Hypoglycermic effect of sunflower seed, soybean and corn oil on Alloxan-induced diabetic Sprague-Dawley rats

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Abstract- Nowadays, cases of diabetes mellitus consistently elevate globally and produce a significant impact to the society, making it one of the most prevalent and life threatening diseases among adult over 50 years of age. Diabetes mellitus is usually associated with uncontrolled elevation of glucose in our body due to an impaired production of insulin by the pancreas. This type of diabetes once acquired can lead to short term prognosis and administration of insulin may be recommended to severe cases. Several chemicals have been studied and revealed that fatty acid specifically linoleic acid can lower excessive amount of glucose in our body. Sunflower seed, soybean and corn contain various types of fatty acid including linoleic acid but percent composition varies in each type of oil. This study determined the effect of sunflower seed, soybean and corn oil on the fasting blood glucose (FBG) levels of Alloxan-induced diabetic Sprague Dawley rats. Results of the study revealed that corn oil produced the highest hypoglycemic effect on the FBG levels of laboratory animals at a concentration of 1ml and 6ml/100g body weight followed by sunflower seed and soybean oil. Statistical analysis of the experimental data however revealed that there is no significant differences on the effect of 1ml/100mg and 6ml/100mg of sunflower, soybean, corn oil and glibenclamide at <0.05 level of significance This implies that the oils used in the study exhibited hypoglycemic properties similar to that of glibenclamide and therefore can be used as an adjunct nutraceutical to lower blood glucose levels. Further testing on a larger population of animal models is recommended.

Keywords- hypoglycemic, sunflower seed oil, soybean oil, corn oil, linoleic acid

INTRODUCTION

The incidence of diabetes mellitus is increasing worldwide (Toumilehto et al., 2001). Globally, there are approximately 200 million people with diabetes. If nothing is done to slow down the epidemic, the number will exceed 333 million by the year 2025 (Knol et al., 2006). The Philippines ranks 10th among countries with the highest diabetes incidence worldwide. An estimated 6 Million Filipinos know they have diabetes. Another 6 Million Filipinos have diabetes but do not know they have it. Health experts believe many more have impaired glucose tolerance (IGT) and are prone to diabetes. Unless we act now to change our lifestyle of too much sweet and fatty foods and too little exercise, our path will lead us to the gaping mouth of a diabetes epidemic.(Source: US Centers for Disease Control and Prevention 2004 press statement)

Usually, type 2 diabetes mellitus results from the interaction between a genetic predisposition and behavioral and environmental risk factors (Toumilehto et al., 2001). Diabetes mellitus is important because it is common, has serious complications and reduces life expectancy by 8-10 years; it is difficult to treat, and expensive to manage. For diabetes physicians, it is considered as a complex and heterogeneous disease with a poorly understood etiology, apart from the fact that there is a strong genetic propensity that becomes overt when exposed to a typical Western lifestyle (Astrup and Finer; 2000).

Several lifestyle factors affect the incidence of type 2 diabetes. Obesity and weight gain dramatically increase the risk, and physical inactivity further elevates the risk, independently of obesity (Hu et al., 2001). Obesity is associated with high blood cholesterol and high risk of for developing diabetes and cardiovascular disease (Wang et al., 2004).The classical symptoms include polyuria, polydipsia, polyphagia, visual blurring, frequent or recurring infections, cuts and bruises that are slow to heal, tingling and or numbness in hands and or feet, drowsiness, nausea, and decreased endurance during exercise (Jimam et al., 2010). Fetal exposure to maternal hyperglycemia can cause permanent fetal changes, leading to malformations,increased birth weight),and an increased risk of obesity , cardiovascular disease, hypertension , and type 2 diabetes(Pettit et al.,2008). Several studies have suggested an association between diabetes mellitus and cognitive impairment, rapid declinein cognitive function, and dementia(Roberts et al.,2008).

Despite the advancement in the understanding and management of DM, the incidence of the disease continues to increase. More rural diabetic patients are now relying much more on traditional remedies, due to the frequent claims by herbal medicine practitioners with regard to the effectiveness of herbal medicines in diabetes mellitus (Jimam et al., 2010). Unfortunately, many patients withtype1 and type2 diabetes will ultimately experience diabetes complications. While there are methods to manage diabetes complications.These complications are generally progressive and often become irreversible; thus, mitigating the risk for their appearance and identifying and treating them early is of outmost importance for clinicians and patients.(Moore et al 2009).

Medicinal plants are commonly used in treating and preventing specific ailments and diseases, and are generally considered to play a beneficial role in healthcare (Abere, 2010).

