prevalent in African communities where people depend mostly on hunting, livestock rearing, farming and fishing for their livelihood and among those that reside and work around riverine area (**Matthews et al., 2015; Shaw et al., 2014** ). The disease is mainly caused by strains of *Trypanosoma brucei* and is estimated to affect around 70 million people in Africa (**Franco et al., 2014; Tesfaye et al., 2012; Simarro et al., 2012**). This disease results in an annual economic loss of about $ 4.5 billion from animal fatality and disease control (**Franco et al., 2014; Tesfaye et al., 2012;Simarro et al., 2012;Simarro et al., 2010; Steverding, 2008).**

African trypanosomiasis results in death if it is untreated. This makes chemotherapy or vaccination compulsory (**Franco et al., 2014**).Unfortunately, investment in the research, development and production of new drugs are not on the priority list of pharmaceutical industry since the disease affects only nations who cannot afford the drugs (**Bouteille and Buguet, 2012**). Therefore there is need for vaccine development that may be attractive to both the pharmaceutical industries and the patients.

One of such targets that is now being considered is *T. brucei brucei* hexokinase, the first key regulatory enzyme of the glycolytic pathway which serves as the only source of ATP and nucleic acid precursors for the parasite in the infective stage and differs from the mammalian host enzyme (**Besterio et al., 2002, Willson et al., 2002**). This enzyme is attracting attention because during the infective stage of the parasites in the mammalian bloodstream, the parasites live in glucose rich environment in the mammalian bloodstream and depend solely on glycolysis because of its poorly developed mitochondrion which lacks the active components of the citric acid cycle and the electron transport chain. This dependence on glycolysis for ATP in addition to the parasite's poorly developed mitochondrial limits the metabolic options available to the bloodstream form (BSF) of this parasites (**Joice et al., 2013; Bringaud et al., 2006; Besterio et al., 2002**).Good understanding of this enzyme can guide in its selective inhibition at different stages of synthesis without interference with the mammalian host hexokinase. This will deprive the parasite of the only source of ATP and nucleic acid precursors. The enzyme is therefore a possible potential target for drugs development against the African trypanosomiasis **(Willson et al., 2002**). This research, therefore, attempted to characterize the hexokinase 1 gene of the *T.b.brucei (*Federe isolate) which has been the subject of several screening campaigns for small molecule inhibitors in some isolates (**Joice et al.,2013**).

## MATERIALS AND METHODS

### Reagents

All reagents used were of analytical grade and purchased from Sigma, BDH POOLE, England, Whatman, USA; Pharmacia Fine Chemicals and Amresco Life Science, Fountain Parkway,Solon. DNA extraction kit was purchased from ZYMO RESEARCH.*Taq* polymerase and High-Fidelity Polymerase Enzyme Mix were bought from Promega, USA and Fermentas, respectively. Molecular size marker was purchased from Roche, Mannheim Germany. Oligonucleotide primers were synthesized by Inqaba biotec Industry, Pretoria South Africa. The GeneAmp PCR System9700 for gene amplification was obtained from Applied Biosystems, Indonesia and the Gel Documentation System was obtained from Synegene® Inc. Indonesia. Sequencing analyses were performed by Macrogen Corp. in the Netherlands.

### Parasite Isolate

*Trypanosoma brucei brucei (*Federe isolate) was obtained from the Veterinary and Livestock Studies Department of the Nigerian Institute for Trypanosomiasis Research, Vom, Plataeu State, Nigeria.

**Cultivations of Trypanosomes in Rats**

Healthy rats were infected by intraperitoneal inoculation of $1x10^4$ parasites/rat in 0.3ml of Puck saline glucose (PSG) (pH 7.8). Parasitemia was monitored daily from the second day after the infection by bleeding the tail of each animal and the blood observed under a light microscope with fields examined and the mobile parasites counted by using the rapid matching method described by Herbert and Lumsden, (1976), in this method, 20µl of blood from the tail vein of rat was placed on a glass slide and covered with coverslip. The number of parasites was determined microscopically at 40× magnification. The microscopic field was compared with a range of standard logarithmic values and the logarithmic values which matched the microscopic observation were therefore converted to antilogarithm where the absolute number of trypanosome per milliliter (ml) of blood was obtained.

**Purification of Bloodstream form *T. brucei brucei* from *Rat***

After 72 hours from the time of inoculation and at peak parasitemia, the circulating blood was withdrawn by cardiac puncture using a syringe loaded with a 0.2ml of PSG containing heparin (5:1) (see the supplementary material). The whole blood was centrifuged at 1,250 × *g* for 15 minutes at 4ºC resulting in separation of the sample into three different layers. The Buffy layer was carefully removed using a Pasteur pipette and $10^7$*T.bruceibrucei* cells per ml were re-suspended in phosphate buffered saline supplemented with 0.1% glucose (pH = 7.8) and thereafter purified using diethyl aminoethyl cellulose (DEAE) anion exchanger column (DE-52, Whatman) as described by Lanham and Godfrey (1970). 5ml of the eluate containing the trypanosomes in centrifuge tubes were centrifuged for 10mins at 1,600 x g in a bench top centrifuge, and the supernatant was discarded. The trypanosome pellets were then washed by gentle re-suspension in 1ml PBS (pH 7.4) and transferred to micro-centrifuge tubes and centrifuged for 5 minutes at 1,600 *x* g, the supernatant was removed, the trypanosomes were pooled, and the pellet was re-suspended in PBS (pH 7.4) at 1 x $10^8$cells/ml for subsequent analysis.

**GENOMIC DNA ISOLATION**

Genomic DNA (gDNA) was extracted from the resulting trypanosome cell pellets using the ZR Fungal/Bacterial DNA MiniPrep (ZYMO RESEARCH) following the manufacturer's instruction.

***Gene Searches* and Primer Design**

Nucleotide sequence for the *T. brucei brucei* hexokinase 1 gene was located through text searches for the gene name in GeneDB (www.genedb.org) and a BLAST (Basic Local Alignment Search Tool) search against the *T. brucei brucei* genome was performed in NCBI databases (www.ncbi.nlm.nih.gov). Primers based on the sequence obtained from GeneDB were designed as shown below to amplify the gene of *T. brucei brucei* hexokinase 1 (Accession Number: XM841212.1) and subsequently sent to Inqaba Biotech Industry, Pretoria, South Africa for synthesis.

