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Research Article

BIOENHANCING EFFECT OF FAGOPYRUM ESCULENTUM (BUCKWHEAT) IN HYPERTENSIVE PATIENTS

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Abstract

Buckwheat (Fagopyrum esculentum) is also known as common buckwheat or kuttu ka atta, which belongs to the polygonaceae family. Owing to the presence of antioxidants and phytochemicals such as quercetin, rutin, epicatechinyldimethyllate, Buckwheat has been shown to be useful for treating hypertension , hyperlipidemia, diabetes, cancer as well as celiac disease. It is rich in complex carbohydrate, about 100 gm of buckwheat provide 343 calories, 3.4 gm lipid, 71.5 gm of carbohydrate & 10 gm of fibre. Buckwheat flour has the highest protein (19.0 gm) content among all cereals. The amino acids in buckwheat protein are well-balanced & rich in lysine, methionine, histidine & tryptophan, which are lacking in wheat & barley. Buckwheat diet products exert a protective effect on the cardiovascular system, by having a positive impact on blood pressure, blood glucose, insulin, lipids etc. Thisstudy aimed to evaluate the therapeutic effects of buckwheat flour on newly diagnosed stage1 and 2 adult hypertensive patients along with the standard of care treatment. About 100 gm of Buckwheat flour was given in the form of flatbread orally for 3 months to the study (case group) subjects. The control group was advised to follow only lifestyle modification and antihypertensive medication amlodipine. Biochemical (lipid profile), anthropometric (weight), and clinical (blood pressure, pulse rate) parameters were recorded at baseline and after 2 weeks, 6 weeks, and 12 weeks for both groups. At the end of 12 weeks, biochemical, anthropometric, and clinical parameters improved in the cases as compared to controls. For the pharmacokinetics study patient blood samples were drawn from the control as well as case group after 7 days from the date of enrolment at different points plots against drug concentration. A pharmacokinetics study performed which indicates that the drug concentration was more in the case group (with buckwheat) as compared to the control group (without buckwheat), showing that buckwheat may increase drugs retention time in the blood more effectively reducing blood pressure and improving the lipid profile. Further studies witha large sample size are required to validate the findings.

Keywords: Buckwheat, Amlodipine, Anthropometric, Biochemical, Pharmacokinetics.

INTRODUCTION

Buckwheat (Fagopyrumesculentum) is derived from the Latin and Greek words, ―fagol‖ (meaning beech) and ―pyrum‖ (meaning nut); ―esculentum‖ means ―edible‖[1]. In India, common buckwheat is known as ogal, while in Nepal, is known as mite phapar[2]. In India, phapar ,titephapar, and bjo are the names given to Tartary buckwheat. Sweet buckwheat is the name given to common buckwheat in China and Nepal, while bitter buckwheat is the name given to Tartary buckwheat. This is most likely due to the taste of the flour, as Tartary buckwheat has a rather harsh after taste[3]. Russai was the world leader in buckwheat production whereas China held the second position followed by Ukraine & France. FAOStat of United Nation 2016) [4]. The largest buckwheat-producing states in India are Jammu and Kashmir, Ladakh, Kargil, Gurez Valley, Uttarakhand, Himachal Pradesh, Chattisgarh, and Uttar Pradesh. Buckwheat contains a lot of phenolic compounds, satisfactory source of vitamins, and exhibits an extraordinary protein content with a well-balanced Amino Acid composition. (Mota C. et al. 2016)[5]. The antioxidant activity of buckwheat bran and hulls is two to seven times higher than that of barley, triticale, and oats, and the phenolic compound content of whole buckwheat is two to five times higher than that of oats and barley (Holasova *et al.,* 2002[6]; Zdunczyk *et al.,* 2006[7]).

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Buckwheat proteins have a highly biologically valuable mix of well-balanced amino acids (Woo *et al.,* 2018[8]). The most prevalent protein in buckwheat grain is globulin followed by glutelin, albumin and prolamin, 43, 3%, 22.7, 18.2% and 0.8% respectively. (Ikeda and Asami 2000)[9] Flavonoids are most abundantly found in buckwheat (Arsi *et al.,* 2008[10]). Buckwheat has active constituent quercetin. Quercetin act as a bio enhancer of calcium channel blocker (Choi JS 2004)[11]. Buckwheat grain (Fagopyrumesculentum Moench) which is a crop belonging to the *polygonaceae* family is an important source of quercetin (Sedej I. et al. 20120)[12]. The amount of quercetin found in buckwheat is 170 mg/100 gm (Bai C. Z. et al 2015)[13] (Nemeth *et al.,* 2007)[14]. There are some nutrients of active constituents found in various food products, which increase the bioavailability of a drug. These are called bioenhancers. Without engaging in any usual pharmacological action, a bioenhancer is a substance that can lower the dose of a medication by increasing its bioavailability and bioefficacy. (Gurpreet *et al.,* 2011)[15]. The use of bio-enhancers in combination therapy has several benefits, including: increasing the bioavailability of medicine results in an increase in its efficacy; - reducing the dosage and risks associated with drug resistance, thereby minimizing adverse drug reactions and side effects; and reducing both intra- and inter-individual variability due to the drug's increased bioavailability (Atal, 2010)[16]. Bio-enhancers must not be harmful to people or animals, be efficient at extremely low concentrations when combined, be simple to construct, and, most essential, must increase drug uptake, absorption, and activity (Gopal *et al.,* 2014)[17].

