EFFECT OF INCORPORATION OF FENUGREEK SEED ON GLYCEMIC INDEX OF CHAPATI

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2021

Effect of Incorporation of Fenugreek Seed on Glycemic Index of *Chapati*

A dissertation submitted to department of Nutrition and Dietetics, Central Campus of Technology, Tribhuvan University, in partial fulfillment of requirement for the degree of B.Sc. Nutrition and Dietetics

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March, 2021

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Approval letter

This ***dissertation*** entitled ***Effect of Incorporation of Fenugreek Seed on Glycemic Index of Chapati*** presented by **Usha Gartoula** has been accepted as the partial fulfillment of the requirement for the **B.Sc. Nutrition and Dietetics**

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Acknowledgements

First and foremost, I thank my supervisor, Dambar B. Khadka (Assistant professor, Central Campus of Technology and Head of department, Nutrition and Dietetics), for his excellent guidance, valuable suggestions, motivation and technical support throughout the work. I must also thank him for the trust, inspiration, and opportunities I have been given throughout the work, for which I am deeply grateful.

Furthermore, I am very much indebted to thank Dr. Dil Kumar Limbu, Campus Chief, Central Campus of Technology, for enormous technical and administrative support in my academic and research work.

I take this opportunity to express gratitude to all of the Department faculty members, batch mates and juniors of Nutrition and Dietetics for their help and support. I also thank my parents and seniors for the continuous encouragement, support and attention. I am too much grateful to all the volunteers participating in GI testing and my dear friend Aakansha, Manjushree, Sadiksha, Tripura and Rejina who helped me with my lab works. I also want to thank all the laboratory staffs, library staffs and all the respected teachers who helped me in my research work. I also express my sense of gratitude to one and all, who directly and indirectly, helped me during my research work.

Date of submission: - March, 2021

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Abstract

This study was aimed to determine whether fenugreek seed powder and fenugreek seed extract could reduce the glycemic response when added to chapati. Through sensory analysis, 7.5% fenugreek powder incorporated chapatti and 10% fenugreek extract incorporated chapatti was found acceptable to taste. 9 healthy human subjects were given glucose (reference food, twice), chapatti (0 %, 7.5 % fenugreek seed powder and 10 % fenugreek seed extract) on 5 different occasions after 10-12 hours fasting. All the test food contained 50 g carbohydrate. Finger prick blood samples were collected at 0, 15, 30, 45, 60, 90 and 120 min after the start of the meal using glucometer.

Addition of 7.5 % fenugreek powder lowered the glycemic index of chapati by 29.8% and glycemic load by 46.72%. Addition of 10% fenugreek seed extract lowered the glycemic index by 2.9% and glycemic load by 12.9%. The glycemic index of chapati with 7.5% fenugreek seed powder (50.71±10.83) is significantly different ($p < 0.05$) than that of control (72.24±6.04). But there is no significant difference ($p > 0.05$) in glycemic index of chapati with 10% fenugreek extract (70.14±8.98) and control. Glycemic load of fenugreek powder incorporated chapati (7.31±1.56) and fenugreek extract incorporated chapati (11.94±1.53) is significantly different from that of control (13.72±1.14). This present finding indicates that glycemic response of chapati can be improved by incorporating fenugreek seed.

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List of Abbreviations

Abbreviations	Full form
BGL	Blood glucose level
IAUC	Incremental area under curve
GL	Glycemic load
GI	Glycemic index
FBG	Fasting blood glucose level
BMI	Body mass index
DF	Degree of freedom
SD	Standard deviation
WHO	World health organization
FAO	Food and agriculture organization
HDL	High density lipoprotein
LSD	Least significant difference

Part I

Introduction

1.1 Background

In present context, there is increased incidence of various chronic metabolic disorders like diabetes, obesity, dislipidemia, cardiovascular disease etc. Diet has been recognized as a corner stone in management of such health condition. Foods such as rice, bread, noodles are part of the cultural identity, meal, and habits (Knight, 2011). Completely removing carbohydrates or implementing low-carbohydrate diets is also not recommended as other health functions may be impaired (Sheard et al., 2004), and Replacement of carbohydrates with animal-based protein and fats may have even more adverse health effects (Seidelmann et al., 2018). Hence, an effective approach toward managing metabolic disorders in Asia would be to improve carbohydrate quality in addition to reducing carbohydrate quantity. Carbohydrate quality and quantity can be represented by the concepts of GI (T. Wolever, 2013).

The GI is a property of the carbohydrates in different foods, specifically the blood glucose-raising ability of the digestible carbohydrates (T. M. S. Wolever et al., 2003). When combined in actual meals, low GI foods produce less fluctuation in blood glucose and insulin levels than high GI foods (Brouns et al., 2005). Recently several studies have explored the ability of several functional food to lower the glycemic index and fenugreek seed have shown similar effect (Wani & Kumar, 2018).

Fenugreek (*Trigonella foenum-graecum L.*) is known to have hypoglycemic, and hypocholesterolaemic effects, Anti-inflammatory effects (Didarshetaban & Saeid Pour, 2013). In the study Losso et al. (2010) several commercial prototypes of fenugreek enriched cereal products, including bread, breakfast cereal, flakes, tortillas, cookies, pastas and other pastries, as functional food for patients with DM or for individual who likes to eat healthy. These products have been very well accepted by consumers through clinical and consumer trial. Since chapati is the most commonly consumed food in South Asia, we incorporated fenugreek in chapati for our study.

Fenugreek has lowering effect on glycemic index when added to rice and wheat diets, due to delayed gastric emptying and increased transit time. In addition fenugreek decrease

glucose absorption and inhibits starch digestion due to the presence of soluble fiber and galactomanns (Sampath Kumar et al., 2011). Some studies even report that water extract of fenugreek shows hypoglycemic effect in rats (Puri et al., 2002). On long term consumption it is also proven to improve insulin sensitivity in diabetic human beings. The mechanism of action and the component of fenugreek responsible for producing hypoglycemic effect are still unclear.

1.2 Statement of problem

Elevated blood glucose or hyperglycemia is a pathological condition which may occur among healthy individuals due to frequent consumption of foods with high GI. Hyperglycemia, if not well controlled by diet, can lead to prediabetes and diabetes mellitus (Nyenwe & Dagogo-Jack, 2011). Recently, several research studies have explored the ability of functional foods to control hyperglycemia (Deng, 2012). Fenugreek seed, a commonly used spice, is a functional ingredient which may have the ability to depress the surge in the postprandial blood glucose and increase satiety (Robert et al., 2014). The ability of fenugreek to reduce glycemia is due to its rich content of the viscous dietary fibre galactomannan (Madar & Shomer, 1990).

Studies by Xue et al. (2007), Hasan and Rahman (2016) and Yadav et al. (2008) showed that water extract of fenugreek can also produce hypoglycemic effect. The exact component and the possible mechanism for producing hypoglycemic effect are still unclear. Chapati is the most commonly consumed food around the world where individual can easily consume at least 2 slices of bread a day. To establish the practicability of extending the use of fenugreek seeds as functional food, the study was conducted by adding fenugreek powder and extract in chapati made from wheat flour and change in glycemic index and glycemic load were observed.

1.3 Objective

1.3.1 General objective

To determine the effect of incorporation of fenugreek seeds powder and water extract of fenugreek on glycemic index of chapati.

1.3.2 Specific objectives

- To prepare fenugreek seeds powder and prepare chapati by incorporating it.
- To prepare water extract of fenugreek seed and use it in preparation of chapati.
- To conduct sensory analysis to determine the best combination for fenugreek seed incorporated chapatti.
- To determine the proximate constituents of prepared chapatis.
- To determine the GI and GL control, fenugreek powder incorporated chapati and fenugreek extract incorporated chapati.
- To compare the GI and GL of fenugreek extract incorporated chapatti and fenugreek powder incorporated chapatti with that of control.

1.4 Significance of study

Various metabolic disorders like diabetes, dislipidemia etc. have become a growing issue in present context. Low glycemic index food is proven to be beneficial for those disorders. Thus strategy to reduce GI of food has become the matter of concern to all. In such this study will help to determine the glycemic index of chapati made from wheat flour incorporated with fenugreek seed's powder and water extract of fenugreek. Since this research includes easily available and commonly consumed spice fenugreek incorporated in the most common staple food of Asians, practicability of this study is higher. It not only proves the hypoglycemic effect of fenugreek but also allows comparing the effect of fenugreek powder and fenugreek extract. Sensory analysis would determine the acceptable quantity of fenugreek seed incorporation in chapati and GI determination would provide evidence of hypoglycemic effect. Thus, study would be practicable in every household to minimize the risk related to metabolic disorders. Further analysis to determine the component responsible for hypoglycemic effect can be done. This research can also be further continued by varying the food to which fenugreek is incorporated. The long term effect of fenugreek consumption in specific disease condition and its response in insulin level can also be studied. Thus this research could be beneficial for both general people and researchers.

1.5 Limitation of study

- This study does not show how varying dose effect the glycemic response.
- This study does not show the long term effect of fenugreek consumption.
- Its effect on insulin sensitivity cannot be illustrated.
- The exact component responsible for hypoglycemic effect cannot be identified.

PART II

Literature review

2.1 Fenugreek plant and seed

Fenugreek (*Trigonella foenum graecum*) is an annual plant belongs to the family *Leguminosae.* It is the famous spices in human food. The seeds and green leaves of fenugreek are used in food as well as in medicinal application that is the old practice of human history. It has been used to increase the flavoring and color, and also modifies the texture of food materials. Seeds of fenugreek spice have medicinal properties such as hypocholesterolemic, lactation aid, antibacterial, gastric stimulant, for anorexia, antidiabetic agent, galactogogue, hepatoprotective effect and anticancer. These beneficial physiological effects including the antidiabetic and hypocholesterolemic effects of fenugreek are mainly attributable to the intrinsic dietary fiber constituent which has promising nutraceutical value. It is well known for its fiber, gum, other chemical constituents and volatile contents. Dietary fiber of fenugreek seed is about 25% which changes the texture of food. These days it is used as food stabilizer, adhesive and emulsifying agent due to its high fiber, protein and gum content. Fenugreek is known for its pleasantly bitter, slightly sweet seeds. The seeds are available in any form whether whole or ground form is used to flavor many foods mostly curry powders, teas and spice blend. Fenugreek seed has a central hard and yellow embryo which is surrounded by a corneous and comparatively large layer of white and semi-transparent endosperm (Wani & Kumar, 2018).

Dietary fiber from fenugreek blunts glucose after a meal. The mechanisms for these effects have not been fully elucidated. Fenugreek seeds contain 45.4% dietary fiber (32% insoluble and 13.3% soluble), and the gum is composed of galactose and mannose. The hypoglycemic effect of fenugreek has been especially documented in humans and animals with type 1 and type 2 diabetes mellitus (Roberts, 2011).

The chemical analysis of fenugreek seed showed that the contents of moisture, fiber, ash, protein, fat and carbohydrates were 4%, 6.50%, 3.20%, 28.55%, 4% and 62.48%, respectively. The physiochemical characteristics of fenugreek seed oil were similar to the most edible oils. The inhibitory effect of fenugreek oil was tested against four microorganisms: three bacteria: Escherichia coli, Staphylococcus aureus, and Salmonella

typhimurium and one mould: Aspergillus niger. The results indicated that the oil has a potent antimicrobial activity against all tested microorganisms. The highest antimicrobial activity among the bacteria was detected against E. coli (Sulieman et al., 2008).

2.1.1 Fenugreek production in Nepal

Fenugreek seeds commonly known as *methi* in are an indispensable spice in Nepali cooking; these brownish-yellow rectangular seeds are very hard and highly aromatic. The seeds have a very strong, unpleasantly bitter taste in the raw stage, but when cooked, they emit a wonderful aroma. Fenugreek (*Trigonella foenum graecum*) is an herbaceous plant grown in North Africa, the Middle East, and South Asia. It is drought tolerant, fixes nitrogen, can act as an over crop, and can grow in a variety of climates. Many of these benefits make fenugreek a conservation agriculture technique. Fenugreek leaves and seeds also have many flavour and nutrition properties that make it a staple in many traditional recipes and medicines.Though currently a marginal crop in Nepal, fenugreek can be grown on diverse land types: October-December in Terai, August-November in mid-hill regions and March-May on hillsides. Though it is a hillside crop, commercial production is limited to urban and semi-urban areas due to proximity to market. Resistant to climatic stress, Trigonella f. is grown in arid regions with moderate to low rainfall, with the possibility of light irrigation during germination and flowering (Bhatta et al., 2014).