FORWARD 5′-ATGTCTAGACGCCTAAACAATATCC-3′
REVERSE 5′-TTACTTGTCGTTCACCACCATTGCG-3′

**POLYMERASE CHAIN REACTION (PCR)**

*T. brucei brucei* hexokinase 1 (TbHK1) sequence from the total genomic DNA of *T. brucei brucei* was amplified by Polymerase Chain Reaction (PCR) using the designed primers. The PCR reaction was performed in a 50µl reaction mixture which contained 27µl of nuclease free water, 10 µl of 5 X phusion buffers (Promega), 1 µl of dNTPS, 1.5µl of DMSO, 2.5 µl of forward primer, 2.5 µl of reverse primer, 0.5 µl of *Taq*DNA polymerase (Promega) and 5.0 µl of the genomic DNA of *T.brucei brucei*. The tubes containing the mixture will be subjected to 30 cycles of amplification in a thermocycler using the following Amplification conditions: Polymerase activation 94°C for 5 min**;** Denaturation 94°C for 30 s**;** Annealing 48°C for 30 s**;** Extension 72°C for 1 min**.** Step 2-4 were repeated for 30 cycles and final extension was carried out at 72°C for 5 minutes. The PCR product was then subjected to 1.5% agarose gel electrophoresis. The results were documented using GelDocumentation System (Synegene® Inc. Indonesia).

**DNA sequencing and Bioinformatics Analysis**

The *T. brucei brucei* (Federe isolate) hexokinase 1 gene amplified was sequenced by Macrogen Corp. in the Netherlands. The sequence obtained *was* analysed using BLAST tools (BLASTN, BLASTX and BLASTP) provided by NCBI (http://www.ncbi.nlm.nih.gov/).The sequence similarity of Tbhk1 and other organism studied were aligning using CLAWSTAL W**.**

