

ORIGINAL ARTICLE**Antihyperglycemic and antinociceptive effects of a commercially produced beta-glucan from *Saccharomyces cerevisiae* in Swiss albino mice****¹Md. Tanvir Morshed, ¹Shahnaz Rahman, ¹Shiblur Rahman, ¹Sharmin Jahan, ²Mohsina Mukti, ²Inin Taznin, ¹Masta Hasenur Reza Chawdhury, ¹Mohammed Rahmatullah**¹Faculty of Life Sciences, University of Development Alternative, Dhaka-1209, Bangladesh.²Department of Pharmacy, North South University, Bashundhara, Dhaka-1229, Bangladesh.Md. Tanvir Morshed, Shahnaz Rahman, Shiblur Rahman, Sharmin Jahan, Mohsina Mukti, Inin Taznin, Masta Hasenur Reza Chawdhury, Mohammed Rahmatullah; Antihyperglycemic and antinociceptive effects of a commercially produced beta-glucan from *Saccharomyces cerevisiae* in Swiss albino mice**ABSTRACT**

Beta-glucans are sugars that are found in the cell walls of bacteria, fungi, yeasts, algae, lichens, and plants, such as oats and barley. They are polysaccharides of D-glucose monomers linked by beta-glycosidic bonds. Their major use is as a nutraceutical and it has been claimed that these compounds can act as effective immune modulators through boosting the immune system. The most active forms of beta-glucans are those comprising D-glucose units with (1,3) links and with side-chains of D-glucose attached at the (1,6) position. In dietary studies with diabetic rodent models, these compounds have been observed to lower the absorption of glucose and so lead to decreasing blood sugar levels. The objective of the present study was to evaluate the anti-hyperglycemic and antinociceptive effects of a commercially available beta-glucan produced from yeast (*Saccharomyces cerevisiae*). In oral glucose tolerance tests with glucose-loaded Swiss albino mice, beta-glucan, administered orally at doses of 30, 60, 120 and 240 mg per kg body weight, inhibited increases in blood sugar levels significantly and dose-dependently. The percent inhibitions at the afore-mentioned four doses were, respectively, 33.5, 35.0, 35.7, and 38.7. In comparison, a standard anti-hyperglycemic drug, glibenclamide, when administered at a dose of 10 mg per kg body weight inhibited increases in blood sugar levels by 40.0%. As such, the results obtained with the commercial beta-glucan were comparable to that of glibenclamide, at least at the highest dose tested. In antinociceptive activity tests with acetic acid-induced gastric pain model in Swiss albino mice, beta-glucan at the afore-mentioned four doses also significantly and dose-dependently attenuated the number of writhings in mice induced by intraperitoneal administration of acetic acid. The percent inhibitions in the number of gastric writhings at the above four doses were, respectively, 36.6, 40.0, 43.4, and 60.0. A standard antinociceptive drug, aspirin, when administered at doses of 200 and 400 mg per kg body weight, reduced the number of writhings by 40.0 and 63.4%, respectively. Thus, the result obtained with beta-glucan at the highest dose was comparable to that of aspirin at the highest dose tested. Together, the results suggest that the commercial beta-glucan used in the present study can be used for both reducing blood sugar in high blood sugar level individuals as well as to alleviate pain.

Key words: Beta-glucan, antihyperglycemic, antinociceptive, *Saccharomyces cerevisiae***Introduction**

Beta-glucans are sugars that are found in the cell walls of bacteria, fungi, yeasts, algae, lichens, and plants, such as oats and barley. They are polysaccharides of D-glucose monomers linked by beta-glycosidic bonds. Their major use is as a nutraceutical and it has been claimed that these compounds can act as effective immune modulators through boosting the immune system. The most active forms of beta-glucans are those comprising D-glucose units with (1,3) links and with side-chains of D-glucose attached at the (1,6) position.

Beta-glucans, differing in molecular weight, have been isolated from many diverse species like *Rhynchelytrum repens*, *Lentinus edodes*, *Grifola frondosa*, *Tremella mesenterica*, *Tremella aurantia*, *Zea mays*, *Agaricus blazei* (mushroom), *Phellinus buammi*, and *Saccharomyces cerevisiae* (yeast) (Rahar *et al.*, 2011). The most common sources of beta-glucans have generally been maize, oats, and yeast. Beta-glucans, isolated from *Agaricus blazei* and their enzymatically hydrolyzed oligosaccharides derivatives have been shown to demonstrate anti-hyperglycemic, anti-hypertriglyceridemic, anti-hypercholesterolemic, and anti-arteriosclerotic

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activity in diabetic rats, indicating overall anti-diabetic activity (Kim *et al.*, 2005). In human subjects, beta-glucan, isolated from oat bran, was found to lower peak values of glycemic responses than the reference glucose load (Mäkeläinen *et al.*, 2007). Aminated beta-1,3-D-glucan reportedly improved wound healing in diabetic db/db mice, suggesting a beneficial effect of beta-glucans in diabetes (Berdal *et al.*, 2007). Indeed, dietary intake of beta-glucans has been shown to reduce risk factors arising from diabetes like high blood glucose level, manifestations of thirst, polyuria, polydipsia, and weight loss (Chen and Raymond, 2008).