Conjugated linoleic acid (CLA) is a group of positional and geometric isomers of conjugated dienoic derivatives of linoleic acid (Kasaoka. et al., 2000). Like nutraceuticals, being minor lipids with supposed functional food status (Benjamin and Spener; 2009) it has been studied extensively due to their ability to modulate cancer, atherosclerosis, obesity, immune function and diabetes in a variety of experimental models (Brown and McIntosh; 2003). diabetes can be caused by too little insulin (type1), resistant to insulin (type 2), or both. Supplementing the diet with conjugated linoleic acid may lead to better disease management in diabetics especially type 2 (Benjamin and Spener., 2009).

Supplementation with conjugated linoleic acid induces a number of physiological effects in experimental animals, including reduced body fat content, decreased aortic lipid deposition, enhanced glucose metabolism and inhibited tumorigenesis(Smedman and Vessby.,2001)

The sunflower (Helianthus annus L.) is a member of the compositae (Asteracea) family in the genus Helianthus (Abitogun et al., 2010). As an oilseed crop,it was introduced in Pakistan in 1960. Sunflower oil is considered as premium oil due to its light color, mild flavor and ability to withstand at high cooking temperatures (Iqbal, et al., 2009). Linoleic acid dominates with the range values of 51.65-67.66%/100g (Abitogun et al., 2010). Due to the health benefits of some polyunsaturated fatty acids, there has been an increasing trend to enrich the food with them or triglycerides(Zarate et al.,2009). Sunflower oil was mixed with other oils rich in monounsaturated fatty acids to extend the shelf life of sunflower(Farag et al.,2010)

The soybean species grow in very different soils and under diverse climate conditions.Such a broad geographical distribution and diverse growing conditions contribute to the plentiful genetic diversity of annual wild soybeans(Dong et al.,2001).Soybean oil obtained from the extraction of oil from it seed is fairly rich in glycerides of the unsaturated fattyacids particularly linoleic and linolenic with few oleic fattyacids, which do not oxidize readily because they contain natural antioxidants(Ashaye and Olusoji.,2006). Protein and oil percentages in soybean,while influenced by both genotype and environmental cues, average approximately 40% and 20%, respectively(Clemente et al.,2009). Soybean oil's utilization is determined by its fatty acid composition, with commodity soybean oil typically containing 13% palmitic acid(16:0), 4% stearic acid (18:0), 20% oleic acid (18:1), 55%linoleic acid (18:2), and 8% linolenic acid (18:3)(Pham et al .,2010).

Corn is the major feedstock for producing fuel ethanol in the United States(Kim and Dane.,2002).Most commercial corn oil is produced by either pressing corn germ (a fraction derived from the kernel, consisting mainly of embryos) or extraction with hexane or a combination of pre-pressing and hexane extraction, followed by refining, bleaching and deodorization (RBD) [1]. About 90% of the commercial corn oil is obtained from corn germ that is a byproduct (co-product) of corn wet milling, a process developed to efficiently remove the starch (*70%) from corn kernels. Like many other seed oils, linoleic acid is the predominantfatty acid in all three types of corn oil [7], withsmaller proportions of oleic, palmitic, and linolenic acids.(Moreau et al., 2009). Corn oils contain 59.8% of linoleic acid of its total fatty acid(Hwang et al., 2009)

In summary, diabetes is an alarming disease that requires significant attention. Several supplements and drugs are used as treatment. Recent studies revealed that linoleic acid helps in lowering glucose level thus possessing hypoglycemic property. Comparison of different oil with linoleic acids has not yet been studied and this study aims to determine which among the three types of oil has the greatest efficacy in lowering blood glucose levels in the laboratory animal models.

MATERIALS AND METHOD

Materials

Sunflower seed oil, soybean oil and corn oil are commercially available in the market. Samples of oils were bought from a supermarket in Batangas City, Philippines.

In this study the following oils were used: Susan Baker Sunflower Seed Oil (Kawsek Inc., Philippines), Simply Soybean Oil (Wilmar Trading PTE Ltd., Philippines), Baguio Corn Oil (Cheng Ban Yek & Co. Inc., Philippines). All oil samples are 100% pure.

Chemicals

Alloxan monohydrate was purchased from Sigma-Aldrich, MO, USA and the Glibenclamide from PT Aventis Pharma, Indonesia.

Experimental animals

Experiments were performed on albino rats weighing between 180-210 grams. The animals were housed in colony cages (four per cage) under conditions of standard lighting, temperature (22±1°C) and humidity for at least one week before the beginning of the experiment, to adjust to the new environment and to overcome stress possibly incurred during transit (Prashanth et al., 2010). A standard pellet diet and water were given ad livitum(Zhou et al., 2009).The baseline blood glucose concentration levels of the overnight fasted rats were measured before the administration of oil(Jimam et al.,2010). The ethical approved for the use of laboratory animal were secured from Bureau of Animal and Industry.