**RESULTS**

The gene sequence gave 1401bp (Accession number: MH198230.1) and the analysis of the sequence in National Centre for Biotechnology Information(NCBI) BASTN revealed its similarity to *Trypanosoma brucei brucei* TREU927 (AJ437577.1); *Trypanosoma brucei Gambiense (FN554973.1); Trypanosoma cruzi* (XM841212.1) hexokinase sequences. The analysis of the sequence gave 87% similarity to those of *T. brucei brucei* (TREU 927) and *Trypanosoma brucei gambiense* and 74% to that of *Trypanosoma cruzi*, the causative agent of American Trypanosomiasis. There was 100% coverage for both *T. brucei brucei* (TREU 927) and *Trypanosoma brucei gambiense* hexokinase sequences with no gap in between while only 38% coverage was observed for *Trypanosoma cruzi* due to the presence of gaps. The result of the comparison is shown in Figure 1 and summarised in Table 1

```
HK1
ATGTCTAGACGCCTAAACAATATCCTCGAACACATCTCGATCCAGGGAAATGATGGTGAG
Tcru   ATGTCCGCTCGCCTGAACAACCTGCTGCAGCACATTGCTGTGAAAGACAAAGACAGCGAC
Tbg    ATGTCTAGACGCCTAAACAATATCCTCGAACACATCTCGATCCAGGGAAATGATGGTGAG
Tb927  ATGTCTAGACGCCTAAACAATATCCTCGAACACATCTCGATCCAGGGAAATGATGGTGAG
*****    ***** *****  * **  * ******  *  *  * *  ** **  * **
HK1    ACTGTGCGTTCTTTTTA------TGTTACGTTGCACGCCCTCACCAACCAACCTTTTCTG
Tcru   ACAATGCGGCACCTGAAACAGCGCATGGCGCTGGCGTCCCTCGCGAACCAATTCACGGTG
Tbg    ACTGTGCGTGCCGTTAAGCGTGATGTTGCAATGGCAGCGCTGACCAACCAATTCACAATG
Tb927  ACTGTGCGTGCCGTTAAGCGTGATGTTGCAATGGCAGCGCTGACCAACCAATTCACAATG
**  ****    *  *      *  *  **    * **  * *******       **
HK1    AGTGTCGAGTCTATGCGACAGATCATGACATACCTCCTGTACGAGATGGTGGAGGGTCTT
Tcru   GGCAAGGACCACCTCAAGCAGCTGATGTTGTACATGGTACACCAGATGATTGAGGGACTG
Tbg    AGTGTGGAGTCTATGCGACAGATCATGACATACCTCCTGTACGAGATGGTGGAGGGTCTT
Tb927  AGTGTGGAGTCTATGCGACAGATCATGACATACCTCCTGTACGAGATGGTGGAGGGTCTT
  *   **    *   *** * ***   *** * *  * ** ***** * ***** **
HK1    GAGGGTCTTCACACCACCCTCTCCATTTTACC-----ATGTACCCGACGCGGACCCTA--
Tcru   GAGGGACGCGAGAGCACTTTGCGTATGCTGCCTTCTTACGTGTACAAAACCGATCCCAGC
Tbg    GAGGGTCGTGAAAGCACCGTCCGCATGTTACCATCTTATGTGTACAAGGCGGACCCTAAG
Tb927  GAGGGTCGTGAAAGCACCGTCCGCATGTTACCATCTTATGTATACAAGGCGGACCCTAAG
***** *   * * *** *   **  * **    * **  * * * ** ** *
HK1    CGCCCTGGCCTTCTTCTCCCCCTTTGACCTCTGTGGTTCCATTTTCTGTGTGTTGCGT
Tcru   AAGGCGACAGGCGTCTTCTACGCACTTGACCTCGGGGGCACGAACTTTCGAGTGCTGCGT
Tbg    CGTGCTACTGGCGTCTTCTACGCACTTGACCTCGGTGGTACCAACTTCCGTGTGTTGCGT
Tb927  CGTTACTGGCGTCTTCTACGCACTTGACCTCGGTGGTACCAACTTCCGTGTGCTGCGC
 **   * * ******* * *  ******** * **  * *  **  * *** ****
HK1    GGC-CATGCAAGGAGGGTCCCGTGGTGTTTTCCTCTCCTTCTGCATTCAAAATCCCCAAA
Tcru   GTAACGTGCAAGGAAGGAAGAGTAGCGGACCGTGTGGACGCCAAGTTTGTGATTCCGCAG
Tbg    GTTGCATGCAAGGAGGGTGCCGTGGTGGATTCCTCTACTTCTGCATTCAAGATTCCCAAA
Tb927  GTTGCATGCAAGGAGGGTGCCGTGGTGGATTCCTCTACTTCTGCATTCAAGATTCCCAAA
*   * ********* **   ** * *         *   **   ** **  *
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```
HK1  TATGCCCTTGAGGGTAACGCCACCGATCTGTTTGGCTTCATTGCATCCAATGTGAAAAAA
Tcru CAGGCACTGCAAGGAACGGCAGAGGATTTGTTTGGCTTTATTGCGCAGAGTGTGAAGAAA
Tbg  TATGCCCTTGAGGGTAACGCCACCGATCTGTTTGACTTCATTGCATCCAATGTGAAGAAA
Tb927 TATGCCCTTGAGGGTAACGCCACCGATCTGTTTGACTTCATTGCATCCAATGTGAAGAAA
     * ** **  * *** *  **   *** ******* *** *****   * ******* ***

HK1  CCCCTGGAACTCTCGTACCATGGAAACTCGTGCACCTGAGGACCTCAATCGCACAGTTCC
Tcru ----------ATGATGGAGCAGAAAGCACCGGAGGACTTGAACCGCACCGTGCC
Tbg  ----------ACCATGGAAACTCGTGCACCTGAGGACCTCAATCGCACAGTTCC
Tb927 ----------ACCATGGAAACTCGTGCACCTGAGGACCTCAATCGCACAGTTCC
               * *****      ****** ******* * ** *****
HK1  TCTTGGGTTTACCTTCAGTTTCCCCGTGGAGCAGACGAAGGTTAACCGTGGTGTGCTTAT
Tcru GCTTGGCTTCACATTTAGCTTTCCAACAGAGCAGAAAGGCGTCGATCACGGTTTCCTGAT
Tbg  TCTTGGGTTTACCTTCAGTTTCCCCGTGGAGCAGACGAAGGTTAACCGTGGTGTGCTTAT
Tb927 TCTTGGGTTTACCTTCAGTTTCCCCGTGGAGCAGACGAAGGTTAACCGTGGTGTGCTTAT
***** ** ** ** ** ** **   *******    ** * *  *** * ** **
HK1  CCGGTGGACGAAGGGCTTCAGC----------GGATGCTATGTGATTGCCCTTCT
Tcru AAAATGGACGAAGGGCTTCTCGACACGGGGTGTGGAGGGGAAGGACGTGGTGGAACTTCT
Tbg  CCGGTGGACGAAGGGCTTCAGCACGAAAGGCGTTCAAGGAAACGATGTGATTGCCCTTCT
Tb927 CCGGTGGACGAAGGGCTTCAGCACGAAAGGCGTTCAAGGAAATGATGTGATTGCCCTTCT
 **************             **    * *** * *  *****
HK1  TCGCGCTGCTTTTGGGAAATTTAGCCTAAGTGTCAATGTTGTGGCTTTGTGCAACGACCC
Tcru GCAGAAGGCATTGAAACGCATGGAAGTGAAGGTGAAAGTGGTGGCACTCTGCAACGACAC
Tbg  TCAGGCTGCTTTTGGGCGAGTGAGCCTGAAGGTGAATGTTGTGGCGTTGTGCAACGACAC