Oat products, which are abundant in beta-glucan has been reported to lower the glycemic index of products or foods, which is beneficial in the control of postprandial glycemia. Streptozotocin-induced diabetic mice, fed oat products containing beta-glucan in their diet for 6 weeks, had significantly decreased fasting blood glucose and glycosylated serum protein (Shen *et al.*, 2011). A synergistic favorable effect of bitter melon (*Momordica charantia*) and beta-glucan isolated from oats has been shown in streptozotocin-induced diabetic rats, when administered for 28 days (Kim *et al.*, 2012). Hypoglycemic and hypocholesterolemic effects of botryosphaeran (a water-soluble exopolysaccharide of the beta-1→3; 1→6 -D-glucan type) from *Botryosphaeria rhodina* has been observed following administration to diabetic rats over 15 days (Miranda-Nantes *et al.*, 2011). Overall, the various reports suggest that beta-glucans isolated from various microbial and plant sources have an overall beneficial effect in diabetic rodent models, as well as in humans. These results point to an anti-diabetic effect of beta-glucans manifested primarily through reduction in blood sugar levels and lowering of glycemic index of foods. However, other complications arising from diabetes have also been reported to be reduced, especially following long-term administration of beta-glucans in diet or when administered alone through gavaging.

An anti-inflammatory and analgesic property of a (1→3),(1→6)-linked beta-glucan isolated from *Pleurotus pulmonarius* has also been shown in a rodent model (Smiderle *et al.*, 2008). It is therefore evident that beta-glucans from different sources possibly possess potential to be used as anti-hyperglycemic and analgesic agents on top of their being used as nutraceuticals, primarily to boost immune functions in the body. The objective of this study was to evaluate the anti-hyperglycemic and antinociceptive potential of a commercially available beta-glucan isolated from *Saccharomyces cerevisiae*.

Materials and Methods

Chemicals:

Glacial acetic acid was obtained from Sigma Chemicals, USA; aspirin, glibenclamide and glucose were obtained from Square Pharmaceuticals Ltd., Bangladesh. WGP® beta-glucan Wellmune was obtained from a nutraceutical shop at Kuala Lumpur, Malaysia. WGP is a registered trade mark of Biothera, Minnesota, USA. The product was manufactured by Pahang Pharmacy SDN.BHD, Selangor, Malaysia (Lot 5979, Batch Number 10N37, expiry date April 2013; this study was done in 2012). As per the information that came with the beta-glucan container, the beta-glucan was prepared from baker's yeast (*Saccharomyces cerevisiae*). The product was distributed as capsules, with each capsule containing 200 mg beta-glucan. The dosage for human consumption was mentioned in the information booklet to be one capsule per day on an empty stomach. Stock solution of beta-glucan was prepared in water (0.06g beta-glucan per ml distilled water).

Animals:

In the present study, Swiss albino mice (male), which weighed between 22-29g were used. The animals were obtained from International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B). All animals were kept under ambient temperature with 12h light followed by a 12h dark cycle. The animals were acclimatized for three days prior to actual experiments. The study was conducted following approval by the Institutional Animal Ethical Committee of University of Development Alternative, Dhaka, Bangladesh.

Antihyperglycemic activity:

Glucose tolerance property of the commercial beta-glucan was determined as per the procedure previously described by Joy and Kuttan (1999) with minor modifications. In brief, fasted mice were grouped into six groups of six mice each. The various groups received different treatments like Group 1 received vehicle (10 ml distilled water/kg body weight) and served as control, group 2 received standard drug (glibenclamide, 10 mg/kg body weight). Groups 3-6 received beta-glucan at doses of 30, 60, 120, and 240 mg per kg body weight. Each mouse was weighed and doses adjusted accordingly prior to administration of vehicle, standard drug, and test samples. All substances were orally administered. Following a period of one hour, all mice were orally administered 2 g glucose/kg of body weight. Blood samples were collected 120 minutes after the glucose administration through puncturing heart. Blood glucose levels were measured by glucose oxidase method (Venkatesh *et al.*, 2004).

Antinociceptive activity:

Antinociceptive activity of beta-glucan was examined using previously described procedures (Shanmugasundaram and Venkataraman, 2005). Briefly, mice were divided into seven groups of six mice each. Group 1 served as control and was administered vehicle only. Groups 2 and 3 were orally administered the standard antinociceptive drug aspirin at a dose of 200 and 400 mg per kg body weight, respectively. Groups 4-7 were administered beta-glucan at doses of 30, 60, 120 and 240 mg per kg body weight, respectively. Following a period of 60 minutes after oral administration of standard drug or beta-glucan, all mice were intraperitoneally injected with 1% acetic acid at a dose of 10 ml per kg body weight. A period of 15 minutes was given to each animal to ensure bio-availability of acetic acid, following which period, the number of writhings was counted for 10 min.

Statistical analysis:

Experimental values are expressed as mean \pm SEM. Independent Sample t-test was carried out for statistical comparison. Statistical significance was considered to be indicated by a p value < 0.05 in all cases.