2.1.2 Uses of fenugreek

In India, fresh *methi* ka saag (the stems and leaves of the plant) is very commonly cooked as a winter vegetable, and the seeds are used year round as a flavoring agent for various dishes (Wani & Kumar, 2018). In Rajasthan they are also used as food. various preparations made are plain *methi* (fenugreek curry), papad *methi* (made with pa-pad), besan (gram flour) *methi*, sweet sour *methi*, *methi raita*, *methi ladoo* and sprouted *methi* (Mathur & Choudhry, 2009). Fenugreek seeds are used as spice for flavoring selected types of cheese, mainly parmesan. Powdered or crushed seeds are added to salads and cottage cheese spreads. Fenugreek seeds are also used to flavor coffee and vanilla extracts. Fenugreek seeds are roasted for direct consumption and are added to broth and tea. Fenugreek leaves are fried in butter, added to salads and used as spice in the powdered form. Fenugreek seeds enhance the flavor and aroma of dishes (Zuk-Golaszewska & Wierzbowska, 2017). In India, normal consumption of fenugreek seeds by adults is

estimated at 0.3 to 0.6g per day. In both humans and animals, diets where the above intake levels were exceeded 50- to 100-fold delivered health benefits.

Due to rich source of natural dietary fiber in fenugreek, it has established itself in the modern food ingredient or functional food. Fenugreek as a hydrocolloid, which is fenugreek gum (soluble fiber of fenugreek), gives textural, appeal, thickening, emulsifying, stabilizing, gelling, and encapsulating properties. So the dietary fiber, more importantly soluble fiber can find their way into nutrition and dairy products, cereal bars, yogurts, and nutritional beverages. The powder of soluble fiber or total dietary fiber can be mixed with juices of fruit, seasonings and other spice mixes. Directly it can be used to formulate tablets or capsules along with the other vitamins and nutrients necessarily needed. It can also be used in milk shakes, dressings, soups, candies and sweets. It has been used to fortify bakery flour for pizza, pizza, cake mix, bread, bagel, muffins, chapati, tortilla and noodles, fried, baked corn chips. Bakery foods such as bread, pizza, cakes and muffins have been prepared by using flour fortified with eight to ten percent soluble dietary fiber. When fiber fortified flour was used for making oil fried snacks, 8–15% of less oil absorption only takes place which is really appreciable in terms of unwanted fat intake (Krishna Kumar & Maliakel, 2008).

2.1.3 Nutraceutical property of fenugreek

Fenugreek has a beneficial effect on cleansing the blood and as a diaphoretic it is able to bring on a sweat and to help detox the body. Due to pungent aroma of fenugreek, that is smelt on the skin and in under-arm perspiration. Fenugreek is also known for its lymphatic cleansing activity though its vital role is to irrigate the cells with nutrients and to remove toxic wastes, dead cells and trapped proteins from the body. Block in the lymphatic system can mean poor circulation of fluid, fluid retention, pain, energy loss and disease, anywhere in the body of a person. Fenugreek maintains mucus conditions of the body, mostly the lungs, by helping to clear congestion. It also acts as a throat cleanser and mucus solvent that also eases the urge to cough. Drinking water in which seeds of fenugreek have soaked helps in softening and dissolving, accumulating and hardening the masses of cellular debris. Fenugreek has been used to relieve colds, bronchial complaints, influenza, asthma, catarrh, constipation, sinusitis, pleurisy, pneumonia, sore throat, laryngitis, hay fever tuberculosis and emphysema (Wani & Kumar, 2018).

2.1.3.1 Lactation aid

Breasts are modified sweat glands and fenugreek has been found to stimulate sweat production as it contains hormone precursor to increase milk formation. Some scientists reported that fenugreek can increase a nursing mother's milk supply within 24–72 h after first taking the herb (Snehlata & Payal, 2012).

2.1.3.2 Immunological activity

A research work on the effect of fenugreek on stimulatory immunomodulatory effect (as evidenced from assay, phagocytosis, cellularity of lymphoid organs of body, late type of hypersensitivity response, plaque forming cell assay, a lymph proliferation and increase in phagocytic index and phagocytic capacity of macrophages significantly) of aqueous extract of fenugreek at three doses (50, 100 and 200 mg per kg) of body weight for ten days on the immune system of Swiss albino mice was studied (Meghwal & Goswami, 2012).

2.1.3.3 Hypoglycemic effect

Dietary fiber from fenugreek blunts glucose after a meal. The mechanisms for these effects have not been fully elucidated. Fenugreek seeds contain 45.4% dietary fiber (32% insoluble and 13.3% soluble), and the gum is composed of galactose and mannose. The latter compounds are associated with reduced glycemic effect. The hypoglycemic effect of fenugreek has been especially documented in humans and animals with type 1 and type 2 diabetes mellitus (Roberts, 2011).

Mechanism of action of an orally active hypoglycemic principle isolated from water extract of seeds of fenugreek was investigated in alloxan induced sub diabetic and overtly diabetic rabbits of different severity cases. Active component was orally administered to the sub diabetic and mild diabetic rabbits (five in each group) at a dose of 50 mg per kg body weight for period of 15 days, and result showed significant attenuation of the glucose tolerance curve and improvement in the glucose induced insulin response. The result suggested that the hypoglycemic effect may be mediated through stimulating insulin synthesis and/or secretion from the beta pancreatic cells. Upon prolonged administration of the same dose of the active principle for 30 days to the severely diabetic rabbits lowered fasting blood glucose significantly, but could elevate the fasting serum insulin level to a much lower extent, which suggests an extra-pancreatic mode of action for the active

principle. The effect may also be by increasing the sensitivity of tissues to available insulin. The hypoglycemic effect was observed to be slow but sustained, without any risk of developing severe hypoglycemia (Puri et al., 2002).

Impact of fenugreek incorporated therapeutic food on blood sugar levels of 24 non-insulin dependent diabetes mellitus patients was investigated in a study. A type of therapeutic food was developed from legumes viz., bengal gram, green gram, horse gram, dry peas and fenugreek seeds. An amount of 30 g of product was supplemented for a period of about one month and was found that both fasting and postprandial blood sugar levels were reduced significantly. So, it was concluded the usefulness of high fiber fenugreek diet in the management of diabetes (Kumari & Sinha, 2012).

The galactomannan-rich soluble fiber fraction of fenugreek seeds may be responsible for the antidiabetic activity. A study on animals evaluated the hypoglycemic effects of the fenugreek seeds on dogs. The seeds (defatted) lowered blood glucose levels, plasma glucagons and somatostatin levels; carbohydrate-induced hyperglycemia also was found to be reduced. Clinical analysis showed that glycemic control was improved in a small study of patients with mild type-2 diabetes mellitus. A reduction in glycosylated hemoglobin levels and increased insulin sensitivity were observed in fenugreek recipients (Snehlata & Payal, 2012).

It is possible that fenugreek lowers lipids because it contains saponins that are transformed in the gastrointestinal tract into sapogenins. Fenugreek seeds contain 25% fiber that can slow the rate of postprandial glucose absorption. This may be a secondary mechanism for its hypoglycemic effect (Basch et al., 2003).

2.1.3.4 Hypocholesterolemic effect

The abnormal deficiency of cholesterol level in the blood is known as hypocholesterolemic problem and oral administration of methanolic and aqueous extracts of seeds at a dose of one gram per kilogram body weight resulted in hypoglycemic effect in mice (Zia, 2001). Fenugreek seeds contain the large amount of fiber galactose and mannoses are the main composition of gum. The latter compounds are associated with reduced cholesterolemia (Roberts, 2011).

The fenugreek extract has been investigated for its effects on blood lipid, and in experimental rats with diabetics. The streptozotocin-induced diabetic rats were administrated by oral intra gastric intubation separately with low dose, middle dose, and

high dose of fenugreek extract, and Metformin HCl for about one and half month (6 weeks). As compared to diabetic group, rats treated with fenugreek extract had lower triglycerides, total cholesterol, and higher HDL cholesterol in a dose-dependent manner (Xue et al., 2007).

2.1.3.5 Antioxidant activity

Fenugreek seed extract with methanol, ethanol, dichloromethane, acetone, hexane and ethyl acetate has a radical scavenging activity (Bhanger et al., 2008). Fenugreek has protective effect on lipid per oxidation and on enzymatic antioxidants (Bhatia et al., 2006).

2.1.3.6 Anticancer effect

Amin et al. (2005) showed that fenugreek seed extract significantly inhibited 7,12-glutathione peroxidase in liver. A selective cytotoxic effect of fenugreek extract in vitro to a panel of cancer cell lines has been observed, including T-cell lymphoma (Alsemari, 2014). A diet containing fenugreek seed powder decreased colon tumor incidence and hepatic lipid peroxidation in 1,2-dimethylhydrazine treated rats and also increased activities of catalase, superoxide dismutase, glutathione S-transferase and fenugreek seed and its main active constituents as new supplements in diet-based preventive/therapeutic strategies to potentially alleviate human diseases remains an important field of study for future investigations (Abdelgawad et al., 2012).

2.1.3.7 Antibacterial and antifungal effect

The antibacterial and antifungal role of fenugreek is recently being shown. In a study by Haouala et al. (2008), it was found that all parts of the fenugreek plant showed antifungal potential and the magnitude of effect varies with plant parts and species of fungus. It could be suggested that fenugreek is an important source of biologically active compounds useful for developing better and novel antifungal drugs (Haouala et al., 2008). The effectiveness of extracts obtained from fenugreek against Helicobacter pylori has been reported by several studies. Laroubi et al. (2007) studied the prophylaxis effect of fenugreek seeds on renal stone formation in rats. The fenugreek can be used in the treatment of patients with calcic urolithiasis.

2.1.3.8 Digestion aid

Spices consumed in diet positively influenced the pancreatic digestive enzymes. Platel and Srinivasan (2000) experimentally showed that capsaicin, piperine, dietary curcumin, ginger, fenugreek and asafetida prominently enhanced pancreatic lipase activity in rats, on feeding rats with spicy diets for eight weeks. Non-starchy polysaccharides increase the bulk of the food and increase the bowl movement. Also, non-starchy polysaccharides assist in smooth digestion whereas high fiber of fenugreek helps in relieving constipation ailments.

2.1.4 Fenugreek extracts

In study conducted by Yadav et al. (2008), five extracts of fenugreek seed with different solvents (water, ethanol, methanol, hexane and chloroform) alone and in combination with glimepiride were tested for hypoglycemic and anti-hyperglycemic activity in rats by screening blood glucose for 6 h. Water extract exhibited highest hypoglycemic and anti-hyperglycemic activity in rats among all the extracts, while hexane and other extracts exhibited least and moderate activity, respectively. Water extract was further studied to dose dependent [200, 100 and 50 mg kg^{-1} body weight] hypoglycemic and anti-hyperglycemic effects alone and in combination with glimepiride (20, 10 and 5 mg kg^{-1} b.wt.). The combination of water extract (200 mg kg^{-1} b.wt.) and lower dose of glimepiride (5 mg kg^{-1} b.wt.) has shown safer and potent hypoglycemic as well as anti-hyperglycemic activity and not created severe hypoglycemia in normal rats, while higher doses (200 mg kg^{-1} b.wt. of water extract and 10 and 20 mg kg^{-1} b.wt. of glimepiride) were generated lethal hypoglycemia in normal rats. The results of present study enforced to say that, the water extract of fenugreek seeds has higher hypoglycemic and anti-hyperglycemic potential and may use as a complementary medicine to treat the diabetic hyperglycemia by significantly reducing dose of standard drugs.

