Emirates Journal of Food and Agriculture. 2015. 27(7): 585-591
doi: 10.9755/ejfa.2015.05.307
http://www.ejfa.me/

REGULAR ARTICLE

Use of polysaccharide extracted from *Tremella fuciformis* berk for control diabetes induced in rats

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ABSTRACT

Exopolysaccharides (EPS) was extracted from *Tremella fuciformis* growth in solid medium contained sorghum seeds. The objective of present work was analyzed EPS and evaluate the effect on the glucose, cholesterol, HDL, triglycerides, glutamic-pyruvic transaminase (GPT), urea level in the plasma of rats with type 1 *diabetes mellitus* (DM1) induced by high sugar diet and streptozotocin. Beta-glucan and total sugar from *T. fuciformis* was determined and the major quantity was alfa linked glucose. Concentration used for animals was 1mmol and 2mmol of EPS. Male Wistar rats were separated in two groups where one was induced *diabetes mellitus* (DM1) with streptozotocin and another with high sugar diet. The rats were allocated as follows: control that received commercial pellet; control that received polysaccharide; diabetic group that received streptozotocin or high sugar diet; diabetic group that received 1mmol or 2mmol from polysaccharide obtained from different *T. fuciformis* isolate. In group that received streptozotocin were evaluated results until 35days but in another group with high sugar diet the results go to 60days. Weight and blood glucose levels were measured once a week and after the period from experiment the animals were euthanized and blood was collected for the analysis of cholesterol, HDL, triglycerides, GPT and urea. Results indicate that EPS is beneficial in control of DM1 when the level from blood glucose is until 130mg/dL accomplished by reducing cholesterol, triglyceride, GPT, urea and increasing HDL cholesterol. When the blood glucose level is above 200mg/dL the action of EPS in reducing is not satisfactory. The *T. fuciformis* solution did not show anti-inflammatory activity.

Keywords: polysaccharide; *Tremella fuciformis; Diabetes mellitus*

INTRODUCTION

Mushrooms have been used as an important source of nutrition and/or therapy throughout the world since ancient times (Chang, 1996). Show high protein value (27-48%), low lipid value (2-8%), and also vitamins (thiamine, riboflavin, and niacin), minerals (calcium, iron, and phosphorus), beta-glucans and compounds with antioxidant activity (Manzi et al., 2001; Zhang et al., 2002, 2009).

Tremella fuciformis (family *Tremellaceae*, ordem *Tremellales*, class *Basidiomycetes*) is probably one of the most beautiful fungi growing in subtropical and tropical areas, or even temperate zones. It was first found in Brazil but has developed to an artificially cultivated species in Taiwan, China and some other countries in Asia (Urben, 2006, 2010). It is clearly associated with *Ascomycetes* in the field, especially *Hypoxylon* spp., however, unlike other *Tremella* mycoparasites in basidiocarps of Basidiomycetes, the real host-relationship of this group is still not investigated.

T. fuciformis is commonly known as the "white auricularia" or "white jelly fungus", and in Japan, *Shirokikurage*, with a jelly-like, translucent fruiting-body which usually grows on deciduous trees in warm climates worldwide and the name is *Tremella mesenterica*. It can now be grown artificially and is being increasingly consumed in Asia.

T. mesenterica contains acidic polysaccharides especially glucuronoxylomannan, readily extracted with hot water giving a smooth and stable solution used in Oriental cuisine. The polysaccharides of this fungus show anti-cancer activity and can enhance immune functions (Hobbs, 1995).

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Received: 02 December 2014; **Revised:** 04 February 2015; **Accepted:** 06 February 2015; **Published Online:** 29 May 2015

Clinical trials have shown it to be effective in treating radio- and chemo-therapy-induced leukopenia, boosting immunological functions and stimulating leukocyte activity (Hu and But, 1987). Kachhawa et al. (2003) proposed a structure of polisaccharide as α-(1→4) e (1→6) in proportion of 2:1.

Fruit bodies from *T. fuciformis* are difficult to obtain and is not cultivated in Brazil. Thus, mycelia of these mushrooms are mainly prepared in substrate (Hang et al., 2001; Urben, 2006) but exopolysaccharides (EPS) has been studied through submerged cultures, all of which have different and interesting biological activities (Zhu et al., 2012). Su-Yun et al. (2010) demonstrated that chemical structures of the polysaccharides from fruit body, spore, and submerged culture of *T. fuciformis* are similar. *T. fuciformis* polysaccharides mainly consist of heteropolysaccharides with α-(1→3)-linked D-mannan as the backbone chain, exhibiting diverse activities such as immunomodulation, antitumor, antioxidation, and antiaging.