Tb927 TCAGGCTGCTTTTGGGCGAGTGAGCTTGAAGGTGAATGTTGTGGCGTTGTGCAACGACAC
 *    ** **      *    * *  ** ** ** ***** * ********** *
HK1  CTTTTGAACTTTAATTTCGCATTACTTTAAGGACCCTGAGGTACAGGTTGGTGTGATTAT
Tcru TGTCGGTACGCTTATCACGAACTACTTCTTTGACCCCGACACGCAGGTTGGCGTAATCAT
Tbg  CGTTGGAACATTAATTTCGCATTACTTTAAGGACCCTGAGGTACAGGTTGGCGTGATTAT
Tb927 TGTTGCCACGATGATTTCGCATTACTTTAAGGACCCTGAGGTACAGGTTGGTGTGATTAT
 *   ** * ** ** * *****   ***** **   ******** ** ** **
HK1  CGGCACTGGTCCAGATGCGTGCTACTTTGACAGGGCCTCTGCTGTGACAAAAGACCGTGC
Tcru CGGCACCGGCTCCAACGCGTGCTACTTCGAGGATGCCTACGCCGTGACGAAGGAGCCCTC
Tbg  CGGCACTGGTTCCAATGCGTGCTACTTTGAGACGGCGTCTGCTGTGACGAAGGACCCTGC
Tb927 CGGCACTGGTTCCAATGCGTGCTACTTTGAGACGGCGTCTGCTGTGACGAAGGACCCTGC
     ******* ** *  * ********** **   ** *  ** ***** ** ** *  *
HK1  CTTTGCTGCTCGTGAGTCAGCACTTACTCCCATCAATATACAAAGCCTCCATTTTGACTC
Tcru AGTGGCCGCGCGCGGTACGACACAGACACCAATCAACATGGAGTGCGGGAACTTTGACTC
Tbg  CGTTGCTGCTCGTGGGTCAGCACTTACTCCCATCAATATGGAAAGCGGCAACTTTGACTC
Tb927 CGTTGCTGCTCGTGGGTCAGCACTTACTCCCATCAGTATGGAAAGCGGCAACTTTGACTC
 * ** ** ** *  *  ***  ** ** **** ** *  **   * *********
HK1  CGAGTACCGGTATGTCCT—CTACAACAAATTTCAACTTGTATATCGACGATCCGTCGTT
Tcru CAAGTACAAGTTTGTGCTGCCGGTCACCGCGTACGACGAGGCGATGGATGCCGTTACGCC
Tbg  CAAGTACCGGTTTGTCCTCCCTACGACGAAGTTCGACTTGGATATTGACGATGCGTCGTT
Tb927 CAAGTACCGGTTTGTCCTCCCTACGACGAAGTTCGACTTGGATATTGACGATGCGTCGTT
     * ***** ** *** **  *   **   * * **  *  ** ** *     **
HK1  AAACAAATGTCAACATGCTCTAGAGAAGAT-ATATCCCGCATGTATCGCGGTAAAATCTC
Tcru GAACCGCAACTTCCAGACGCAAGAGAAGATGGTCTCCGGCATGTATCTTGGGGAAATCAG
Tbg  GAACAAGGGTCAACAGGCGCTGGAGAAGATGATATCCGGCATGTACCTCGGTGAAATCGC
Tb927 GAACAAAGGTCAACAGGCGCTGGAGAAGATGATATCCGGCATGTACCTCGGCGAAATCGC
***       ** * *  ********  * *** ******** *  **  *****
HK1  CCGCCGCGTTATTGTGCACCTTTCGTCTATAAGCCGCCTTC------CTGCGGCACTCCA
Tcru TCGCCGCATGATAGCGCACCTTGCCGAGTTGCATTGCCTCCCAAGTGCTCTTGCATCCAA
Tbg  CCGCCGCGTTATTGTGCACCTGTCGTCTATTAACTGCCTTC------CTGCGGCACTGCA
Tb927 CCGCCGCGTTATTGTGCACCTGTCGTCTATTAACTGCCTTC------CTGCGGCACTGCA
****** * *** * ******* *    *    **** *     **  ***   *
HK1  GACGGCTTTTGGACAACCGGGTGTCGTTTTAATCCCCATTTCCCCGGAATCACCAGTGCT
Tcru GATGGC-------CAAGCCGTGGAGCTTTGAGACGAAATTT-ATGGGCATGATATCCGCA
Tbg  GACTGC-TTTGGGCAACCGGGGGTCGTTTGAGTCCCGATTT-GCCGGGATGATCAGTGCT
Tb927 GACTGC-TTTGGGCAACCGGGGGTCGTTTGAGTCCCGATTT-GCCGGGATGATCAGTGCT
    ** **     *** * * *  *** * *  ****   ** ** *    **
HK1  TCACCTTAAGCCCCGACTTCAGTTCACTCGCAGCACGATCCAGAAGGTGTGTGGTGTTGA
Tcru G-ACCGCATGCCGGGTCTGCAGTTCACGCGGCAAGTGTTCCAAGAGCTTTTCCAAGTCGA
Tbg  G-ACCGTATGCCCGGACTTCAGTTCACTCGCAGCACGATCCAGAAGGTGTGTGGTGTTGA
Tb927 G-ACCGTATGCCCGGACTTCAGTTCACTCGCAGCACGATCCAGAAGGTGTGTGGTGTTGA
 *** * ***  * ** ********* **     * *****  ** * *    ** **
HK1  CGTGCAGTCAATTGAAGACCTTCGCATCATTCGCGATGTGTGCCGCCTTGTCCGTGGGAG
Tcru CGTGACGGATGTTGCAGACCTGCACGTGATCCGTGATGTGTGCTGCCTGGTGCGCGGCCG
Tbg  CGTGCAGTCAATTGAAGACCTTCGCATCATTCGCGATGTGTGCCGCCTTGTCCGTGGGAG
Tb927 CGTGCAGTCAATTGAAGACCTTCGCATCATTCGCGATGTGTGCCGCCTTGTCCGTGGGAG
****  *   *** ******* * * * ** ********** **** ** ** **  *
HK1  GGCTGCGCAACTCTCTGCTTCCTTCTGCTGCGCTCCACTGGTTAAGACTCAAACACAGGG
Tcru CGCTGCGCAGATCAGTGCCATGTTCTGCAGTGCGCCACTTGTGAAGACAAGGAAAGAGGG
Tbg  GGCTGCGCAACTCTCTGCTTCCTTCTGCTGCGCTCCACTGGTTAAGACTCAAACACAGGG
Tb927 GGCTGCGCAACTCTCTGCTTCCTTCTGCTGCGCTCCACTGGTTAAGACTCAAACACAGGG
      *********  **  ***   ******* * ** ****** ** ******   * * ****
HK1  CCGTGCAACTATTGCAATTGACGGCTCCGTGTTTGAGAAGATTCCGTCATTCCGCCGCGT
Tcru CCGTGCGACGGTCGCGATCGATGGCTCCGTGTTTGAGAAGACACCCTCATTCCGCCGCCT
Tbg  CCGTGCAACTATTGCAATTGACGGCTCCGTGTTTGAGAAGATTCCGTCATTCCGCCGCGT
Tb927 CCGTGCAACTATTGCAATTGACGGCTCCGTGTTTGAGAAGATTCCGTCATTCCGCCGCGT
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******* **  * ** ** ** ********************  ** ************ *
HK1  CTTGCAGGACAACATCAACCGTATCCTTGGCCCTGAGTGCGATGTCAGGGCCGTTCTCGC
Tcru GCTGCAGCAAAACATGAACGCCATTCTCGGTCCTGGGTGTGATGTCACGACGGCACTCGC
Tbg  CTTGCAGGACAACATCAACCGTATCCTTGGCCCTGAGTGCGATGTCAGGGCCGTTCTCGC
Tb927 CTTGCAGGACAACATCAACCGTATCCTTGGCCCTGAGTGCGATGTCAGGGCCGTTCTCGC
****** * ****** ***   ** ** ** ***** *** ******** * * *  *****
HK1  AAAGGATGGCAGTGGAATTGGTGCTGCATTTATTTCCGCAATGGTGGTGAACGACAAGTAA
Tcru AAGGGACGGCAGCGGCATTGGCGCCGCTTTTATCTCCGCGCTGGTTGTGAACGACAAATAA
Tbg  AAAGGATGGCAGTGGAATTGGTGCTGCATTTATTTCCGCAATGGTGGTGAACGACAAGTAA
Tb927 AAAGGGTGGCAGTGGTGTCGGTGCGGCACTTATCTCCGCTATCGTTGCTGACGGGAAGTGA
      ** **  ****** **  * ** ** **  **** ******  * ** *   ***  ** * *
```