Induction of diabetes

Rats were injected with freshly prepared solution of alloxan monohydrate in normal saline intraperitoneally at a dose of 150 mg/kg body weight. Because alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 20% glucose solution (5 to 10 mL) orally after 6 hours. The rats were then kept for the next 24 hours on 5% glucose solution bottles in their cages to prevent hypoglycemia. After 3 weeks, rats with moderate diabetes having glycosuria and hyperglycemia (i.e. blood glucose of 200 to 300 mg/dL) were chosen for the experiment (Ananthan et al., 2003).

Determination of oil doses

Six groups of male rats comprising of 4 per group were given the vehicle for oil reconstitution (control), 2, 4, 6, 8, 10 g/kg of the oil using orogastric tube (CU.FNC-16-3) (Ozolua et al., 2009). Mortality was observed for the purpose of dose determination that the rats are capable of receiving not for the toxic effect. Our oils are edible and do not have any toxic effect in human.

Experimental procedure

Five groups of rats, six in each received the following treatment schedule (Ahmed et al., 2010).

Group I: Normal control (saline).
Group II: Alloxan (150 mg/kg.ip)+ Sunflower seed oil
Group III: Alloxan (150 mg/kg.ip)+ Soybean oil
Group IV: Alloxan (150 mg/kg.ip) + Corn oil
Group V: Alloxan (150 mg/kg.ip) + Standard drug, Glibenclamide(5mg/kg, p.o).

Collection of Blood Sample and Blood Glucose Determination

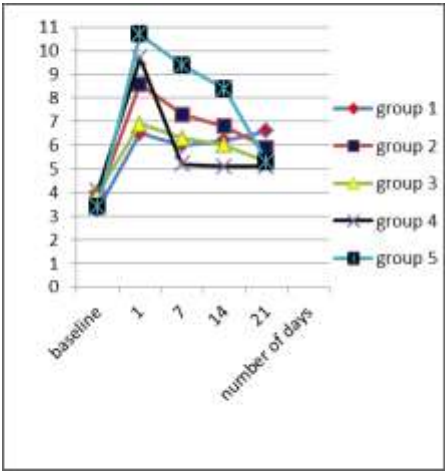
Blood samples were drawn from the tail tip of rat at weekly intervals till the end of the study (i.e., 2 weeks). Fasting blood glucose estimation and body weight measurement were done on day 1, 7, and 14 of the study. Blood glucose estimation can be done by electronic glucometer using glucose test strips. (Ahmed et al., 2010).

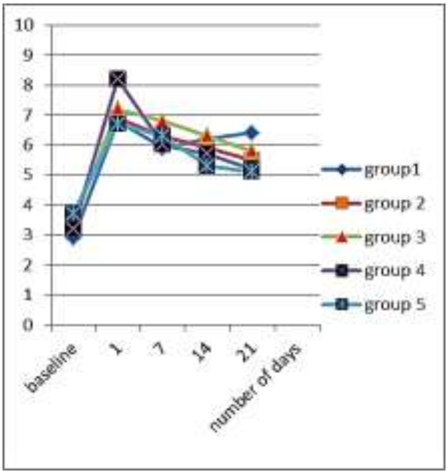
Statistical analysis

Each result was expressed as means ± Standard Error. The grouped data was evaluated statistically using one-way analysis of variance (ANOVA) (Zhou et al., 2009).

RESULTS

After an intraperitoneal injection of alloxan monohydrate, the drug successfully elevated the fasting blood glucose level of the rats resulting to induction of diabetes. A baseline was established by measuring the fasting blood glucose level prior to administration of alloxan monohydrate that served as the initial basis of the experiment. From 2.9mmol/L and 3.3mmol/L to 6.8mmol/L and 6.5mmol/L in group 1, 3.6mmol/L and 3.8mmol/L to 6.9mmol/L and 8.6mmol/L in group 2, 3.4mmol/L and 4mmol/L to 7.2mmol/L and 6.9mmol/L in group3, 3.2mmol/L and 4.1mmol/L to 8.2mmol/l and 9.7mmol/L in group 4 lastly from 3.7mmol/L and 3.4mmol/L to 6.7mmol/L and 10.7mmol/L On the day 7 of the experiment, corn oil produced the greatest efficacy in lowering blood glucose level of laboratory animals both in 1ml/100g and 6ml/100g doses. On the day 14 of the experiment the positive control, glibenclamide greatly decreased FBG level than commercially available oils in both doses. On the last week of the experiment still, glibenclamide proved its efficiency in depressing FBG level of animal model. Overall, those rats treated with corn oil exhibited the greatest hypoglycemic effect. Other oils also demonstrated hypoglycemic property but not as significant as the corn oil.