In the present study we analyzed the fungal EPS production in a solid medium, and evaluate the effect polysaccharides on the blood sugar, cholesterol, HDL, triglycerides, glutamic-pyruvic transaminase (GPT), urea level in the plasma of rats with type 1 diabetes mellitus (DM1) induced by high sugar diet or streptozotocin.

MATERIALS AND METHODS

Preparation of polysaccharide

Tremella fuciformis received from Embrapa-Brasília and from São Paulo (Table 1), were cultured in potato dextrose agar (PDA) for 8-day old and then transferred to plastic bag containing sorghum seeds. The bags were incubated for 45days for micelial growth in chamber with controlled temperature (27±1^{0}C) and dark. For produced polysaccharides, a solid medium was made that involved 100g of sorghum seeds (Embrapa variety 308) that was first cooked in water and after crushed in a blender in 400mL of water and boiled again. The mixture was filtered through sieve, gauze, cotton cloth, completely to 500mL water and supplemented with 0.5g of agar. After boiled was transferred to bottles and sterilized. Seeds sorghum with mycelium was inoculated to bottle with solid medium and incubated for 20 days in chamber with controlled temperature (25 ± 1^{0}C) and dark. After period was removed EPS with water, observed presence of spores, centrifuged (3000 xg/10 min) and precipitated the EPS to end with 70% of alcohol. Solvent was evaporated and beta-glucan was determined by method of Lever (Lever, 1972) involved enzyme beta-glucanase (Sigma). Standart in test was used glucose and laminarin that said one unit of enzyme can be liberated 1 μM of glucose/min at 37°C (Van Hoof et al., 1991). For total sugar was used method Anthrona (Dische et al., 1947, Dische, 1962).

Animals

Animals were obtained from the University Nove de Junho (UNINOVE) animal lodging facility. The UNINOVE Ethics Committee for Animal Research approved the protocols used in this study (process numbers: 34/2010 and 20/2012).

The study was carried out in 60 Male Wistar rats, and separated in two groups. In first group, 30 rats aged five weeks, weighing 210 to 240g and induced diabetes with streptozotocin. In second group 15 rats aged two weeks, weighing 50g to 60g and treated with high sugar diet. The animals were kept in polypropylene cages (two to three animals per cage) covered with metallic grids in a room maintained at 23 ^{0}C, 55 ± 10% relative humidity and a 12-h light/dark cycle. The animals had free access to food and water for one week before beginning the study. Food and water consumption were measured every two days. Blood glucose levels and weight were measured twice a week, always at 14:00 p.m.

First group: 35 animals were fasted for 12 hours and chemical diabetes was induced in a part (30 rats) through an intraperitoneal injection of streptozotocin (50 mg/kg) (STZ - Sigma). The STZ solution was prepared immediately prior to injection by dissolving the drug in a fresh, cold citrate buffer, pH 4.5. After 72 hours, blood glucose levels were measured using a portable glucose meter (Accu Check Active). For such, the distal part of the tail was gently snipped; the first blood drop was discarded and the second was absorbed by a test strip inserted in the glucose meter. Rats were considered diabetic when the blood glucose level was at least 150 mg/dL. The four sub groups were composed as follows: 1) C= control that received commercial pellet; 2) D= diabetic group that received streptozotocin and commercial pellet; 3) 1mmol= Diabetic group that received oral Tremella (EPS) 1mmol from Ch (China); 4) 1mmol= Diabetic group that received oral Tremella (EPS) 1mmol from Br (Brazil); 5) 2mmol= Diabetic group that received oral Tremella (EPS) 2mmol from Ch (China); 6) 2mmol= Diabetic group that received oral Tremella (EPS) 2mmol from USA; 7) 2mmol= Diabetic group that received oral Tremella (EPS) 2mmol from Br (Brazil). The groups were evaluated for 35 days.

In the second group Animals were allocated to five sub-groups and a part (15 rats) received for 10 weeks high sugar diet (HSD). The treatments were formed: a) control group (Control); b) Control with Tremella (EPS) 2mmol treated (CTrem); c) diabetic group that received higher

sugar diet (Diabetic); d) diabetic and treated with Tremella (EPS) 1mmol (Trem 1mmol); e) diabetic and treated with Tremella (EPS) 2mmol (Trem 2mmol). The rats were fed a commercially available rat pellet diet *ad libitum* throughout the experimental period. Control groups were supplied with normal drinking water *ad libitum* and groups with high sugar diet were supplied with biscuit, wafer, chocolate and 20% of Frutose solution.