Hasan and Rahman (2016) observed that10 g/40ml liquid dosage form of fenugreek seed has a significant effect on reducing the blood sugar level and it is proven as better than other dosage form of fenugreek. From some other study it has been seen that it requires minimum ten consequent days to have a physical effect of fenugreek in case of other dosage form like tablet, capsule, crushed seeds, powders etc. But we have observed effect within five days which ensures the outstanding strength of fenugreek liquid dosage

form.

Fenugreek seeds have previously been shown to have hypoglycemic and hypocholesterolemic effects on type 1 and type 2 diabetes mellitus patients and experimental diabetic animals. The fenugreek extract has now been investigated by Xue et al. (2007) for its effects on general properties, blood glucose and blood lipid, and hemorheological parameters in experimental diabetic rats. Streptozotocin-induced diabetic rats were administrated by oral intragastric intubation separately with low dose (0.44 g/kg.d), middle dose (0.87 g/kg.d), high dose (1.74 g/kg.d) of fenugreek extract, and Metformin HCl (0.175 g/kg.d) for 6 weeks. Compared with diabetic group, rats treated with Trigonella foenum-graecum extract had an increase in body weight and a decrease in kidney /body weight ratio.

2.2 Wheat

Wheat is an annual grass growing to between ½ to 1 ¼ meters in height, with a long stalk that terminates in a tightly formed cluster of plump kernels enclosed by a beard of bristly spikes. It is grown all over the world for its highly nutritious and useful grain, as one of the top three most produced crops, along with corn and rice . It is used in the production of bread, biscuits, feeds, confectionary, amongst much utilization (Oyewole, 2016).

2.2.1 Fenugreek suitability in wheat flour products.

Kasaye and Jha (2015) assessed flour of fenugreek supplemented at 5, 10, and 15% levels with wheat flour for the production of bread and biscuit to improve nutritional profile. The nutritive values in terms of protein, fiber, ash as well as calcium, magnesium, iron and zinc content of bread and biscuit increased as a result of the supplementation of germinated fenugreek flour to wheat flour. The sensory evaluation of the bread samples made by supplementation at 5 and 10% and 5% levels for biscuit were rated acceptable.

In the study conducted by Hooda and Jood (2004) Wheat flour was separately substituted with fenugreek flour (raw, soaked, and germinated) at 5–20% levels for product making. Nutrient analysis of the blends, product development, and their acceptability were carried out. Replacement of wheat flour with fenugreek flour increased the protein, fat, lysine, minerals, and dietary fibre contents proportionately to the level of substitution. Among the composite flours, the blends containing germinated fenugreek flour were found superior in nutritional quality compared to others. However, products, viz., bread, biscuits,

noodles, and macaroni prepared from the wheat–fenugreek blends at 10, 15, and 20% levels, were found organoleptically acceptable.

Hooda and Jood (2005) prepared biscuits from the blends containing different proportions (0%, 5%, 10%, 15% and 20%) of raw, soaked and germinated fenugreek seed flour were evaluated for width, thickness, spread ratio and sensory characteristics. The thickness of fenugreek supplemented biscuits increased, whereas width and spread ratio of biscuits decreased with the increasing level of fenugreek flour. The sensory results showed that a maximum of 10% fenugreek flour can be incorporated to prepare acceptable quality biscuits. Addition of raw, soaked and germinated fenugreek flour to wheat flour increased the contents of protein (10.5%, 10.4% and 11.0%), lysine (2.15, 2.20 and 2.25 g/100 g protein), dietary fibre (12.7%, 11.3% and 10.9%), total Ca (58.3, 57.1, 57.7 mg/100 g) and total iron (7.40, 7.26 and 7.36 mg/100 g), respectively, at 10% level of substitution. These biscuits can be safely stored in polypropylene bags upto 1 month without altering their organoleptic properties.

Al-Gemeai conducted a study with the objective to evaluate the effect of fortification of biscuits with 10%, 20% and 30% of fenugreek seed that germinated for 2, 3, 4, and 5 days. The Organoleptic properties results showed that a maximum of 10% germinated fenugreek flour for 2 and 3 days can be incorporated to prepare acceptable quality biscuits.

2.2.2 Chapati

Chapati is a baked product made from whole wheat flour, is the staple diet of majority of the population of India and its subcontinent. Whole wheat flour is mixed with water into a dough and the dough is normally given a minimum rest period of 15-30 min, before it is sheeted to a thickness of about 2 to 3 mm. The dough thus sheeted is cut into a diameter of 12 to 15cm and baked on a hotplate at 220°C and finally puffed on a live flame for few seconds. It is generally consumed hot along with other adjuncts. Complete and full puffing, soft and pliable textures as well as whitish brown color with dark brown spots are some of the important attributes of good quality chapati. The amount of water used during chapati production depends on the type of flour and bread. Approximately 50% water results in finely textured, light chapati (Mir et al., 2014).

2.3 Glycemic index and glycemic load

Elevated blood glucose or hyperglycemia is a pathological condition which may occur among healthy individuals due to frequent consumption of foods with GI. Hyperglycemia, if not well controlled by diet, can lead to prediabetes and diabetes mellitus (Kolb & Eizirik, 2011; Nyenwe & Dagogo-Jack, 2011).

The GI is defined as the incremental blood glucose area following the test food, expressed as the percentage of the corresponding area following a carbohydrate equivalent load of a reference product. With white bread as a reference, GI ranges from less than 20 to approximately 120 percent (initially, the reference food was glucose, but more recently it has been white bread) (Jenkins et al., 1981).

The food may be classified into one of three categories depending on its mean GI value. Foods are classified as low GI if the GI value is less than 55; moderate GI if between 55-69; and high GI if greater than 70. The categories are arbitrary and do not necessarily related to healthy food choices. For example, foods with a high sugar, high fat content such as ice-cream, have a low GI, whereas many fruits have a medium GI. Carbohydrates that broke down quickly during digestion, releasing glucose quickly into the blood have the high GI. Carbohydrates that broke down slowly, releasing glucose gradually into the blood have low GI. Foods with high GI are rapidly digested, absorbed and result is marked fluctuation in blood sugar level. Thus, foods with high glycemic indexes are proposed further as a dietary factor that favors the development of chronic disease (Jenkins et al., 2002; Teixeira et al., 2008) . Low GI foods, produce gradual rise in blood sugar and insulin levels and have proven benefits for health (Mulholland et al., 2009; van Baak & Astrup, 2009) . Low GI diets have been shown to improve both glucose and lipid levels. Also reduce insulin levels and insulin resistance (Pal et al., 2008). They have benefits for weight control because they help to control appetite and delay hunger (Philippou et al., 2009).

The glycemic load (GL), which assesses the total glycemic effect of the diet and proves very useful in epidemiological studies, is the product of the dietary glycemic index and total dietary carbohydrate (Liu et al., 2000). Glycemic load represents both quality and quantity of carbohydrates and interaction between the two. One unit GL is approximately equal to glycemic effect of 1g glucose (Beulens et al., 2007). Typical diet has approximately 100 GL units per day. The range varies from

60-180. GL provides a measure of total glycemic response to a food or meal. The University of Sydney defines low, medium and Glycemic loads as follows: Low Glycemic load: 0 to 10. Medium Glycemic load: 11 to 19. High Glycemic load: 20 and over. Table 2.1 shows glycemic index and glycemic load of some commonly consumed foods.

Table 2.1 Glycemic indexes and glycemic load of some commonly consumed foods

Food	Glycemic index	Serving size	Glycemic load
Corn flakes	93(high)	30g	23(high)
Milk	41(low)	250ml	5(low)
Instant rice	89 (high)	150g	43(high)
Ice cream	57 (medium)	50g	6(low)
Coca cola	63(medium)	250ml	16(medium)
Wheat bread	71(high)	30g	10(low)
Carrot	35(low)	80g	2(low)
Spaghetti	46(low)	180g	22(high)
Apple	39(low)	120g	6(low)
Lentil beans	29(low)	150g	5(low)
Honey	61(medium)	25g	12(medium)

(Atkinson et al., 2008)

2.3.1 Measuring the GI

To determine a food's GI rating, measured portions of the food containing 10-50 g of carbohydrates are fed to 8-12 healthy people after an overnight fast. Finger prick blood samples are taken at 15-30 minutes intervals over the next two hours. These blood samples are used to construct a blood sugar response curve for two hours period. The area under the curve is calculated to reflect the total rise in blood glucose level after eating the test food. GI rating in percentage is calculated by dividing the area under the curve for the test food by the area under the curve for the reference food (having same amount of carbohydrates) and multiplying by 100 (Arya & Shalini, 2009). The incremental area under the blood glucose response curve (IAUC) is the sum of the areas between blood collection time-points calculated using the trapezoidal rule. Areas below baseline are ignored. The equation given by T. M. Wolever and Jenkins (1986) is as follows:-

$$\text{Area} = \left(A + B + C + \frac{D}{2}\right)t + \frac{(D + E)T}{2} + \frac{E^2T}{2(E + F)}.$$

Where A,B,C,D and E represent positive blood glucose increment values from baselines, F equals the first negative increment value, t equals the 15-minute time interval between blood samples and T is the 30-minute time interval (T. M. Wolever & Jenkins, 1986). The blood glucose concentration often falls below fasting levels, which results in a negative blood glucose increment value.

After launching the concept of GI, several studies have used various procedures for measuring GI values. Typically, the protocol used has been adapted from the original procedure described by (T. M. S. Wolever et al., 2003) , which is in line with the protocol recommended by the FAO/WHO. The FAO/WHO expert report, published in 1998, has been referred to as the international standard. The protocol of determining GI values based on the recommendation is briefly summarized as follows FAO/WHO (1998):

- At least 8 - 10 subjects should be studied.
- The portion of the study meal (the test or the reference food) should contain 50 g of available carbohydrate, and it should be given to the participant after a 10 to12 h overnight fast.
- The study meals should be tested in random order on separate days.
- The reference food can be either white bread or a glucose solution, and the reference food should be tested at least two times in each subject.
- Either capillary or venous blood sampling can be used.

Recent recommendations suggest that the amount of available carbohydrate be 25 g with foods having a low carbohydrate density to avoid a large test meal size (Brouns et al., 2005). The recommendation of the FAO/WHO (1998) also demonstrates how the GI can be applied to mixed meals or diets by calculating the weighted GI value of a meal or diet.

2.3.2 Effect of glycemic index on health

Two studies (one that used the third National Health and Nutrition Examination Survey database and the other a British study) showed a negative relation between

GI and high density lipoprotein (HDL) cholesterol, suggesting that low GI diets may preserve HDL cholesterol and have a potentially positive effect in reducing CHD risk (Jenkins et al., 2002; Parikh et al., 2005). After consuming a low GI diet for 1 month, patients with hyperlipidemia showed reduced low density lipoprotein (LDL) cholesterol and triacylglycerol concentration (in those with higher triacylglycerol concentration), despite no significant difference in body weight (Jenkins et al., 1987).

In relation to diabetes outcome, health professional's studies showed an inverse relation between GI and the risk of developing diabetes by using a validated food-frequency questionnaire. In addition, increased food frequency, as a model for mimicking the slow digestion of low GI food, has been shown to reduce glycemic and insulinemic responses over the course of a day in diabetic subject (Jenkins et al., 1992). GI may have relevance to cancer prevention. In addition, insulin resistance and insulin like growth factors have been implicated in the so-called diet related cancers: colon, breast, and prostate. A case control study showed a direct association between dietary GI and colon cancer risk. A sedentary lifestyle in conjunction with a high GI diet increase risk relative to a sedentary lifestyle with a low dietary GI an Italian case control study reported that the dietary GI was related to colorectal cancer. The same relation of GI and disease was also shown for breast cancer. Prostate and ovarian cancers, among other forms of cancer, may be influenced by the dietary GI (Jenkins et al., 2002).
Some studies that used low GI meals showed an improved second meal carbohydrate tolerance that reminiscent of the Staub-Traugott effect (i.e. in which the first meal improves the glucose tolerance of the second meal) and related the improved postprandial glycemia of the second standard meal to lower FFA concentrations (Jenkins et al., 1982) . The cause of this second meal effect is probably that a prolong absorption phase following breakfast will favor a more efficient suppression of free fatty acids, thus improving insulin sensitivity at the time of the next meal. This mechanism has been implicated as partly responsible for the long-term benefits of low GI foods (Bjorck et al., 2000) .