**Figure1** Alignment of the gene sequences of *Trypanosoma brucei* brucei hexokinase with other hexokinase sequences. Tcru: *Trypanosoma cruzi* (AJ437577.1); Tbg: *Trypanosoma brucei gambiense* (FN554973.1); Trypanosoma *brucei brucei* (TREU927) ( XM841212.1). Asterisks indicate where all the organisms share identical or highly conserved sequence.

**Table 1** Summary of the Comparison of hexokinase 1 Sequence with some trypanosomes Sequences in the NCBI Data Base (DB) using NCBI BLASTN

| Species | Query Cover | Identity | NCBI Accession Number |
|---|---|---|---|
| *T. brucei Gambiense* | 100% | 87% | FN554973.1 |
| *T. brucei brucei* TREU927 | 100% | 87% | XM841212.1 |
| *Trypanosoma cruzi* | 38% | 74% | AJ437577.1 |

The NCBI TBLASTP analysis gave 464 translated amino acids, which is close to 471 amino acids of *T. brucei brucei* hexokinase 1 (XP_822456.1) in the NCBI data bank and 474 amino acids sequence earlier reported (Willson *et al*., 2002). Further analysis of the sequence and aligned using CLUSTAL W software, the result of comparison is shown in Table 2 and Figure 2. It revealed that translated amino acids from the hexokinase 1 gene is 68% similar to that of *T. brucei gambiense* and *T. brucei brucei* TREU927. On the other hand, there is only 48% and 26% similarity between it and that of Trypanosoma cruzi and Human Glucokinase (Hexokinase IV) (Figure 2 and Table 2).

```
                10        20        30        40        50        60        70        80        90       100
        ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
HK1             MSRRLNNILEHISIQGNDGETVRAVKRDVAMAALTNQFTMSVESMRQIMTYLLYEMVEGLEGR---
ESTVRMLPSYVYKADPKRATGVFYALDLGGTNFR
Gluk            ----------
MLDDRARMEAAKKEKVEQILAEFQLQEEDLKKVMRRMQKEMDRGLRLETHEEASVKMLPTYVRSTPEGSEVGDFLSLDLGGTNFR
Tcru            MSARLNNLLQHIAVKDKDSDTMRHLKQRMALASLANQFTVGKDHLKQLMLYMVHQMIEGLEGR---
ESTLRMLPSYVYKTDPSKATGVFYALDLGGTNFR
Tbg             MSRRLNNILEHISIQGNDGETVRAVKRDVAMAALTNQFTMSVESMRQIMTYLLYEMVEGLEGR---
ESTVRMLPSYVYKADPKRATGVFYALDLGGTNFR
TbU927          MSRRLNNILEHISIQGNDGETVRAVKRDVAMAALTNQFTMSVESMRQIMTYLLYEMVEGLEGR---
ESTVRMLPSYVYKADPKRATGVFYALDLGGTNFR

                110       120       130       140       150       160       170       180       190       200
        ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
HK1             VLRVACKEGA----
VVDSSTSAFKIPKYALEGNATDLFGFIASNVKRTMETRAPEDLNRTVPLGFTFSFPVEQTKVNRGVLIRWTKGFSTKGVQGNDVIA
Gluk            VMLVKVGEGEEGQWSVKTKHQMYSIPEDAMTGTAEMLFDYISECISDFLDKHQMK--
HKKLPLGFTFSFPVRHEDIDKGILLNWTKGFKASGAEGNNVVG
Tcru            VLRVTCKEGR----
VADRVDAKFVIPQQALQGTAEDLFGFIAQSVKKMMEQKAPEDLNRTVPLGFTFSFPTEQKGVDHGFLIKWTKGFSTRGVEGKDVVE
Tbg             VLRVACKEGA----
VVDSSTSAFKIPKYALEGNATDLFDFIASNVKKTMETRAPEDLNRTVPLGFTFSFPVEQTKVNRGVLIRWTKGFSTKGVQGNDVIA
TbU927          VLRVACKEGA----
VVDSSTSAFKIPKYALEGNATDLFGFIASNVKKTMETRAPEDLNRTVPLGFTFSFPVEQTKVNRGVLIRWTKGFSTKGVQGNDVIA

                210       220       230       240       250       260       270       280       290       300
        ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
HK1             LLQAAFG-
RVSLKVNVVALCNDTVGTLISHYFKDPEVQVGVIIGTGPDACYFDRASAVTKDRAFAARESALTPINIQSLHFDSEYRYVL---KFQL-VYR
Gluk            LLRDAIKRRGDFEMDVVAMVNDTVATMISCYYEDHQCEVGMIVGTGCNACYMEEMQNVELVEGDEGR----
MCVNTEWGAFGDSGELDEFLLEYDRLVDE
Tcru            LLQKALK-
RMEVKVKVVALCNDTVGTLITNYFFDPDTQVGVIIGTGSNACYFEDAYAVTKEPSVAARGTTQTPINMECGNFDSKYKFVLPVTAYDEAMD
A
Tbg             LLQAAFG-
RVSLKVNVVALCNDTVGTLISHYFKDPEVQVGVIIGTGSNACYFETASAVTKDPAVAARGSALTPINMESGNFDSKYRFVLPTTKFDLDIDD
TbU927          LLQAAFG-
RVSLKVNVVALCNDTVGTLISHYFKDPEVQVGVIIGTGSNACYFETASAVTKDPAVAARGSALTPINMESGNFDSKYRFVLPTTKFDLDIDD

                310       320       330       340       350       360       370       380       390       400
        ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
HK1             RSVVKGQQALEKMISGMYLGEIARRVIVHLSSINCLPA-ALQTALGNRGSFESRFAGMIK----
PRLQFTRSTIQKVCGVDVQSIEDLRIIRDVCRLVRG
Gluk            SSANPGQQLYEKLIGGKYMGELVRLVLLRLVDENLLFHGEASEQLRTRGAFETRFVSQVESDTGDRKQIYN--ILSTLGLRPS-
TTDCDIVRRACESVST
Tcru            VTPNRNFQTQEKMVSGMYLGEISRRMIAHLAELHCLPS-
ALASKMAKPWSFETKFMGMISADRMPGLQFTRQVFQELFQVDVTDVADLHVIRDVCCLVRG
Tbg             ASLNKGQQALEKMISGMYLGEIARRVIVHLSSINCLPA-
ALQTALGNRGSFESRFAGMISADRMPGLQFTRSTIQKVCGVDVQSIEDLRIIRDVCRLVRG
TbU927          ASLNKGQQALEKMISGMYLGEIARRVIVHLSSINCLPA-
ALQTALGNRGSFESRFAGMISADRMPGLQFTRSTIQKVCGVDVQSIEDLRIIRDVCRLVRG
```