Euthanasia and sample collection

At the end of the experimental period, the animals were anesthetized with a lethal dose of a cocktail containing ketamine (1 mg) and xylazine (5 mg). Thoracotomy was performed. Blood was collected from the left ventricle and centrifuged. The plasma was removed and stored at -20°C for no longer than three days before the assay. Total cholesterol, triglycerides, HDL-cholesterol, urea, creatinine, and Glutamin pyruvate transaminase, were measured using biochemical test kits (Labtest Diagnostica®).

Statistical analysis

Statistical analysis was performed using the Assistat program and involved ANOVA, and Student's t-test. Statistical significance was determined by p-values <0.05 and <0.01.

Anti-inflamatory evaluation in rats

The method used is based in Basile et al. (1989) and Wadt (2000). Male Wistar rats weighing 150-180g were obtained from UNINOVE bioterio (creation room). The animals were kept in polypropylene cages (three animals per cage) covered with metallic grids in a room maintained at 23ºC, 55+10% humidity, 12h light and 12h dark cycle, water and feed *ad libitum* for one week before the start of the study. Groups of five rats were anesthetized and submitted to cotton pellets implantation method at the dorsal region.

Negative controls were water, ethanol 70% in dosage 1mL/kg and for positive control was used dexamethasone in dosage 0,2 mg/kg (1mL/Kg). Further the solution of *Tremella fuciformis* was given by oral gavage in dosage 1mL/kg for seven days.

The animals were sacrificed by anesthetic excess (xylazina/ketamine) and the cotton pellet was removed, dried and weighted in analytic balance and the mass difference was statically evaluated by the Anova/Tukey method.

RESULTS AND DISCUSSION

Analysis of *Tremella fuciformis*

The chemical composition and efficiency of extraction processes of polysaccharides in fruiting bodies from *T. fuciformis* were not completely clear. Wu et al. (2008) demonstrated that chemical structure of the polysaccharides consists of a linear backbone of (1→3) α-D-mannan (with side chains composed of glucuronic acid, xylose and fucose) backbone chain to which β-(1→2)-linked D-xylose residues are attached at the C-2 position. The estimated ratio of mannose, fucose, xylose, and glucuronic acid is 9:1:4:3 that makes glucoronic acid accounted for 17.6% of the polysaccharides content in *T. fuciformis* (De Baets and Vandamme, 2001; Gao et al., 1996, 1997; Tsing, 2006; Wu et al., 2008; Kakuta et al., 1979; Khondkar, 2009).

In Brazil, is difficult to find fruiting bodies and work in labor it´s possible. Results when obtained EPS from solid medium, confirmed that total sugar was alfa-glucose and by method of Lever have trace of quantity of beta-glucose linked. Was used various solid medium but the best was make with sorghum seeds (variety 308-Embrapa).

The isolates of *Tremella fuciformis* from Brazil, China and United States produced EPS in solid medium and were present spores from fungi. The spores were broadly ellipsoid with 7-9 x 6-7 μm, smooth, hyaline according to Berkeley (1856). Polysaccharides after precipitation with alcohol presented white color. Table 1 shows concentration of glucose linked beta-(1,3) (1,6) and total sugars from 10 preparations. In total sugar the concentration was millimol that corresponded to alfa-glicose.

Induction of diabetes

There are many models to induce diabetes like the use of a high fructose, or sucrose (HSD). High concentration in the diet requiring a long induction time and in this way raising the Project cost (Singla et al., 2009; Song et al., 2007; Srinivasan et al., 2005). To avoid this situation there are some chemical inducers, commonly streptozotocin (STZ) and alloxan. Many authors used for the induction of both type 1 and type 2 diabetes in animals where STZ has been found to be a better chemical inducer than alloxan (Szkudelski, 2001). Kiho et. al. (1994) used polysaccharide solution from fruiting bodies of *T. fuciformis* and give to mice for treated diabetes when induced diabetes with STZ.