2.3.3 Factors affecting glycemic index

The various factors affecting glycemic index according to Thorne et al. (1983) are as follows:-

- Gelatinization of starches: - Gelatinization of starches occurs when the starchy food is exposed to liquid and heat (i.e. cooking). The water binds with the starch in the presence of heat and expands the starch granules.
- Particle size: - Intact grains such as whole wheat, barley, whole corn and whole rye have much lower GI values than flours (tiny particles) made from the same grains.
- Processing: - Milling, beating, grinding, mixing, mashing and refining foods raise the GI of that food. For this reason most processed foods will have a higher GI than the same unprocessed food.
- The chemical composition of the starch: - Starches, such as rice, can have different types of starch structures which affect their digestibility. Some types of rice such as Basmati rice have higher amylose content. Amylose is made up of long straight chains of glucose molecules which are packed closely together, which are more difficult to digest. Other rice, with higher amylopectin content, is much easier to digest and thus has a higher GI. Amylopectin are branched chains of glucose that do pack closely together and are thus much less dense and easier to digest.
- Fibre: type and content: - Foods containing soluble fibre, such as oats and legumes, have a lowering effect on the GI because they delay gastric emptying. Insoluble fibre found in digestive bran, on the other hand, has very little effect on the digestibility and absorption of the carbohydrate foods. For example South African standard brown bread and white bread both have high GI values.
- Protein and fat: - The presence of protein and fat in food may lower the GI. However, it is not advisable to add fat to lower the GI of foods for health reasons. Excess protein tends to wear out the body's insulin; and fat has the effect of decreasing the effectiveness of insulin. Protein also overtaxes the kidneys and high protein intakes can lead to osteoporosis, arthritis and gout.
- Anti nutrients: - Phytates, lectins and polyphenols (tannins) normally slow digestion and thereby decrease the GI. These are found in many vegetables and fruits.
- Acidity: - Higher Acidic foods have lower GI. For example, beetroot salad with a vinegar dressing will have a lower GI than hot cooked beetroot without the dressing.
- Cooking: - Cooking usually increases the digestibility of the food, and would raise the GI of that food.

- Resistant starch: - When starches are cooked and then cooled, the crystalline structure within the food changes to resistant starch which is more difficult to digest. Thus cold cooked starches, (e.g. boiled, cold potatoes in a potato salad) have a lower GI.
- Speed of eating: - Studies have shown that blood glucose levels rise less rapidly when eating more slowly.

PART III

Materials and methods

3.1 Materials required

3.1.1 Fenugreek seeds

250g Fenugreek seeds were purchased from Baraha department store, Dharan during the month of September, 2020. It is also known as *methi*. It was manufactured by Goyal Masala and food products with the brand name *Cookme*. It was packaged on august 2019 and was best for use before 9 months from the date of packaging.

3.1.2 Wheat flour

5 kg packet of wheat flour was purchased from Baraha department store Dharan with brand name *Hulas* during the month of September, 2020. It is commonly known as *aata.* According to the labeling in the package, it was manufactured in august 25, 2019 and was suitable for consumption till 120 days of manufacture. Nutritional facts per 100 gm included carbohydrate (70-72%), protein (7-10%), fat (1.7%), minerals (2.7%), fiber(1.9%), calcium (48 mg), iron (4.9 mg), moisture (12%) and energy (341 kcal).

3.1.3 Chemicals and reagents

All chemicals and reagents required for analysis were provided by Central campus of technology, Dharan. Chemicals used include Diethyl ether or petroleum ether, Acetone, H_2SO_4, NaOH, Phenolpthalein indicator, Methyl orange indicator, Boric acid, mixed indicator solution (Bromocresol green & methyl red indicator), Catalyst mixture (SeO_2, K_2SO_4 & $CuSO_4.5H_2O$) and HCl.

3.1.4 Glasswares and equipment

Standardized and calibrated glass ware and equipments were used.

- Dr Morpen gluco one Glucometer,
- Lancets,
- Blood glucose test strips,
- Cooking arrangements,

- Hot air oven,
- Desiccators,
- Soxhlet Extraction apparatus,
- Buchner filter assembly,
- Crucible,
- Suction pump,
- Muffle furnace,
- Whatman filter paper,
- Kjeldhal digestion and distillation set,
- Titration equipment,
- Glass material equipment,
- Heating arrangement,
- Weighing balance,
- Ashless filter paper,
- Refrigerator
- Water bath

All the equipment facilities were provided by Central Campus of Technology (CCT), Dharan-14.

3.2 Methodologies

3.2.1 Processing of raw material

- Fenugreek seeds were cleaned manually for the presence of any extraneous material. Then it was subjected to solar drying for 3 to 4 hours and grinded to powder in electric grinder.
- For preparing water extract of fenugreek seeds, the fenugreek seeds powder were boiled in water in 1:10 ratio (g/ml) in water bath at 100ºc for 1 hour, then it was filtered by using Buchner funnel.

3.2.2 Formulation and Experimental design

Using design expert, 5 samples of each fenugreek powder incorporated chapati and fenugreek extract incorporated chapati were made which was then coded alphabetically as

given in table 3.1. In the studies Kasaye and Jha (2015), Hooda and Jood (2005) andAl-Gemeai maximum of 10g fenugreek have shown to produce better oraganoleptic quality of product made from wheat fenugreek blend. Thus variations were determined by keeping the fenugreek powder concentration 0g as minimum and 10g as maximum. The variations in extract were made by differing the amount of fenugreek powder used for preparation of extract where water was added in the ratio 1:10(w/v). Table 3.1shows different formulations for fenugreek powder and extract incorporated chapati.

Table 3.1 Different formulation of chapati

Fenugreek powder incorporated chapati				**Fenugreek extract incorporated chapati**			
Code	Wheat flour(g)	Fenugreek powder	Water(ml)	Code	Wheat flour(g)	Extract with fenugreek powder concentration	volume makeup (ml)
A	100	0	50	V	100	0	50
B	97.5	2.5	50	W	100	2.5	50
C	95	5	50	X	100	5	50
D	92.5	7.5	50	Y	100	7.5	50
E	90	10	50	Z	100	10	50

3.2.3 Preparation of chapati

Chapati was prepared as suggested by Mir et al. (2014) control chapati was prepared by mixing wheat flour and warm water in the ratio of 2:1(gm/ml). Then, mixture of flour and water was kneaded to soft dough. The dough was covered by soft cloth and left for 30 min. The dough was kneaded again and converted to small balls. Each ball were pressed into a flat circle, dipped into dry wheat flour so that the flour coated both sides, rolled into a flat disc, placed on a preheated pan and puffed over the live flame. To prepare fenugreek powder incorporated chapati, the wheat flour was mixed with fenugreek seed powder in different proportion and kneaded with warm water in ratio (2:1) and chapati was made similarly. For fenugreek extract incorporated chapati, fenugreek extract prepared was made up to the required volume with warm water and used for kneading the dough and chapati was prepared in similar way.

3.2.4 Sensory evaluation

Sensory evaluation was performed by ranking test for overall acceptance. The evaluation was carried out by 10 panelists. Sensory evaluation was carried out in individual booth with adequate light and free from obnoxious odors. Each panelist was provided with samples coded random numbers and evaluation card. They were provided with potable water for rinsing after tasting each sample. Verbal communication among the panelist was prohibited. Panelist evaluated the sample for overall acceptability (according to bitterness) and ranked them from 1 to 5 giving rank 1 to the best one (Rangana, 1986). The result was then taken for statistical analysis.

3.2.5 Proximate analysis

The proximate analysis of both fenugreek powder incorporated chapati and fenugreek extract incorporated chapati with the best formulation obtained after sensory analysis along with the control sample was done. Chemical composition of samples for moisture, ash, crude protein, crude fat, and crude fiber contents were determined as per the methods described by Rangana (1986). Total carbohydrate contents was determined by indirect method (subtracting the sum of all other contents from 100 g sample)

3.2.5.1 Determination of moisture content

The moisture content of the selected samples was done by hot air oven method. The sample was weighed before and after drying and the difference in weight was measured and expressed in percentage.

3.2.5.2 Determination of total ash

The total ash content was determined by incinerating the samples in muffle furnace at temperature not exceeding 525ºc for 5-6 hours. The weight of ash was measured and data were presented as percentage dry basis.

3.2.5.3 Determination of crude fat

The fat content was determined by using soxhlet apparatus. The calculated data were presented as percentage dry basis. Crude fat content is determined by extracting the fat from the sample using a solvent, then determining the weight of the fat recovered.

3.2.5.4 Determination of crude fiber content

Crude fiber was determined gravimetrically after chemical digestion and solubilization of other materials present. The results were expressed in percentage. During the acid and subsequent alkali treatment, oxidative hydrolytic degradation of the native cellulose and considerable degradation of lignin occur. The residue obtained after final filtration is weighed, incinerated, cooled and weighed again. The loss in weight gives the crude fiber content.

3.2.5.5 Determination of protein content

The protein content was determined by kjeldahl method. The results were expressed in percentage dry basis. In this method, the total nitrogen in the sample is liberated at high temperature, the nitrogen is released into a strong acid and the content is measured after neutralization and titration. Following the nitrogen determination, crude protein content is calculated using a conversion factor.

3.2.5.6 Determination of total carbohydrate content

Total carbohydrate contents of samples will be calculated by difference method as follows:
% Carbohydrate = 100 – (% Moisture + % Ash + % Protein + % Fiber + % Fat)

3.2.6 Determination of glycemic index

3.2.6.1 Standardization of Glucometer

Standardization of glucometer was done by correlating the value of blood glucose obtained from lab test and that from glucometer. For this a blood glucose testing lab was visited in early morning and with the help of the lab technician and other staff, people visiting the clinic on the particular day for blood glucose monitoring were tested by both using glucometer and laboratory analysis. The blood samples for both the test were drawn at same time. The results were then statistically analyzed for determining the accuracy.

3.2.6.2 Selections of subjects

Nine healthy individuals between the ages of 21 and 30 years and BMIs between 18.5 and 24.9 kg/m^2 were selected for this study. Recent medical reports were studied to make sure that subjects do not have cardiac disease, diabetes mellitus, and thyroid problems.

Individuals with wheat allergy, smokers, alcohol consumption or those taking drugs that can influence the blood glucose were excluded from participating. The subjects were students of central campus of technology. The consent of the subject was considered. Subjects were not allergic to the test foods i.e. wheat flour and fenugreek was confirmed by past histories of their dietary intake.

3.2.6.3 Experimental setup

Glycemic index test was done following the protocols of FAO/WHO (1998). Subjects were studied after 10–12 h overnight fasts. Study subjects arrived at the dietetics laboratory at 7 am with an interval of 2 days between each experiment. Subjects were allowed to rest for 10 min before starting the experiment. Fasting finger prick blood sample was tested and then one of the test foods was ingested within 10–13 min; further finger prick blood samples were tested at 15, 30, 45, 60, 90 and 120 min after the first bite of the food. Glucose (50 g of Glucolin™ dissolved in 250 ml water) was the reference food. Glucose was tested twice by each subject. The test foods would consist of control chapati, fenugreek powder incorporated chapati and fenugreek extract incorporated chapati. The amounts of test food given to the subjects were calculated in such a way that each contained 50 g carbohydrates and were served with 250 ml of water. Capillary blood samples were analyzed for plasma blood glucose using Dr Morpen Gluco one glucometer.

3.2.6.4 Data collection and analysis

The data obtained from all the subjects at different time interval was recorded, after the intake of glucose, control, fenugreek powder incorporated chapati and fenugreek extract incorporated chapati separately. The average of data obtained from all subjects at each time interval was calculated. The average glucose response was plotted in the graph against the time interval. Area under the curve was calculated geometrically, ignoring the area below fasting level. Area under the curve would be calculated for reference food (i.e. glucose), control sample (chapati), fenugreek powder incorporated chapati and fenugreek extract incorporated chapati. The area under the curve gives the glucose response of each food. Now the glycemic index was calculated by the formula,

$$\text{Glycemic index} = \frac{\text{glusose response of test food}}{\text{glucose response of standard food}} \times 100$$

The amount of carbohydrate present in one serving of chapati was calculated by unitary method and Glycemic load was determined using formula,

$$\text{Glycemic load} = \frac{\text{GI} \times \text{carbohydrate content(g) per portion}}{100}$$

In this way glycemic index and glycemic load of control, fenugreek powder incorporated flatbread and fenugreek extract incorporated flatbread was calculated. Then, the result would be compared for determining the change in glycemic index and glycemic load on incorporation of fenugreek seed powder and extract on chapati. The data was analyzed statistically using SPSS version 20.