Results and Discussion

The beta-glucan used in the present study showed significant and dose-dependent lowering of blood glucose levels in glucose-loaded mice at all the tested concentrations. At doses of 30, 60, 120 and 240 mg per kg body weight, the percent lowering in blood glucose levels were, respectively, 33.5, 35.0, 35.7 and 38.7. By comparison, a standard anti-hyperglycemic drug, glibenclamide, when administered at a dose of 10 mg per kg body weight reduced blood sugar in mice by 40.0%. Thus the beta-glucan, at least at the highest experimental dose tested, lowered blood glucose, which was comparable to that of glibenclamide. The results are shown in Table 1.

Table 1: Effect of beta-glucan on blood glucose level in hyperglycemic mice following 120 minutes of glucose loading.

Treatment	Dose (mg/kg body weight)	Blood glucose level (mmol/l)	% lowering of blood glucose level
Control (Group 1)	10 ml	6.80 \pm 0.24	-
Glibenclamide (Group 2)	10 mg	4.08 \pm 0.42	40.0*
Beta-glucan (Group 3)	30 mg	4.52 \pm 0.42	33.5*
Beta-glucan (Group 4)	60 mg	4.42 \pm 0.25	35.0*
Beta-glucan (Group 5)	120 mg	4.37 \pm 0.39	35.7*
Beta-glucan (Group 6)	240 mg	4.17 \pm 0.30	38.7*

All administrations were made orally. Values represented as mean \pm SEM, (n=6); * $P < 0.05$; significant compared to hyperglycemic control animals.

The blood glucose lowering capability exhibited by beta-glucan could be due to the reported ability of beta-glucan to lower the glycemic index of carbohydrates (Mäkeläinen *et al.*, 2007). The exact mechanism remains to be elucidated, but one possible factor behind the observed blood glucose lowering may be decreased absorption of glucose from the gut (Bhowmik *et al.*, 2009). However, other mechanism(s) cannot be ruled out, like potentiating insulin secretion from the pancreas (Farjou *et al.*, 1987). It is to be noted that at least one published study has found evidences to that effect in case of beta-glucan (Mäkeläinen *et al.*, 2007).

In antinociceptive activity tests, beta-glucan at doses of 30, 60, 120 and 240 mg per kg body weight inhibited the number of writhings in mice induced by intraperitoneal administration of acetic acid, respectively, by 36.6, 40.0, 43.4 and 60.0%. The percent reductions in the number of writhings were both significant as well as dose-dependent. The results are shown in Table 2. The standard antinociceptive drug, aspirin, when administered at doses of 200 and 400 mg per kg body weight, reduced the number of writhings by 40.0 and 63.4%, respectively. Thus, beta-glucan showed considerable antinociceptive activity, which was directly comparable to that of aspirin.

Table 2: Antinociceptive effect of beta-glucan in the acetic acid-induced gastric pain model mice.

Treatment	Dose (mg/kg body weight)	Mean number of writhings	% inhibition
Control (Group 1)	10 ml	5.00 \pm 0.52	-
Aspirin (Group 2)	200 mg	3.00 \pm 0.73	40.0*
Aspirin (Group 3)	400 mg	1.83 \pm 0.83	63.4*
Beta-glucan (Group 4)	30 mg	3.17 \pm 0.48	36.6*
Beta-glucan (Group 5)	60 mg	3.00 \pm 0.52	40.0*
Beta-glucan (Group 6)	120 mg	2.83 \pm 0.48	43.4*
Beta-glucan (Group 7)	240 mg	2.00 \pm 0.45	60.0*

All administrations (aspirin and beta-glucan) were made orally. Values represented as mean \pm SEM, (n=6); * P < 0.05; significant compared to control.

Anti-inflammatory and analgesic property of a (1 \rightarrow 3),(1 \rightarrow 6) -linked beta-glucan isolated from *Pleurotus pulmonarius* has also been shown in a rodent model (Smiderle *et al.*, 2008). In this case, the beta-glucan was from *Saccharomyces cerevisiae*. We have not elucidated the exact mechanism behind the antinociceptive activity of the beta-glucan, but it may be hypothesized that this activity has to do with possible inhibition of cyclooxygenases, which synthesize prostaglandins [mainly prostacyclins (PGI₂) and prostaglandin- (PG-E)], which has been shown to be responsible for excitation of Delta-nerve fibers, leading to the sensation of pain (Reynolds, 1982; Rang and Dale, 2003).

Overall, the results obtained from the commercially produced beta-glucan isolated from *Saccharomyces cerevisiae* suggest that besides its intended use as a nutraceutical (with possible immunomodulatory benefits), the product may also be used by diabetic patients for lowering blood sugar and by people affected with pain. The results further suggest that more studies should be carried out with beta-glucans from different sources [particularly the (1 \rightarrow 3),(1 \rightarrow 6) type] to fully determine the pharmacological and beneficial effects of these type of glucans.

Funding:

Authors of this study have no financial interest in any of the product(s) or manufacturer(s) mentioned in this article.

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