Table 1: Concentration of beta-glucan linked-(1,3)(1,6) and total sugars from preparations of samples from *Tremella fuciformis*

Sample	Origem	mg Beta-glucan/mL	mmol total sugar
Br	Brazil (Embrapa-Brasilia)	153.6 +/- 0.625a*	28,0a
Ch	China (CC 308/Embrapa Brasilia)	126.4 +/- 0.666b	23,8b
USA	USA (P53- Edison de Souza)	154.9 +/- 0.577a	24,6b

*Different letters on columns indicate statistically significant differences among groups (p<0.01; ANOVA+Student's test)

On the present project, it has been used to models being one with HSD and other with STZ.

Induction of diabetes with Streptozotocin

In the control group (C), weight increased throughout the experimental period, with no significant differences between the treated groups with fungi. Significant differences were found in the comparison of C with the D and treated with sample from USA ($p<0.05$) (Fig. 1). The results indicated that ingestion of polysaccharides from *Tremella* (samples from China and Brazil) maintain weight but isolate from USA increased weight.

Blood glucose was examined one day before the beginning of the experiment, and the concentration was within the normal range (112 to 120 mg/dL). Three days after diabetes induction, increases in glucose concentration were found in the D group and also in all treatments. Glucose was measured once a week until 36days. Letter b in Fig. 2 shows that treatment with polysaccharide of fungi from China (1mmol) have better effect as in concentration of 2mmol (indicate by letter a). Samples from USA (2mmol) have better effect in control diabetes as compared with China but samples from Brazil was the most interesting because decreased until 400g/dL when used 2mmol of concentration.

For rat and human the results show that the reduction is not so significantly, the diabetes still high (Fig. 2), without a doubt the action of *Tremella* was note as the observed by Mascaro et al. (2014), when demonstrated that *Agaricus sylvaticus* is potentially beneficial in the control of DM1 by reducing blood glucose from 480mg/dL to 100mg/dL in 4 weeks.

Induction of diabetes with high sugar diet (HSD)

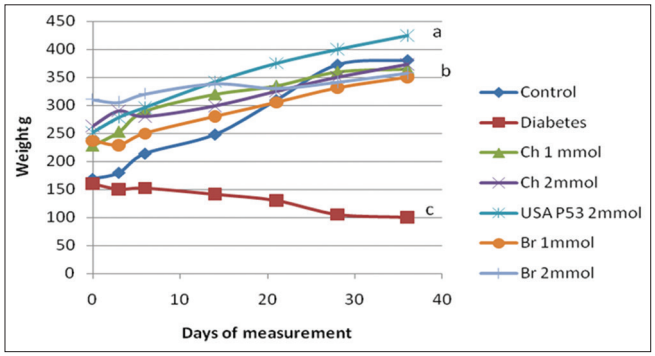

Fig 1. Body weight of rats submitted to treatments 1) C = control that received commercial pellet; 2) D = diabetic group that received streptozotocin and commercial pellet; 3) 1mmol= Diabetic group that received oral Tremella 1mmol from Ch (China) or Br (Brazil); 4) 2 mmol= Diabetic group that received oral Tremella 2mmol from Ch (China), USA and Br (Brazil). Measurements were taken once a week for until 36days. Weight values are mean of 5 animals. Streptozotocin was made in time 0day and obtained response after 3 days. Different letters in line indicate statistically significant difference among all groups ($p<0.05$ ANOVA).

As the glucose level from Brazilian samples were better, the doses were used in rats with induced diabetes using a diet based on a high sugar concentration, based in cookies, biscuits and a solution of 20% sucrose. This was done for 3 months. After this period, it was administered in a group of animals the amount of polysaccharide from the isolated group chosen for 60 days.

The Fig. 3 demonstrated that the weight from the animals submitted to treatment with polysaccharides and, the controls, did not show a significantly difference at the level of 5%. When these animals were compared with the DM1,

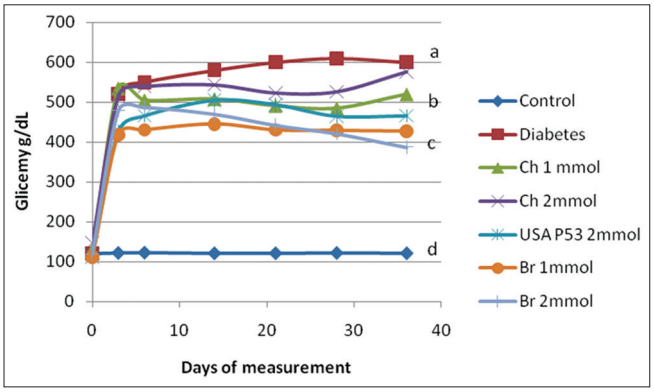

Fig 2. Blood glucose levels in rats submitted to treatments: 1) C = control that received commercial pellet; 2) D = diabetic group that received streptozotocin and commercial pellet; 3) 1mmol= Diabetic group that received oral Tremella 1mmol from Ch (China) or Br (Brazil); 4) 2mmol= Diabetic group that received oral Tremella 2mmol from Ch (China), USA and Br (Brazil). Measurements were taken once a week for until 36 days. Weight values are mean of 5 animals. Streptozotocin was made in time 0 day and obtained response after 3 days. Different letters in line indicate statistically significant difference among all groups ($p<0.05$ ANOVA).