3.2.7 Logistical and Ethical considerations

Ethical approval was taken from the Nepal Health Research Council (NHRC), a statutory and autonomous body, under the government of Nepal for conducting the research. Written permission was taken from the Department of Nutrition and Dietetics, Central Campus of Technology to conduct the research. Verbal permission along with written Consent was taken from the Subject to obtain the blood samples and other necessary information .The purpose of the study was informed to participants prior to the study. Privacy and confidentiality of the respondents will be maintained throughout and after the study period.

Part IV

Results and discussion

A locally available and commonly consumed spice, fenugreek seed was used in this study. Here fenugreek seed powder and fenugreek seed extract was incorporated in chapati which is a common staple food in Asia. And their nutritional value, overall acceptability, glycemic index and glycemic load was determined.

4.1 Raw material analysis

All the raw materials used in the research were analyzed for proximate constituents. The proximate constituents of fenugreek seed and wheat flour are shown in table 4.1.

Table 4.1 Nutrient composition of raw material

Parameters	Wheat flour	Fenugreek seeds
Moisture (%)	12	13.7
Crude protein (%db)	8	26.2
Crude fat (%db)	1.7	5.8
Crude fiber(%db)	1.9	7.2
Carbohydrate(%db)	70	44.1
Ash(%db)	2.7	3.1

4.2 Sensory analysis

To determine the acceptable amount of fenugreek powder and extract incorporation in chapati, sensory analysis was done by varying the percentage of fenugreek seed powder used. The data obtained from the sensory analysis by ranking test was analyzed using SPSS version 20. The results obtained are presented in fig 4.1 and fig 4.2.

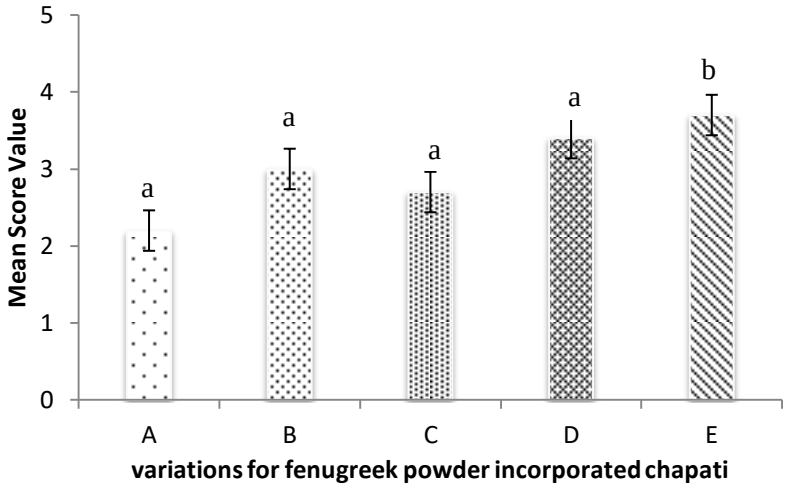

Fig 4.1: mean sensory score of fenugreek powder incorporated chapati

Fig 4.1 shows that formulation B, C and D are not significantly different from that of A (control) whereas the formulation E is significantly different ($p<0.05$) from that of A. If we consider mean value, formulation C has the higher rank after control so it can be considered as best. But in this study except the formulation E none of the formulation showed significant difference with the control. Since higher the fenugreek concentration greater would be the effect on glycemic index instead of selecting the best one we selected the maximum acceptable dose. This sensory analysis showed that fenugreek concentration up to 7.5% as maximum is not significantly different in acceptability then that of control. Thus the study was conducted by incorporating 7.5% fenugreek seed powder in the chapati.

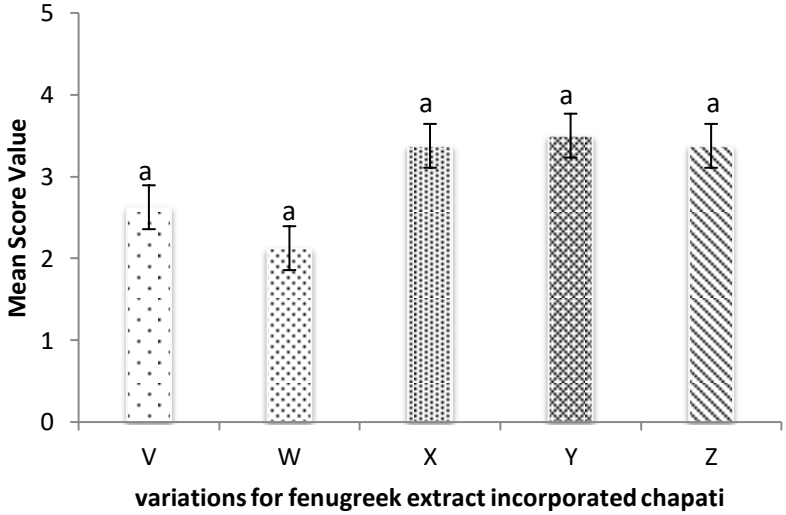

Fig 4.2: mean sensory score of fenugreek extract incorporated chapati

Fig 4.2 shows that formulation B, C, D and E are not significantly different ($p<0.05$) from that of A (control). If we consider mean value, formulation B has the higher rank after control so it can be considered as best. But in this study none of the formulations showed significant difference with the control. Since higher the fenugreek concentration greater would be the effect on glycemic response. This sensory analysis showed that fenugreek concentration up to 10% as maximum is not significantly different in acceptability then that of control. Thus, this study was conducted by incorporating 10% fenugreek seed extract in the chapati.

4.3 Analysis of product

Chemical analysis of selected sample from sensory analysis i.e. chapati with 7.5% fenugreek seed powder and chapati with 10% fenugreek extract and control sample was done and result is presented in table 4.2.

Table 4.2 proximate constituents of final product

Parameters	**Fenugreek powder incorporated chapati**	**Fenugreek extract incorporated chapati**	**Control chapati**
Moisture (%)	29.4	25.6	24.4
Crude protein(%db)	18.71	15.41	9.75
Crude fat(%db)	0.57	0.54	0.79
Crude fiber(%db)	2.1	0.36	0.37
Ash (%db)	1.13	1.1	1.32
Carbohydrate(%db)	48.09	57	63.36

Table 4.2 shows the proximate constituents of control chapati, fenugreek powder incorporated chapati, and fenugreek extract incorporated chapati. This shows that protein content and fiber content of chapati increased on addition of fenugreek seed powder, whereas the carbohydrate content decreased. On incorporation of fenugreek extract there is no much difference in fiber content but there is increase in protein content and decrease in carbohydrate content. Other constituents like moisture increases in the test foods, ash and fat content slightly differ. This shows that chapati with fenugreek extract lacks fenugreek fiber. The carbohydrate content determined was used to calculate the amount of chapati

that should be given to each subject for determination of glycemic response. For determination of glycemic response each subject must consume exactly 50 gm of carbohydrate.

4.4 Evaluation of glycemic response

4.4.1 Standardization of glucometer

For the standardization of glucometer the data obtained from lab test and glucometer test where analyzed using ms excel 2007. The covariance was found to be positive and correlation coefficient was 0.98. This shows that the glucometer is suitable for determining the glycemic response

4.4.2 Characteristics of subject

The anthropometric and clinical characteristics of subject are presented in the table 4.5. All participants were student of Central campus of technology. A total of 9 healthy subjects were studied, 5 males and 4 females fulfilling the criteria recommended by FAO/WHO (1998) for the measurement of GI of foods. Their characteristics are presented in table 4.3.

Table 4.3 Characteristics of subjects

Characteristics	Mean ± SD
Age (years)	21.67±1.22
Weight (kg)	58.36±8.9
Height (cm)	163.77±7.01
BMI (kg/m^2)	21.68±2.18
Waist circumference (cm)	78.7±6.76
FBG level (mg/dl)	88.27±3.97

4.4.3 Amount of nutrient given to each subjects

The amount of test food given to each subject was calculated in such a way that every subject ingests 50g carbohydrate. The nutritional value of food provided to the subjects is presented in table 4.4.

Table 4.4 Amount of nutrient given to each subjects

	Control	Fenugreek powder incorporated chapati	Fenugreek extract incorporated chapati
Amount(g)	78.9	103.96	87.7
Energy (kcal)	23.34	283.11	258.27
Carbohydrate (g)	50	50	50
Protein (g)	7.69	19.45	13.51
Fat (g)	0.62	0.59	0.47
Fiber (g)	0.29	2.18	0.32

In the study conducted by Robert et al. (2016) nutritional analysis of chapati and chapati with 10% fenugreek seeds powder having 50g carbohydrate was calculated whose result showed that chapati with fenugreek have higher energy, higher protein and higher fiber content. It also showed that greater quantity is needed to provide 50g of carbohydrate. This result is proportionate to our study result conducted with 7.5% fenugreek seed powder. Our study was first to analyze the chapati with fenugreek extracts. Fenugreek extracts also increased the protein and energy value of chapati.

4.4.4 Change in blood glucose

The mean and standard deviation of blood glucose level of 9 subjects after consumption of reference, control and test foods were calculated using Ms Excel 2007.

Table 4.5 Average blood glucose level at each time interval.

Time (min)	Glucose(mg/dl)	Control chapati (mg/dl)	Fenugreek powder incorporated chapati (mg/dl)	Fenugreek extract incorporated chapati(mg/dl)
0	87.22±3.93	88.75±5.069	88.33±3.64	88.78±5.069
15	122.11±9.943	99.56±6.89	96.56±4.035	106.11±10.08
30	139.11±13.448	113.89±10.179	111.67±9.138	114.56±11.85
45	119.78±7.436	114.22±7.19	107.56±6.803	112.11±5.51
60	108.56±6.692	109.22±6.996	99.78±6..399	107.33±6.062
90	99±9.179	101.89±6.585	95.56±6.5	100.33±4.153
120	88.33±7.089	96±5.612	91.89±3.06	92.22±4.738

4.4.5 Observed IUAC, GI and glycemic load value of test sample

The incremental area under the curve was determined geometrically by plotting blood glucose level on y-axis and time period on x-axis in the graph. The area below the fasting level was excluded. GI was determined by dividing the IAUC of each food with reference food and multiplying by 100. Glycemic load was calculated by multiplying GI with amount of carbohydrate present in one serving and dividing it by 100. (T. M. S. Wolever et al., 2003). The data were compared using SPSS vs. 20.

Table 4.6 Mean incremental area under curve

IAUC	**Mean ±SD (sq. units)**
Glucose	2640±862.24
Control	1881.667±535.59[a]
Fenugreek powder incorporated chapati	1289.16±304.73[b]
Fenugreek extract incorporated chapati	1812.5±486.7[a]

The table 4.6 shows that mean IAUC is highest in glucose and lowest in fenugreek powder incorporated chapati. There is significant difference (p-value <0.05) between control and fenugreek powder incorporated chapati but there is no significant difference (p>0.05) between fenugreek extract incorporated chapati and control.

Table 4.7 Mean glycemic index

GI	**Mean ±SD**
Control	72.24±6.04[a]
Fenugreek powder incorporated chapati	50.71±10.83[b]
Fenugreek extract incorporated chapati	70.14±8.98[a]

The table 4.7 shows the glycemic index value of control chapati, fenugreek powder incorporated chapati and fenugreek extract incorporated chapati. Result showed that GI of fenugreek powder incorporated chapati is significantly different (p value < 0.05) than control, But GI of fenugreek extract incorporated chapati does not show significant difference (p value > 0.05) with control. This suggests that the glycemic index of chapati changes significantly on addition of fenugreek seed powder but not in the addition of fenugreek extract. Food can be classified as high (GI>70), medium (56<GI<69) and low (GI < 55). In our study control food and fenugreek extract incorporated chapati falls under

high GI category where as fenugreek powder incorporated chapati falls under low GI category.

