Brewer’s spent grain (BSG) is the by-products during the beer brewing, which is rich in proteins and expected to be further utilized in food and medicine industry. Alcalase was employed to hydrolyze the BSG protein, and the hydrolysates were isolated by ultrafiltration with the cutoff of 5000 kDa. The permeate containing BSC peptides with molecular weight lower than 5000 kDa showed a good inhibitory activity on α-glucosidase with a maximum inhibitory rate of 21.4%. The hypoglycemic activity of BSG peptides in streptozocin induced diabetic mice was studied. The results indicated that administration of BSG peptides through the oral route in mice had no acute toxicity. Blood glucose tests showed that the glucose levels in diabetic mice were all within the normal range of lower than 11.1mmol/L after treated with BSG peptides for 28 days. Meanwhile, the treatment with BSG peptides in doses of 250 mg/kg and 500 mg/kg produced significant reductions in blood glucose of 54.4% and 60.0% with no obvious differences (P > 0.05) compared to the positive control mice. And the serum total cholesterol (TC) and triglycerides (TG) of diabetic mice were significantly lower (P < 0.05) than the model control. The pancreas/body weight ratio increased markedly (P <0.05) to 5.08, 5.07 and 5.17 mg/g respectively after treated with doses of 125 mg/kg, 250 mg/kg and 500 mg/kg for 28 days,. The similar changes could be found in serum insulin concentration. It was indicated BSG peptides had significant hypoglycemic effect and was promising in utilization in functional foods.