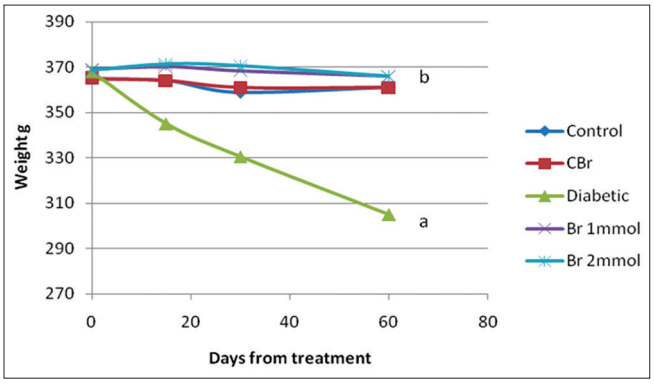

Fig 3. Body weight of rats submitted to treatments 1) Control = control that received commercial pellet; 2) Diabetic = diabetic group that received high sugar diet and commercial pellet; 3) CBr= control that received polysaccharide from Tremella isolate from Brazil and commercial pellet; 4) Br 1mmol= Diabetic group that received oral Tremella 1mmol from Br (Brazil); 4) Br 2mmol= Diabetic group that received oral Tremella 2mmol from Br (Brazil). Measurements were taken once a week for until 60days. Weight values are mean of 5 animals. In time 0day correspond to 3 month of high sugar diet. Different letters in line indicate statistically significant difference among all groups ($p<0.05$ ANOVA).

was observed a weight decrease. This was confirmed on Fig. 4 where the glicemy level from control rats was 90 to 100g/dL. Yet, rats submitted to polysaccharides treatment had a decrease, this is, the level came from 138 to 98 g/dL, when compared with DM1 rats with the level of 149 to 241g/dL.

Without a doubt, the diabetes can be controlled with the *Tremella fuciformis* polysaccharide until the glicemy level of 130g/dL, because above this the reduction was extremely low.

The problem found in these experiments is that in the animals with STZ, the diabetes after 3 weeks is really high, being necessary to discard the rats. On the other model involving diet, the time to evaluate the animals was higher being able to use the polysaccharide at the maximum of 60 days, similar data was described by Wilson and Islam (2012) in animal models, however the monetary cost has been being too high.

Biochemical analysis of plasma

Table 2 displays the results of the biochemical tests at the time of euthanasia.

Creatinine and urea are kidney function markers and demonstrated that all animals when induced diabetes with STZ or HSD, no significant differences were observed for creatinine.

The increased levels of urea in animals DM1 may be due to increased protein catabolism, a situation not seen in animals EPS and Control (Table 2).

Glutamic pyruvic transaminase (GPT) levels increased in DM1 with HSD group and showed greater activity when compared with other groups. The same is seen in the STZ group. This indicates a possible liver damage caused by HSD and STZ, because the own EPS in the control animal shown the same activity from the animal without treatment. However the STZ group, even treated with EPS showed elevated levels indicating hepatotoxicity of streptozotocin (Table 2).

In animals with the two types of diabetes induction, cholesterol and triglycerides occurred an increase, while the HDL had a decreased. Comparing with the other groups submitted to treatment, both the cholesterol and triglycerides decreased, while occurred a slightly increase in the HDL (Table 2). This is justified by the fact that *diabetes mellitus* cause a metabolic syndrome, changing not only glucose metabolism but also lipid and