Here the glycemic index of chapati decreased by 29.8% on addition of fenugreek seeds powder. This may be due to its high fiber content. This result showed resemblance with the study Robert et al. (2016) which says that each g of fenugreek seed powder can be expected to lower the GI by 4.2%.

Here the glycemic index of chapati is reduced by 2.9% on addition of fenugreek extract. This shows that on addition of fenugreek extract there is only slight reduction in the glycemic index. This may be due to lack of fiber content.

Table 4.8 Mean glycemic load

	Grams of carbohydrate in 1 serving(30g)	**Glycemic load (Mean ±SD)**
Control	19	13.72 ± 1.14^{a}
Fenugreek powder incorporated chapati	14.42	7.31 ± 1.56^{b}
Fenugreek extract incorporated chapati	17.1	11.94 ± 1.53^{c}

The table 4.8 shows grams of carbohydrate present in control and each test foods with their glycemic load. Glycemic load depends upon amount of carbohydrate present in each serving. The amount of carbohydrate is lower in both fenugreek extract and fenugreek powder incorporated chapati compared to that of control. Lower glycemic load are considered to be beneficial to health. Fenugreek powder incorporated chapati falls under low glycemic load food (0-10) where as fenugreek extract incorporated chapati and control chapati falls under medium glycemic load food (10-20). Thus, fenugreek powder incorporated chapati is good for health compared to control and fenugreek extract incorporated chapati. P-value shows that there is significant difference in glycemic load between control and both fenugreek extract and powder incorporated chapati. This suggests that fenugreek incorporation significantly affect the glycemic load of chapati.

4.4.6 Glycemic index curve

The blood glucose responses of reference, control and test foods were plotted against different time period and the curve obtained is shown in the figure 4.3.

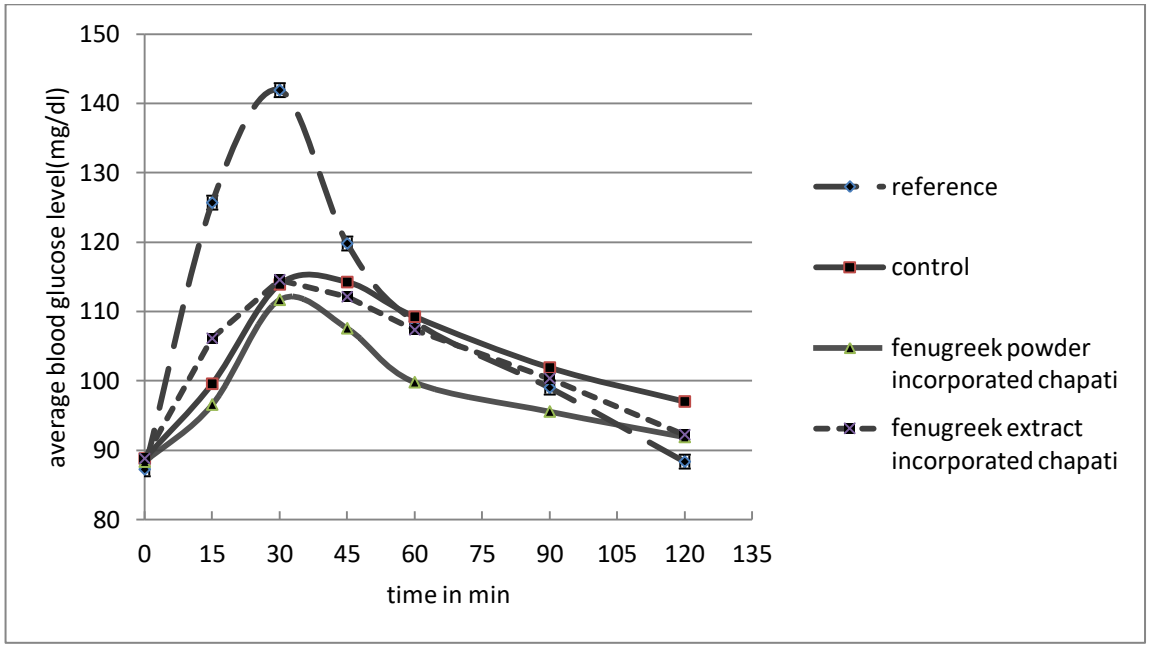

Fig 4.3 Average blood glucose response curves

Fig 4.3 shows that the rise in blood glucose is highest in reference food and lowest in fenugreek powder incorporated chapati. Fenugreek extract incorporated chapati and control chapati showed similar rise in blood glucose level. This shows that the blood glucose response slower down on addition of fenugreek powder but is not much affected on addition of fenugreek extract.

It has been shown that various plant products may act on blood glucose through different mechanisms. Some have insulin like activity (Collier et al., 1987) and others may increase number of Beta cells in the pancreas by activating regeneration of these cells (Shanmugasundaram et al., 1990). The plant fiber may interfere with digestion, absorption and metabolism of the carbohydrates. Over the past two decades of intensive research in dietary fiber, its necessity to the human diet and the positive effects on several diseases have been firmly established and its use in the treatment of several diseases including diabetes is now routine. In recent years, glycemic index of foods received considerable attention because of its usefulness in formulating diets for diabetics. It has been shown that different carbohydrate sources raise the blood glucose to different extent when fed in equivalent amounts. It has been suggested that the difference is due to differences in

digestion and absorption of the various foods.

Data from our present study showed that adding fenugreek seed powder to chapatis significantly reduced the postprandial glycemic response in healthy individuals. This hypoglycemic action may be due to the presence of galactomannan in the fenugreek seed powder. Galactomannan, a water soluble fibre, can inhibit glucose absorption and delay gastric emptying (Hannan et al., 2007). In this study addition of 7.5% fenugreek seed powder to the flour significantly increased the fibre content of the chapatis. Earlier studies have shown that addition of fenugreek seed powder to wheat flour has significantly increased the viscosity and overall the physicochemical properties of foods (Srivastava et al., 2012). Thus fenugreek could have increased the viscosity of chapatis, resulting in decreased absorption of glucose in the gastrointestinal tract. Viscous fibers, due to their water holding capacity, can delay gastric emptying by forming a gel matrix (Würsch & Pi-Sunyer, 1997). This gel matrix thickens the contents of the small intestine, thereby decreasing the rate of nutrient absorption, and impeding the association between food and digestive enzymes. Moreover, viscous fibres can increase the thickness of the unstirred water layer thus slowing the rate of diffusion of glucose and cholesterol to the absorptive surfaces of the intestinal villi (Edwards et al., 1988).

In our study, chapattis incorporated with fenugreek extracts have also reduced the glycemic index by 2.9% and glycemic load by 12.9%. The extract could not produce hypoglycemic effect as effectively as that of powder. This may be due to lack of fenugreek fiber in extract incorporated chapati. There is no significant difference in the glycemic index value of extract incorporated chapati and control chapati but the glycemic load differ significantly. Glycemic load have positive relation with amount of carbohydrate present in one serving and on proximate analysis it was found that carbohydrate content decreased on addition of fenugreek extract. This shows that fenugreek extract is also beneficial for health. The result suggests that not only fenugreek fiber but there may be other components in fenugreek that have potential to produce hypoglycemic effect. Thus, further study can be done preparing better edible extracts from fenugreek and study its hypoglycemic effect. The exact component responsible for the effect can also be identified.

In the study conducted by Puri et al. (2002) mechanism of action of orally active hypoglycemic principle isolated from water extract of seeds of fenugreek was investigated in alloxan induced sub diabetic and overtly diabetic rabbits of different severity cases. The result showed hypoglycemic effect on long term consumption which may be due to extra

pancreatic mode of action. Our study is the first to study whether it produces effect in short term basis or not i.e. by GI lowering effect. But the result obtained was not so convincing about this fact. Fenugreek extract could not lower the GI of chapati significantly as that of powder.

Hasan and Rahman (2016) prepared water extract of fenugreek seeds in two ways soaking it in the ratio 1:4 overnight and boiling the combination in same ratio at 100ºc for 30 min. These extracts were given to 20 diabetic individuals for 90 days with regular monitoring of blood glucose level. Both extracts were equally capable in reducing blood sugar level. Hence in our study we used boiled fenugreek extract to prepare chapati but the result did not show the significant GI lowering action. Thus the hypoglycemic effect of fenugreek extract may not be due to GI lowering but due to its effect on insulin sensitivity. This suggests that fenugreek extract may be effective only if consumed regularly for long period of time. Although the mechanism of action of fenugreek is not known accurately, numbers of compounds have been isolated from fenugreek seed extract and their actions on blood sugar reduction have been reported. Our study could not observe the change in insulin level due to limited facility. Thus further study must consider the measurement of insulin levels along with glycemic response.

High and quick increase in postprandial blood glucose levels is strong signals to the pancreas to increase insulin secretion. Over a period of time, frequent elevations in blood glucose and excessive insulin secretion may increase the risk of developing type 2 diabetes mellitus and cardiovascular disease. Hence low GI foods that are developed by the addition of fenugreek, if consumed regularly, can depress the surge in postprandial blood glucose and may be helpful in reducing the risk of type II diabetes and cardiovascular disease. As chapati is popular food among the world population, incorporation of fenugreek seed powder in chapati can have health benefits. The limitation of using fenugreek in the daily diet is its bitterness. But in this study, through sensory analysis we noted that the chapati with 7.5% fenugreek seed powder is acceptable to consumer making it suitable for consumption on regular basis.

Conclusions and recommendations

5.1 Conclusions

Present work was conducted by preparing chapati with fenugreek seeds powder and fenugreek seed extract and studied its effect on immediate glycemic response in human subject. The following conclusions were drawn.

- The sensory evaluation showed that 7.5% of fenugreek seed powder and 10% of fenugreek extract at maximum can be incorporated to wheat flour for making chapati with taste acceptable to the consumer.
- Proximate analysis showed that addition of fenugreek seed powder increases the moisture, protein and fiber content of chapati. Thus reducing the carbohydrate content.
- On addition of fenugreek extract moisture and protein content increased. Thus reducing the carbohydrate content. But not as significantly as fenugreek seed powder. No change in fiber content was observed by the incorporation of extract.
- Addition of fenugreek seed powder lowered the glycemic index of chapati by 29.8% and glycemic load by 46.72%.
- Addition of fenugreek seed extract on chapati lowered the glycemic index by 2.9% and glycemic load by 12.9%

5.2 Recommendations

- Further study of glycemic index by varying the concentration of fenugreek seed powder can be conducted.
- Further research including insulin measurement along with glycemic response can be done.
- Extract preparation with different methods and its effect on glycemic index can be studied and its chemical analysis can be done to determine the responsible component.
- Fenugreek can be incorporated in other food like rice and change in glycemic response can be compared with present study.

Summary

Diabetes, obesity, dislipidemia and CVD have become a growing issue of world population. Diet has been recognized as corner stone in their management. Low glycemic index foods have proven to be beneficial for such health condition. In this study attempt was made to reduce the GI of chapati by adding a functional component (fenugreek).

Chapati, a baked product prepared from whole wheat flour is the staple food of majority of population in South Asia. Fenugreek is a pleasantly bitter, slightly sweet spice used in almost every household. Fenugreek seeds produce many medicinal benefits such as hypocholesteromic, antidiabetic, antibacterial, gastric stimulating, galactoguge, lactation aid, hepatoprotective and anticancer effect. Hypoglycemic effect is mainly due to its high fibre content. Some studies showed that fenugreek extract could also produce hypoglycemic effect, which raises a question whether it is only fenugreek fiber or there are other responsible components.

In present study, chapati with fenugreek seeds incorporated in two different forms were prepared the most acceptable combination was determined through sensory analysis. It was found that replacement of maximum 7.5% wheat flour with fenugreek powder and replacement of water used to prepare dough with 10% fenugreek extract in chapati had same acceptability as that of control. The nutrient analysis of these combination showed that protein content increased and carbohydrate decreased in both combination whereas fiber content increased with addition of fenugreek powder but remained unchanged in addition of fenugreek extract.