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          410       420       430       440       450       460       470       480       490
     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
HK1       RAAQLSASFCCAPLVKTQTQG-----RATIAIDGSVFEKIPSFRRVLQDNINRILGPECDVRAVLAKDGSGIGAAFISAMVVND------
Gluk      RAAHMCSAGLAGVINRMRESRSEDVMRITVGVDGSVYKLHPSFKERFHASVRRLT-
PSCEITFIESEEGSGRGAALVSAVACKKACMLGQ
Tcru      RAAQISAMFCSAPLVKTRKEG-----RATVAIDGSVFEKTPSFRRLLQQNMNAILGPGCDVTTALARDGSGIGAAFISALVVNDK-----
Tbg       RAAQLSASFCCAPLVKTQTQG-----RATIAIDGSVFEKIPSFRRVLQDNINRILGPECDVRAVLAKDGSGIGAAFISAMVVNDK-----
TbU927    RAAQLSASFCCAPLVKTQTQG-----RATIAIDGSVFEKIPSFRRVLQDNINRILGPECDVRAVLAKDGSGIGAAFISAMVVNDK-----
```

**Figure 2** Comparison of the Amino Acid Sequences of the *Trypanosoma brucei brucei* hexokinase *(Federe isolate)* with the hexokinases of *T. brucei gambiense* (XP_011777423.1) - Tbg; *T.brucei brucei* TREU927 (XP_822456.1) - TbU927; T. cruzi (AAL93565.1) -Tcru; Human Glucokinase (NP_000153.1) - Gluk

**Table 2** Summary of the Comparison of hexokinase 1 Gene Product (Protein) and hexokinase of some common trypanosomes and Human Glucokinase NCBI BLASTP

| Organisms | Query Cover | Identity | NCBI Accession Number |
|---|---|---|---|
| *T. brucei Gambiense* | 99% | 68% | XP_011777423.1 |
| *T.bruceibrucei* TREU927 | 99% | 68% | XP_822456.1 |
| *Trypanosoma cruzi* | 99% | 48% | AL93565.1 |
| Human Glucokinase | 91% | 26% | NP_000153.1 |

The NCBI Conserved Domains analysis of *T. b. brucei* hexokinase 1 protein aligned to members of hexokinase 2 super family that have Accession Number cl27242 as shown in Figure 3. The actual alignment results was detected with superfamily member (Accession Number PTZ00107).The conserved domains for glucose were represented by the amino acids in blue colour, ATP in purple and glucose 6-phosphate in red respectively. The plus sign indicate the binding sites of the sugar moiety of G6P. The letter "a" indicates the binding sites of the phosphate moiety of G6P. The amino acids residues marked ''C'' are present in the conserved hydrophobic channel. The putative, linear B-cell epitopes with higher conservations are marked with green over-line. The putative connecting the two domains are respectively marked with blue over-line

```
L.b     MASRVNNLMNHLAIRDSDSEEMRYIKQRLA-LASLSTQFTMASEKMKQLTMYMVYEMVEG  60
L.i     MATRVNNLLSHIAIRDSDSEEMRYIKQRLA-LASLATQFTMSSEKMKQLTMYMIHEMVEG  60
L.m      MAARVNNLLSHIAIRDSDSEEMRYIKQRLA-LASLATQFTMSSEKMKQLTMYMIHEMVEG  60
HK1      MSRRLNNILEHISIQGNDGETVRSFY--VT-LHALTNQPFLSVESMRQIMTYLLYEMVEG 60
T.bb     MSRRLNNILEHISIQGNDGETVRAVKRDVA-MAALTNQFTMSVESMRQIMTYLLYEMVEG  60
C.p      ----------MEEENQAKRLFDLYEEYFFLSNLKLLELVDDFHKSLEDG    60
C.m      ----------MEVRKYRSSDEWIELYRPYFCLSKAKLQDLVEDFLNSLICG    60

L.b     LEGRP----------STVRMLPSYVYTSDPAKATGVYYALDLGGTNFRVLRVSLRSGK    120
L.i     LEGRP----------STVRMLPSFVYTSDPAKATGVYYALDLGGTNFRVLRVSLRGGK    120
L.m      LEGRP----------STVRMLPSFVYTSDPAKATGVYYALDLGGTNFRVLRVSLRGGK    120
HK1      LEGLH----------TTLSILPCTRRG---PYALAFFSPFDLCGSIFCVLRGHARRVP 120
T.bb     LEGRE----------STVRMLPSYVYKADPKRATGVFYALDLGGTNFRVLRVACKEGA    120
C.p      LENHANRLKIDKYHSNYKPFKMLDSCVDRLPTGKEKGVYYAIDMGGTNLRCVRVNLLGNG  120
C.m      LESHNGITSAN--ICIQKPLKVLDSCIFNLPSGKEQGIHYAIDMGGTNLRCVRVELRGNG  120
                              c+c  a

L.b     VDDRIDSKFVIPKSALTGN----------SANLFDFIA    180
L.i     VDDRTDSKFVIPKSALVGD----------ATDLFDFIA    180
L.m      VDDRTDSKFVIPKSALVGD----------ATDLFDFIA    180
HK1      WCFPLLLHSKSPNMPLRVT----------PPICLASLHPMKN          180
T.bb     VVDSSTSAFKIPKYALEGN----------ATDLFGFIA    180
C.p      QSETKFKKVKLSEMKVSSTGKVVNSNKISK--------VDQEVNIFDKTVSSETMFNSIA    180
C.m      ESFVTYKKMALNDLRISKHKAIENNINIETGDNLFFNSDIQSFSILDKLASATDMFDAIS  180

L.b     QSVKKMM----------SE----------NAPE  240
L.i     QSVKKMM----------SE----------NAPD  240
L.m      QSVKKMM----------SE----------NAPD 240
HK1      PWNSRTM----------ET----------RAPE 240
T.bb     SNVKKTM----------ET----------RAPE  240
C.p      VFFNEFLDECGDLNDLTNDN----------N        240
C.m      NFFYDFLVSCGDINERILEFRDNNEAYTTFNSKIITKPLSTNLNKNNENQLQKYHEKDLK  240
         c  c