Furthermore, it has been noted that there is no significant differences on the effect of 1ml/100mg and 6ml/100mg of sunflower, soybean, corn oil and glibenclamide at <0.05 level of significance (Table 1). The normal control group (0.95% NSS) showed an inconsistent effect from decreased FBG levels on the day 7 and increased FBG levels in succeeding days of the experiment.

Table 1
Comparison of oils and control in terms of fasting blood glucose level

	Sum of squares	df	Mean square	F	sig
Between groups	4.406	4	1.151	0.650	0.629
Within groups	97.382	55	1.771		
Total	101.987	59			

DISCUSSION

This study proved that the oils from sunflower, soybean and corn are capable of lowering blood glucose level therefore exhibiting hypoglycemic property.

Five groups of rats were used, each group consist of 3 rats and received 2 different doses (1ml/100g and 6ml/100g).The first group was given with 1ml/100g dose, 2ml/100g dose to the second, 4ml/100g dose to the third and 6ml/100g dose to the fourth and 8ml/100g dose to the last group. Upon reach the 8ml/100g dose the rats can no longer tolerate the oil and rejected it so the administration of oil was stopped. We used the minimum dose (1ml/100g) and the maximum dose that the rat can tolerate (6ml/100g). Fasting blood glucose levels were checked in weekly interval to determine the progress of the study.

Group 1 also known as the NSS group served as the negative control of the study. The results of this group must be constant starting from day 1 up to last day of the experiment but unfortunately on the first week of FBG determination a decreased of 0.9mmol/L was observed in 1ml/100g dose and 0.5mmol/L in 6ml/100g dose. In the following week an increased of 0.3mmol/L (1ml/100g) and 0.2mmol/L (6ml/L) was demonstrated and finally in the last week of the study, there was an additional increased of 0.2mmol/L (1ml/100g) and 0.4mmol/L (6ml/100g) ruling out that the elevation on the first week is just a physiologic effect.

The second group known as the sunflower oil group also contributes in lowering Fasting blood glucose level. Prior to administration of ALX, FBG level is 3.6mmol/L(1ml/100g) and 3.8mmol/L (6ml/100g) then it increased up to 6.9mmol/L (1ml/100g) and 8.6mmol/L (6ml/100g) after induction of diabetes. Diabetic rats were now treated with sunflower oil and a decreased of 0.6mmol/L (1ml/100g) and 1.3mmol/l (6ml/100g) were observed and an additional decreased of 0.4mmol/L and 0.4mmol/L in the second and third week of the experiment at the dose of 1ml/100g also 0.5mmol/L and 0.9mmol/L declined in 6ml/100g dose.

The soybean oil group exhibited the least potency in lowering FBG level among the oils included in the study. From 3.4mmol/L and 4mmol/L in first FBG determination it increased to 7.2mmol/L and 6.9mmol/L after injection of ALX. During the period of experiment a 1.4mmol/l reduction in FBG level was observed in 1ml/100g dose and 1.6mmol/L in 6ml/100g dose.

The group treated with corn oil has the greatest efficacy in lowering fasting blood glucose level. From 3.2mmol/L and 4.1mmol/L it increased to 8.2mmol/L and 9.7mmol/L after administration of ALX. During the first week of experiment at a dose of 1ml/100g the FBG decreased by 2.2mmol/L, 0.3mmol/L in the second week and 0.5mmol/L in the last week with a total decline of 3mmol/L. At a dose of 6ml/100g from FBG level of 9.7mmol/L it reduced to 5.2mmol/L in the first week of the experiment then decreased to 5.1mmol/L and no change was noted in the third week. Total decline is 4.6mmol/L. Even though it showed no decline on the third week it exhibited the maximum potency in lowering FBG level.

The fifth group was treated with glibenclamide and served as the positive control of the experiment. Induction of diabetes is done by injecting ALX followed by FBG determination same with the other groups. As expected with the positive control, it lowered the FBG level starting from the first week up to the last week of the experiment. It decreased the FBG level by 1.6mmol/L (1ml/100g) and 5.4mmol/L (6ml/100g) during the whole period of the experiment.

CONCLUSION

Corn oil produced the highest hypoglycemic effect on the fasting blood glucose (FBG) levels of laboratory animals at a concentration of 1ml and 6ml/100g body weight followed by sunflower and soybean oil. Statistical analysis of the experimental data however revealed that there is no significant differences on the effect of 1ml/100mg and 6ml/100mg of sunflower, soybean, corn oil and glibenclamide at <0.05 level of significance This implies that the oils used in the study exhibited hypoglycemic properties similar to that of glibenclamide and therefore can be used as an adjunct nutraceutical to lower blood glucose levels

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