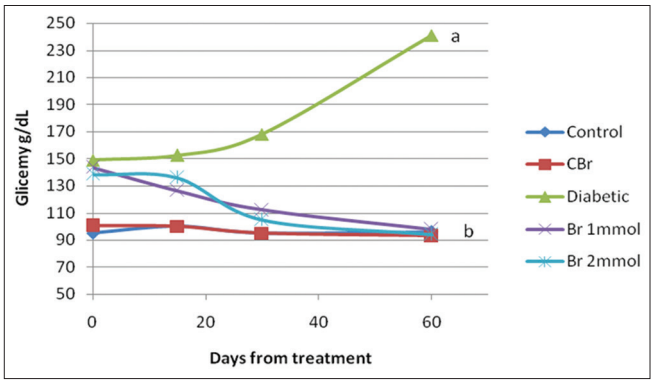

Fig 4. Blood glucose levels in rats submitted to treatments: 1) Control = control that received commercial pellet; 2) Diabetic = diabetic group that received high sugar diet and commercial pellet; 3) CBr= control that received polysaccharide from Tremella isolate from Brazil and commercial pellet; 4) Br 1mmol= Diabetic group that received oral Tremella 1mmol from Br (Brazil); 4) Br 2mmol= Diabetic group that received oral Tremella 2mmol from Br (Brazil). Measurements were taken once a week for until 60days. Weight values are mean of 5 animals. In time 0day correspond to 3 month of high sugar diet. Different letters in line indicate statistically significant difference among all groups ($p<0.05$ ANOVA).

Table 2: Biochemical results of plasma from rats submitted to treatments

Treatements	GPT *	Creatinine	Urea	Cholesterol	Triglycerides	HDL
Diabetes with streptozotocin						
Control	70.0d**	0.3a	44.4d	66c	54e	39.50a
CBr	79.0c	0.3a	46.0d	55e	60d	36.80b
Diabetic	104.0a	0.3a	57.1a	83a	74a	33.80c
Br 1 mmol	90.0b	0.3a	52.8b	59d	62c	36.05b
Br 2 mmol	103.0a	0.3a	51.9c	69b	64b	37.01b
Diabetes with high sugar diet						
Control	30.0a	0.10a	24.8b	65c	69.5a	31.9b
Diabetes	39.0b	0.15a	33.3a	70a	82.5b	28.7b
Ch 1 mmol	30.0a	0.10a	19.5c	68b	68.0a	34.5a
Ch 2 mmol	29.5a	0.10a	25.0b	68b	67.5a	39.7a
USA P53 2 mmol	30.0a	0.15a	26.8b	68b	69.0a	35.0a
Br 1 mmol	30.0a	0.10a	27.0b	67b	69.0a	39.0a
Br 2 mmol	30.0a	0.10a	27.2b	67b	69.0a	39.0a

*Biochemical tests in mg/dL. **Values are means from 5 animals. Different letters on columns indicate statistically significant differences among groups with STZ or HSD ($p<0.05$; ANOVA+Studen't test)

Table 3: Anti-inflammatory test in rats submitted to treatments

Group	N° animals	Average	Standard deviation	Significance
Water	5	0.3733	0.0085	
dexamethasone	5	0.2852	0.0114	***p<0.001
Ch 2 mmol	5	0.3617	0.0199	Ns p>0.05
Br 2 mmol	5	0.3629	0.0136	Ns p>0.05

***=Extremely significant, Ns p>0.05=Not significant

protein metabolism. The results can be accordance to Cheng et. al. (2002) when used fed diets for rats containing different levels of *T. fuciformis* dietary fiber and showed that cholesterol levels were significantly decreased concluding that fungi supplement altered the intestinal physiology of the rats.

Anti-inflammatory assay

In anti-inflammatory test the aqueous solution of *Tremella fuciformis* showed no anti-inflammatory activity in the tested dosage. Probably, because the *Tremella's* solution has high sugar concentration, however they are not from substances with anti-inflammatory potential (Table 3).

CONCLUSION

In conclusion present findings demonstrate that EPS from *Tremella fuciformis* be beneficiated the control of DM1 when blood glucose is until 130mg/dL that can be accomplished by reducing cholesterol, triglyceride, GPT, urea and increasing HDL cholesterol. When the level of blood glucose is higher than EPS from *T. fuciformis*, the action for reduction is not rapidly and function is not satisfactory. The solution of *T. fuciformis* did not show anti-inflamatory activity in the tested dosage.

ACKNOWLEDGEMENTS

This work was supported by CNPq (Grant #474681/2013). Dra. Arailde Urben (Embrapa –Brasilia) and Edison Souza for providing isolates from *Tremella fuciformis* and for Flávio Dessaune Tardin, Pesquisador da Embrapa Milho e Sorgo (Mato Grosso), for providing sorghum seeds.

Author contributions

All authors contributed equally in this article.

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