L.b     DLEKRVPLGFTFSFPVDQKAVNKGLLIKWTKGFST-----KNVEGNDVVELLQGSLRRMH 300
L.i     DLEKRVPLGFTFSFPVDQKAVNKGLLIKWTKGFST-----KNVEGNDVVELLQASLRRVR  300
L.m      DLEKRVPLGFTFSFPVDQKAVNKGLLIKWTKGFST-----KNVEGNDVVELLQASLRRVR 300
HK1      DLNRTVPLGFTFSFPVEQTKVNRGVLIRWTKGFS----------GCYVIALLRAAFGKFS  300
T.bb     DLNRTVPLGFTFSFPVEQTKVNRGVLIRWTKGFST-----KGVQGNDVIALLQAAFGRVS  300
C.p      VGNLPLEVGFTFSFPIVQSKIASAKLVIWTKEIETGRLTDDPVEGKDIGDLLNLAFKRNG 300
C.m      YQSNYFSVAFTFSFPTTQLSIANAHLISWTKGIETGRATLEPVEGYDVGNLLNSAFNRNK  300
           C c c          c          c  c

L.b     INVNVVALCNDTVGTLVARYFV----------DTNAQVGVIIGTGSNA    360
L.i     VNVNVVALCNDTVGTLVARYFV----------DTDVQVGVIIGTGSNA    360
L.m      VNVNVVALCNDTVGTLVARYFV----------DTDVQVGVIIGTGSNA   360
HK1      LSVNVVALCNDPF-TLISHYFK----------DPEVQVGVIIGTGPDA   360
T.bb     LKVNVVALCNDTVGTLISHYFK----------DPEVQVGVIIGTGSNA   360
C.p      VPAQCKCVLNDTVGTLISAMYDLNINNYCDNNHSLKVNFPNNISENQPLIGIVVGTGVNA  360
C.m      IPAHCKCIINDTVGTLLSAMYDLNSNMVGNNMSSYSNNSTPRQLTSNPVIGIVIGTGINA 360
           +                          a
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L.b     CYFERASAVTKDPAVCARGNAVTPINMECGNFDSKYKYALPTTVYDDEMDAITPNRDHQR 420
L.i     CYFERASAVTKDPAVSARGNAVTPINMECGNFDSKYKYALPITVYDDEMDAITPNRENQR 420
L.m     CYFERASAVTKDPAVSARGNAVTPINMECGNFDSKYKYALPITVYDDEMDAITPNRENQR 420
HK1     CYFDRASAVTKDRAFAARESALTPINIQSLHFDSEYRYVLYN-KFQLVYRRSVVKQMSTC 420
T.bb    CYFETASAVTKDPAVAARGSALTPINMESGNFDSKYRFVLPTTKFDLDIDDASLNKGQQA 420
C.p     CYLEPNSSNF--------GYKGVIINTECGDFYS---TKLPITDCDYSMDWFSDNRGEQI 420
C.m     CYIEPMSLFY--------GYKGVIINTECGDFTC---SNLPITDCDLVLDWFSDNRGDQQ 420

L.b     QEKIVSGMYLGEISRRMIVHLAQLGCLPRDLVDGLGKPWAFESKHMGM-VAADQMP---- 480
L.i     QEKLVSGMYLGEISRRLIVHLAQLGCLPRGLVDGLCRPWAFESKHMGM-IAADQMP---- 480
L.m     QEKLVSGMYLGEISRRLIVHLAQLGCLPRGLVDGLCRPWAFESKHMGM-IAADQMP---- 480
HK1     SREDISRMYRGKISRRVIVHLSSISRLPAALQTAFGQPGVVLIPISPESPVLHLKP---- 480
T.bb    LEKMISGMYLGEIARRVIVHLSSINCLPAALQTALGNRGSFESRFAGM-ISADRMP---- 480
C.p     FEKMISGTYLGEISRLLIINFLKNKTPEIFFQK-----NSLKTEHIAKIISHFNDNHQKY 480
C.m     FEKMISGTYLGELCRLLFIRVLQDKAPSIFFQS-----KIITTEDIANIASFDEDN---- 480

L.b     GLQFTRELIKRVAGVN---VTDVADLHTIREICCLVRNRAAQQAAVLSAAPMLK-TRTQG 540
L.i     GLQFTRELIKRIAGVD---VTDMSDLHTIREACCLVRNRAAQQGAVFTAAPMLK-TRTQG 540
L.m     GLQFTRELIKRIAGVD---MADISDLHTIRETCCLVRNRAAQQGAVFTAAPMLK-TRTQG 540
HK1     RLQFTRSTIQKVCGVD---VQSIEDLRIIRDVCRLVRGRAAQLSASFCCAPLVK-TQTQG 540
T.bb    GLQFTRSTIQKVCGVD---VQSIEDLRIIRDVCRLVRGRAAQLSASFCCAPLVK-TQTQG 540
C.p     NQNHDLKSIENYLKETFSSNLDHNSTYIIAKISQMVLMRAASLVSAIIAAFFKRFNKPRN 540
C.m     -TSINIDKIQRFIKSKYNVILDTSSCNTIKTMAIFVLKRAAGLVATVIAALIKKIENFQN 540

L.b     LATVAVDGSVYEKVPSFQRLYQECITGILGPTSN---AKVVLQKDGSGVGAAMICALAAN 600
L.i     LATVAVDGSVYEKTPSFQRLYQECITSILGSTSN---VKVVLQRDGSGVGAAMICALAAN 600
L.m     LATVAVDGSVYEKTPSFQRLYQECITSILGSTSN---VKVVLQRDGSGVGAAMICALAAN 600
HK1     RATIAIDGSVFEKIPSFRRVLQDNINRILGPECD---VRAVLAKDGSGIGAAFISAMVVN 600
T.bb    RATIAIDGSVFEKIPSFRRVLQDNINRILGPECD---VRAVLAKDGSGIGAAFISAMVVN 600
C.p     QITIAIDGSVWTKIPKFQKYVKDSLSSLIQESGYLGSIHFYESDDGSGRGAAILASTTVN 600
C.m     GITVAVDGSVWTRVPKFQNYTKENLNLILGE-QVSQFIQFYEADDGSGKGAAILAATMD- 600
          + a                    a