Immediate glycemic response was determine by giving reference food(glucose), control chapati, chapati with 7.5% fenugreek powdered and chapati with 10% fenugreek extract to 9 subjects after 10 to12 hr overnight fast. Blood glucose level at different time interval was measured by using glucometer and glycemic index was determined geometrically. The result showed that incorporation of 7.5% fenugreek seed powder lowered the GI of chapati by 29.8% and GL by 46.72%. Similarly incorporation of fenugreek extract lowered GI by 2.9% and GL by12.9%.

From this we can conclude that fenugreek extract could not lower the immediate glycemic response as effectively as fenugreek powder. Thus, fenugreek fiber is the major component responsible for producing immediate hypoglycemic effect.

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Appendices

Appendix A
Approval letter from Nepal Health Research council

Government of Nepal
Nepal Health Research Council (NHRC)
Estd. 1991

Ref. No.: 2176.

Date: 16 February 2021

Ms. Usha Gartoula
Principal Investigator
Central Campus of Technology
Dharan

Ref: Approval of thesis proposal

Dear Ms. Gartoula,

This is to certify that the following protocol and related documents have been reviewed and granted approval by the Expedited Review Sub-Committee for implementation.

ERB Protocol Registration No.	884/2019 MT		**Sponsor Protocol No**	NA
Principal Investigator/s	Ms. Usha Gartoula		**Sponsor Institution**	NA
Title	Effect of incorporation of fenugreek seed on glycemic index of flatbread			
Protocol Version No	NA		**Version Date**	NA
Other Documents	1. Data collection tools		**Risk Category**	Low risk
Expedited Review	Proposal	√	**Duration of Approval** 15 February 2021 to 15 February 2022	**Frequency of continuing review**
	Amendment			
	Re-submitted			
	Meeting Date: 15 February 2021			
Total budget of research	**NRs 44,000.00**			
Ethical review processing fee	**NRs 1,000.00**			

Investigator Responsibilities :

- Any amendments shall be approved from the ERB before implementing them
- Submit progress report every 3 months
- Submit final report after completion of protocol procedures at the study site

Tel: +977 1 4254220, Fax: +977 1 4262469, Ramshah Path, PO Box: 7626, Kathmandu, Nepal
Website: http://www.nhrc.gov.np, E-mail: nhrc@nhrc.gov.np

Ref. No.: 2176.

- Report protocol deviation / violation within 7 days
- Comply with all relevant international and NHRC guidelines
- Abide by the principles of Good Clinical Practice and ethical conduct of the research

If you have any questions, please contact the Ethical Review M & E Section at NHRC.

Thanking you,

Dr. Pradip Gyanwali
Member-Secretary
(Executive Chief)

Tel: +977 1 4254220, Fax: +977 1 4262469, Ramshah Path, PO Box: 7626, Kathmandu, Nepal
Website: http://www.nhrc.gov.np, E-mail: nhrc@nhrc.gov.np

Appendix B

Informed consent form

Namaste,

I Usha Gartoula student of Bsc Nutrition and Dietetics in Central Campus of technology, Dharan am going to conduct a research work for a part of dissertation. The topic of the study is "Effect of incorporation of fenugreek on glycemic index of chapati"

I have been told that this research is for dissertation and mine participation is voluntary. I reserve full right to withdraw from this study at my own initiative at any time without having to give reason. Confidentiality would be maintained and would be shared only for academic purpose. I hereby give consent to participate in the above study. I am also aware that I can withdraw this consent at any later date, if I wish to.

I consent to:

- Attending the glycemic index facility for five separate mornings on alternate days following an overnight fast.
- Consuming the test food
- Providing seven blood samples obtained by finger pricking over two hours on each glycemic index test day.
- I know that:
  - The data may be published but my name will not be disclosed.
  - My participation is voluntary.
  - I am free to withdraw at any time without any disadvantage.
  - I agree to take part in this project
  - I have signed this form before my participation in the study.

Signature of participant:

Date:

Contact no:

I hereby state that all the study procedure were explained in detail and all the questions were fully answered to the above mentioned participant.

Investigator's signature:

Name: Usha Gartoula

Email: ushagartoula19@gmail.com

Appendix C

Sensory analysis score card

Sensory analysis of chapati by ranking test

Name of the panelist: …….

Date: ……..

Name of the product: chapati

Dear panelist, you are given 5 samples of chapati, please conduct the sensory analysis and rank them according to your preference or intensity of aroma/ taste of the product.

RANK	SAMPLE CODE
1	
2	
3	
4	
5	

Comments (if any)

……

……..……..

……

……

……..

Signature

Appendix D

Sensory evaluation

Table D-1 Sensory score of fenugreek powder incorporated chapati

control	formulations	Mean difference	Std. error	Sig.	95%confidence interval	
					Lower bound	Upper bound
A	B	-.8000	.61896	.203	-2.0466	.4466
	C	-.5000	.61896	.423	-1.7466	.7466
	D	-1.2000	.61896	.059	-2.4466	.0466
	E	-1.5000*	.61896	.019	-2.7466	-.2534

Table D-2 Sensory score of fenugreek extract incorporated chapati

control	formulations	Mean difference	Std. error	Sig.	95%confidence interval	
					Lower bound	Upper bound
V	W	.50	.699	.479	-.92	1.92
	X	-.75	.699	.291	-2.17	.67
	Y	-.88	.699	.219	-2.30	.55
	Z	-.75	.699	.291	-2.17	.67

Appendix E

Table E1 LSD table for IAUC

Multiple Comparisons

Dependent Variable: IAUC

Tukey HSD

(I) Group	(J) Group	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
CONTROL	GLUCOSE	-758.3333*	274.88192	.045	-1503.0879	-13.5788
	FNUPOWDER	592.5000	274.88192	.158	-152.2545	1337.2545
	FNUEXTRACT	69.1667	274.88192	.994	-675.5879	813.9212
GLUCOSE	CONTROL	758.3333*	274.88192	.045	13.5788	1503.0879
	FNUPOWDER	1350.8333*	274.88192	.000	606.0788	2095.5879
	FNUEXTRACT	827.5000*	274.88192	.025	82.7455	1572.2545
FNUPOWDER	CONTROL	-592.5000	274.8892	.158	-1337.2545	152.2545
	GLUCOSE	-1350.8333*	274.88192	.000	-2095.5879	-606.0788
	FNUEXTRACT	-523.3333	274.88192	.247	-1268.0879	221.4212
FNUEXTRACT	CONTROL	-69.1667	274.88192	.994	-813.9212	675.5879
	GLUCOSE	-827.5000*	274.88192	.025	-1572.2545	-82.7455
	FNUPOWDER	523.3333	274.88192	.247	-221.4212	1268.0879

Based on observed means.

The error term is Mean Square(Error) = 340020.313.

*. The mean difference is significant at the .05 level.

Table E2 LSD table for GI

Multiple Comparisons

Dependent Variable: GI

Tukey HSD

(I) Group	(J) Group	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
CONTROL	GLUCOSE	-27.7526*	3.60974	.000	-37.5327	-17.9725
	FNUPOWDER	21.5311*	3.60974	.000	11.7510	31.3112
	FNUEXTRACT	2.1049	3.60974	.936	-7.6752	11.8850
GLUCOSE	CONTROL	27.7526*	3.60974	.000	17.9725	37.5327
	FNUPOWDER	49.2837*	3.60974	.000	39.5036	59.0638
	FNUEXTRACT	29.8575*	3.60974	.000	20.0774	39.6376
FNUPOWDER	CONTROL	-21.5311*	3.60974	.000	-31.3112	-11.7510
	GLUCOSE	-49.2837*	3.60974	.000	-59.0638	-39.5036
	FNUEXTRACT	-19.4262*	3.60974	.000	-29.2063	-9.6461
FNUEXTRACT	CONTROL	-2.1049	3.60974	.936	-11.8850	7.6752
	GLUCOSE	-29.8575*	3.60974	.000	-39.6376	-20.0774
	FNUPOWDER	19.4262*	3.60974	.000	9.6461	29.2063

Based on observed means.

The error term is Mean Square (Error) = 58.636.

*. The mean difference is significant at the .05 level.

Table E3 LSD table for GL

Multiple Comparisons

Dependent Variable: GL

Tukey HSD

(I) Group	(J) Group	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
CONTROL	GLUCOSE	-36.2730*	.58298	.000	-37.8525	-34.6935
	FNUPOWDER	6.4137*	.58298	.000	4.8342	7.9932
	FNUEXTRACT	1.7326*	.58298	.027	.1531	3.3121
GLUCOSE	CONTROL	36.2730*	.58298	.000	34.6935	37.8525
	FNUPOWDER	42.6867*	.58298	.000	41.1072	44.2662
	FNUEXTRACT	38.0056*	.58298	.000	36.4261	39.5851
FNUPOWDER	CONTROL	-6.4137*	.58298	.000	-7.9932	-4.8342
	GLUCOSE	-42.6867*	.58298	.000	-44.2662	-41.1072
	FNUEXTRACT	-4.6811*	.58298	.000	-6.2606	-3.1016
FNUEXTRACT	CONTROL	-1.7326*	.58298	.027	-3.3121	-.1531
	GLUCOSE	-38.0056*	.58298	.000	-39.5851	-36.4261
	FNUPOWDER	4.6811*	.58298	.000	3.1016	6.2606

Based on observed means.

The error term is Mean Square(Error) = 1.529.

*. The mean difference is significant at the .05 level.