L.b     QK  602
L.i     KK  602
L.m     TK  602
HK1     DK  602
T.bb    DK  602
C.p     TH  602
C.m     --  602
```

**Figure 3** Amino acids in the conserved domains of *T.b.brucei* hexokinase 1(Hk1) as aligned to some members of hexokinase 2 super family (Accession Number cl27242). Leishmania braziliensis(L.b, XP_001564741.1);Leishmania infantum(L.i,XP_001465335.1);Leishmania major (L.m,XP_001682957.1);Trypanosoma brucei brucei (T.bb,XP_822456.1);Cryptosporidium parvum ( C.p,XP_627719.1); Cryptosporidium muris (C.m,XP_002140303.1)

## DISCUSSION

The open reading frame (ORF) of *T. b. brucei* hexokinase 1 (*TbbH*K-1) consisted of 1401 bp encoding 464 translated amino acids. This gene size was larger than the 1350 base pairs obtained for hexokinase from *Clonorchis sinensis* (**Chen et al., 2014**), but closer to that of *T. cruzi* (1416 bp) (**Cáceres et al., 2003**). BLASTx showed that the deduced amino acid sequence of *Tbb*HK 1, shared 68%, 68%, 48% and 26% identity with *T. brucei gambiense* (XP 011777423.1), *T.bruceibrucei* (TREU927: XP822456.1), T. *cruzi* (AAL93565.1) and Human Glucokinase(NP 000153.1) respectively. These 464 translated amino acid sequence was slightly shorter than the number of the translated amino acids of *T. brucei brucei* (TREU927: XP822456.1) which is the reference standard for *T. brucei brucei* hexokinase in the NCBI data bank (471 amino acids) and (474 amino acids) obtained by Willson *et al.*,(2002).

A sequence alignment of the amino acids sequence also demonstrated that *T. brucei brucei* hexokinase 1 shared common evolutionary relationships (**Bork et al., 1993**). As observed in hexokinase from other organisms, the polypeptide also contained the amino acid sequence for ATP-binding site $^{88}$ALDLGGTNFR$^{96}$, $^{232}$VGVIIGTGSNAC$^{243}$, and $^{417}$AIDGSV$^{422}$, with all the conserved amino acid preserved as described by **Cáceres *et al.* (2003)** and **Bork *et al.* (1992**) except one in position 92 where Glycine to Cysteine (transition or substitution) occurs. The amino acids sequence that constitute hexokinase prosite signature$^{156}$LGFTFSFPTEQKG VDHGFLIKWTKGF$^{181}$ were also conserved (**Cáceres et al., 2003; Schirch & Wilson, 1987**). There were multiple binding sites for glucose, sugar and phosphate constituents of Glucose 6- phosphate in the amino acid sequence of *T. brucei brucei* hexokinase 1 (Mulichak *et al.*,1998).The translated amino acids from the hexokinase 1 gene carry some unique features which include peroxisomal targeting signals of type 2 (PTS-2) sequences ($^{4}$RLxxLxxHI$^{12}$) in the N-terminal which is important for the translocation of the enzyme to glycosomes (**Blattner et al., 1995**). All the hexokinase sequences aligned contain the PST-2 sequences except Human Glucokinase (Hexokinase IV). This shows that all the enzymes are glycosome resident enzymes except Human Glucokinase which lacks the PST sequence. In addition to the PST-2 sequence, the result also showed that *T. brucei brucei* (Federe strain) hexokinase 1 also contained "NTD25" sequence which is the next 25 amino acids after the peroxisome targeting sequence-2 sequence in *T. brucei brucei* hexokinase (**Flynn et al., 1998**). This sequence has been shown to direct *T. brucei brucei* hexokinase to the mitochondria/basal bodies in Procyclic forms and the flagellum in Blood Stream Forms where it serves alternative or additional functions in this exta-glycosomal locations before the NTD25 is finally removed (**Harris, 2015; Joice et al., 2013**). **Harris (2015)** in his work showed that misprocessing of the PTS2 of *T. brucei brucei* hexokinase leading to its premature removal can divert this glycosome bound enzyme to an alternative destination, that is, removal of PST-2 can mis-localize the enzyme which is harmful to the parasites. He also observed that mutation of serine 13 which is the first amino acid in NTD 25 to alanine had no effect on either processing or cellular localisation but mutation of conserved arginine 23 to have a deadly effect on cell morphology in both BSF and PF cells. In BSF parasites, it resulted in the failure of the cell to properly divide while PF cells had a flagella defect, with multiple flagella emanating from each cell and concluded that apart from its function *T. brucei brucei* hexokinase has other critical role in the parasite apart from glycolysis.

The NCBI Conserved Domains analysis of *T.b. brucei* Hexokinase 1 protein aligned to members of Hexokinase 2 super family that have Accession Number cl27242 as shown in Figure 3 showed that the TbbHK1 protein sequence depicted that it has a close relationship with the members of the family and the amino acids peculiar to this family well preserved (**Marchler-Bauer et al., 2017**). The two structurally similar domains in this family are also preserved (**Marchler-Bauer et al., 2015**).

## CONCLUSION

From this study it can be seen clearly that *T. brucei brucei* hexokinase 1 is uniquely different from its mammalian forms but conserves the amino acids in domains and motifs peculiar to the Hexokinase 2 super family (Accession Number cl27242 and EC:2.7.1.1). The unique difference between *T. brucei brucei* (Federe isolate) hexokinase 1 and its mammalian forms revealed potential for its selective inhibition without interference with the host hexokinase. This may be a possible avenue for immunisation or drug development against this disease.

ETHICAL APPROVAL
Animal experiments were carried out in accordance with the instructions for the care and use provided by the University of Jos, Nigeria.

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## SUPPLEMENTARY MATERIAL

Phosphate Buffered Saline (PBS)
To 800 ml of distilled water add
- 488.8mg of $NaH_2PO_4$
- 488.8mg of $NaH_2PO_4$
- 2.55g of NaCl
- 8.08g of $Na_2HPO_4$
- 15g of D-glucose

The volume was then adjusted to 1000ml with distilled $H_2O$ and the pH to 7.8 with 5N NaOH. It was sterilized by autoclaving and the pH rechecked before use. Each syringe used for blood collection contained 100µl of heparin mixed with 500µl of PSG