Appendix F

Blood glucose response curve of each subject

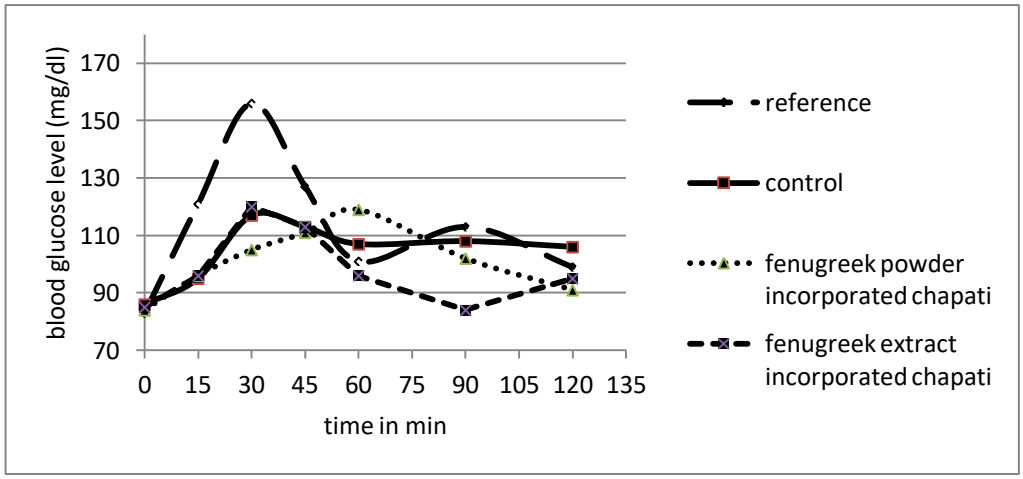

Fig F1: Mean blood glucose response of subject 1

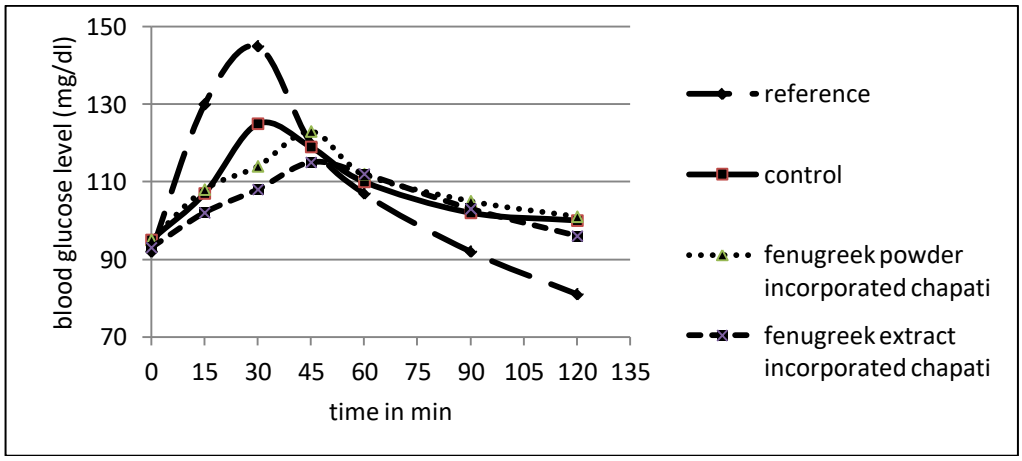

Fig F2: Mean blood glucose response of subject 2

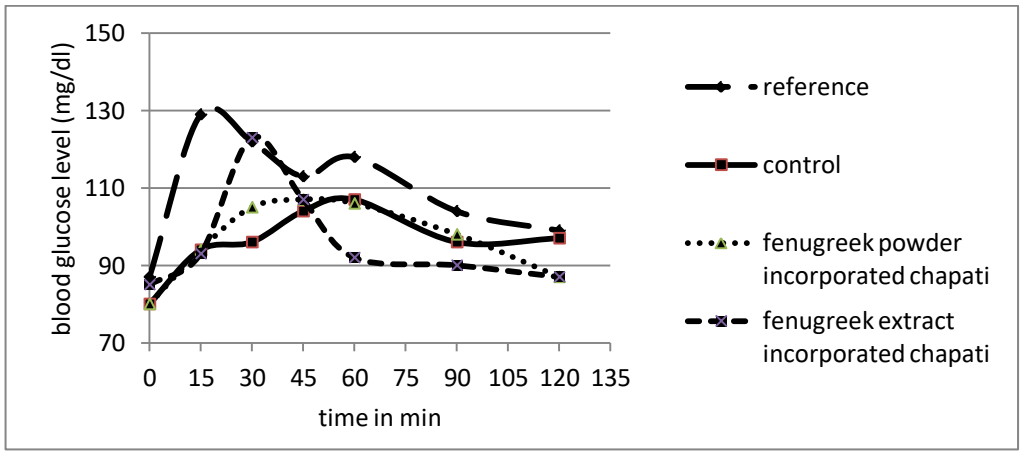

Fig F3: Mean blood glucose response of subject 3

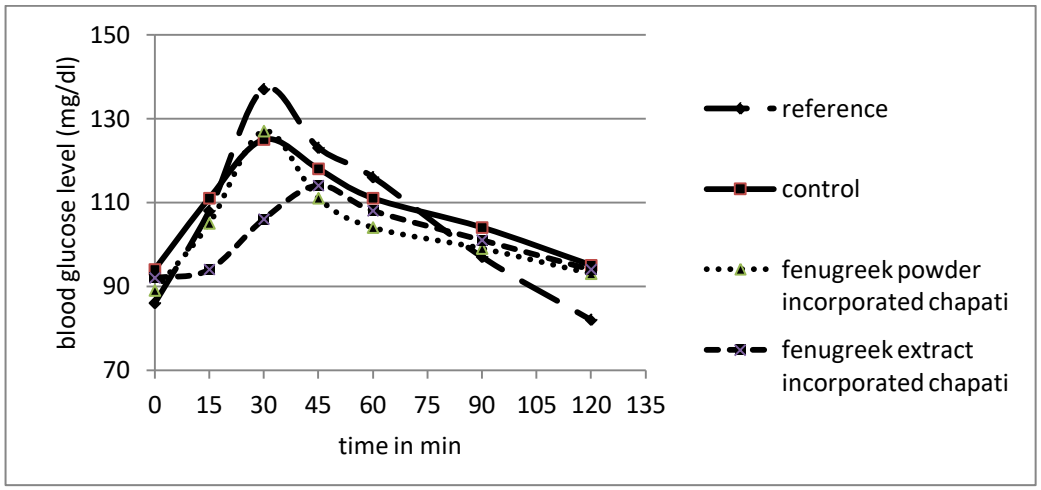

Fig F4: Mean blood glucose response of subject 4

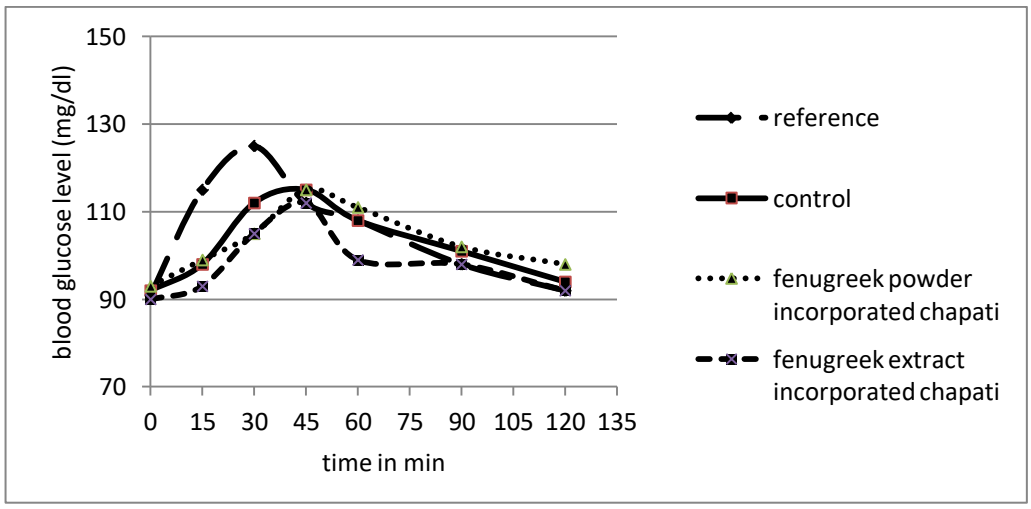

Fig F5: Mean blood glucose response of subject 5

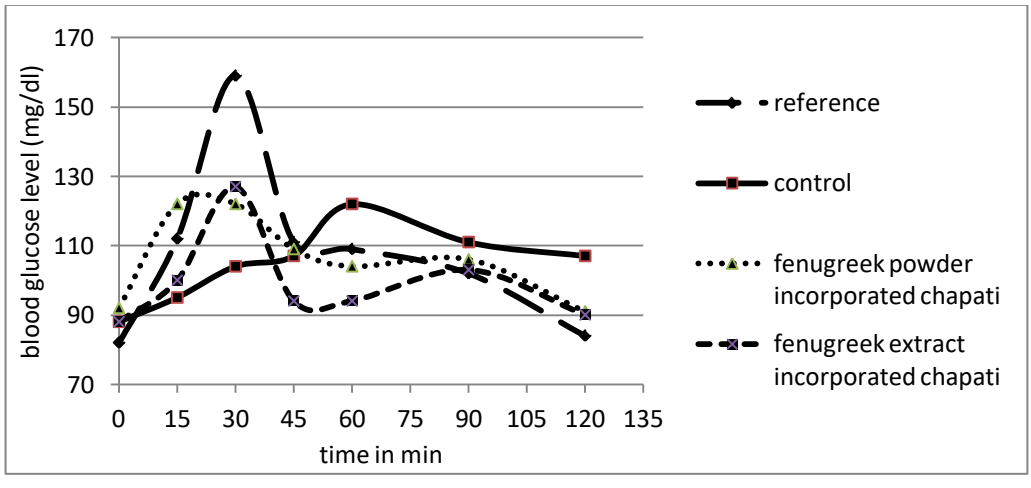

Fig F6: Mean blood glucose response of subject 6

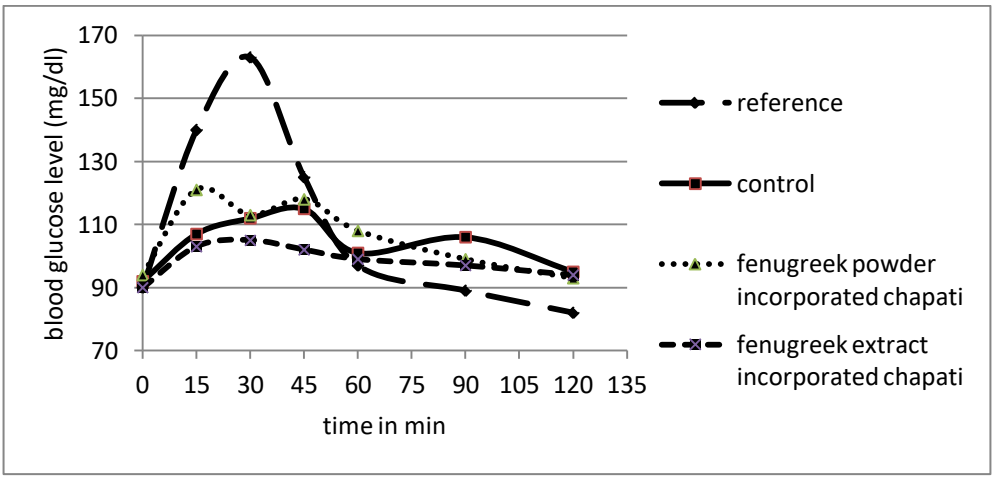

Fig F7: Mean blood glucose response of subject 7

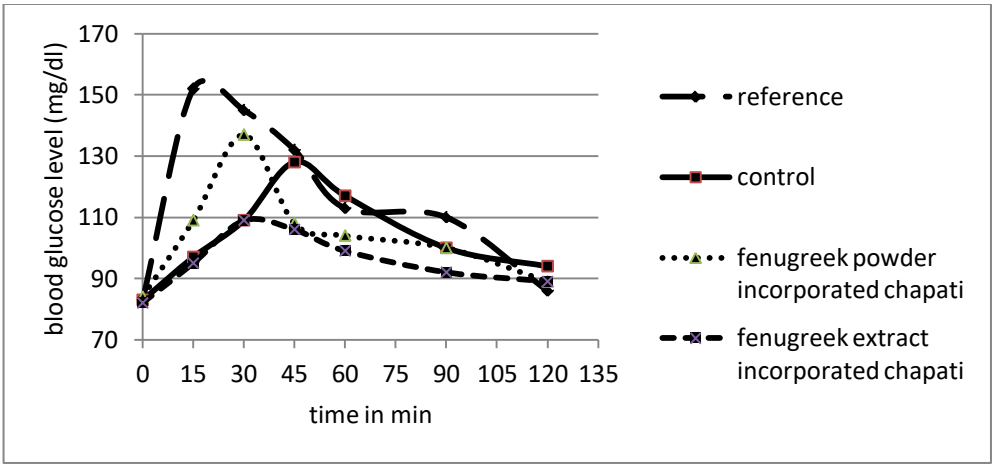

Fig F8: Mean blood glucose response of subject 8

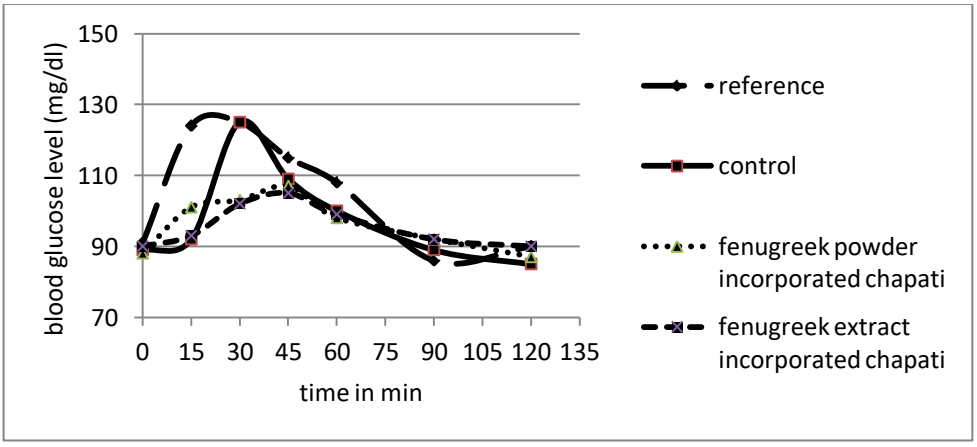

Fig F9: Mean blood glucose response of subject 9

Appendix G

Photo gallery

Sensory analysis

Protein determination

Glycemic index determination

Chapati