Quercetin(2-(3,4-dihydroxy phenyl)-3,5,7-trihydroxy-4Hchromen-4-one) is a flavonoid, an aglycone having antioxidant, radical scavenging, anti-inflammatory, anti-hypertensive, anti-atherosclerotic, anti- cancer properties, and anti-viral effects (Nijveldt *et al.,* 2001)[18] . Quercetin increases the bioavailability, blood levels, and efficacy of some drugs like diltiazem, digoxin, and epigallocatechin gallate (Kesarwani *et al.,* 2013)[19]. Flavonoids present in Tartary and common buckwheat include rutin, quercetin, kaempferol, orientin, isoorientin, vitexin, isovitexin, and vitexin, isovitexin. Quercetin inhibit CYP-450 enzyme and its isoenzyme CYP3A4. Cytochrome P450 are essential for production of cholesterol, steroids, prostacyclins. It is necessary for the detoxification of foreign chemicals and metabolism of drugs. Every part of the buckwheat plant contains flavonoids, including the seeds, hulls, leaves, stems, blossoms, and roots. Flavonoid content in buckwheat varies depending on growth time, species, and genotype. Flavonoids from Tartary, in general. Buckwheat has a higher antioxidant content than ordinary buckwheat. Normal buckwheat grain has 4 to 13 mg of flavonoids per gram, while grains of Tartary buckwheat contain 40 mg. Total flavonoid content of tartary buckwheat leaves, flowers, and stems of over 100 mg/g. Replacement diets with buckwheat products exert a protective effect on the development of cardiovascular disease by reducing blood pressure, blood glucose, insulin lipids, etc (Sofi *et al.,* 2016)[20]. Health benefits of buckwheat include reduction of plasma cholesterol level, neuroprotection, anticancer, anti-inflammatory, antidiabetic effects, and improvement in reducing blood pressure (Gimenez, 2015) [21], (Zhang *et al.,* 2012) [22].

MATERIALS AND METHODS

The present study was conducted on hypertensive patients attending OPD/IPD at Era's Lucknow Medical College & Hospital, Era University, Lucknow‖ after taking upright clearance from the Institutional Ethics Committee‖. The study includes one intervention phase spanning a total of 3 months. According to the "Joint National Committee," (JNC)[23] total of 126 hypertensive outpatient and inpatient clinic attendees were studied. Adults within the lifespan range defined by the hypertensive recommendations (18 years or older). These patients were divided into two groups Control (Only Amlodipine) & Case (Amlodipine plus buckwheat). 100 gm of buckwheat flour per day in two divided dosages in the form of buckwheat roti was given for 3 months. After 7 days of patient‘s enrollment blood samples were drawn randomly from 40 respondents (20 from each group) at different time points for the pharmacokinetics study. Participants provided written informed consent, and the research was conducted in accordance with the principles outlined in the Helsinki Declaration (II). Demographic (Age, Height, Weight, Sex), Anthropometric (Waist Circumference, Hip Circumference, Waist Hip Ratio, BMI), Physical Parameters (BP, pulse rate, Disease and family History, Dietary Intake (Veg/Non-Veg, Smoking/Nonsmoking, Alcohol/No Alcohol, etc) of each subject were recorded on predetermined proforma. All subjects were provided with intensive nutritional education at baseline and during follow-up.

Blood sampling for lipid profile

About 3 ml fasting venous blood samples were collected and blood serum was split bycentrifugation at 3000 rpm and were stored at -80 ^{o}C until analyzed for lipid profile.

Blood sampling for pharmacokinetics study

Donors' blood was drawn using single-use, disinfected syringes. At each collection, 2 mLof blood was taken. We took blood samples before (0h), during (6h), and after (24h) treatment. After being centrifuged at 4000 rpm for 15 minutes at room temperature toseparate serum, the blood samples were kept at -80 ^{o}C until the serum concentration of Amlodipine could be measured.

Investigations:

Fasting blood samples were taken for lipid profile ―(Total cholesterol, Low-density Lipoprotein(LDL), High-density Lipoprotein(HDL), Triglycerides, Very low-density Lipoprotein (VLDL) level)‖ at standard and at the end of three months.

Estimation of Serum Lipid Profile

LDL, HDL, VLDL, Total Cholesterol, and TG were estimated by using the Wintrobe methodat hospital lab ELMC&H.

Estimation of Serum for pharmacokinetics study

Estimation of Serum for pharmacokinetics study done by LC-MS/MS method.

For pharmacokinetics study

- **Drug and reagents**

Amlodipine besylate (AMD) was purchased from TCI Chemicals (Tokyo, Japan). The organic solvents such as methanol, acetonitrile and formic acid of LC-MS/MS grade were purchased from Thermo Fisher Scientific (Waltham, MA, USA). The ultrapure water was procured from inhouse Merck Millipore Milli-Q system. Drug free blank serum was purchased from licensed blood bank (Lucknow, Uttar Pradesh). The sample extraction was performed using Waters Oasis® HLB (1cc/30mg, Milford, MA, USA) cartridge.

- **Preparation of Calibration standards**

The mother stock solution of AMD was prepared in methanol with the final concentration of1 mg/mL. On further dilution with methanol, a working stock of 20 µg/mL was prepared

AUC

From which working standards for calibration and quality control samples were prepared, respectively. The nine-point rectilinear calibration curve was plotted by using blank serum spiking with respective amounts of AMD working solution and processed likewise as that of clinical samples. The linearity range was specified from 1.56 to 400 ng/mL. The quality control (QC) samples were prepared at three levels from a given working stock solution in methanol. High-QC (300 ng/mL), Mid-QC (75 ng/mL), and Low-QC (9.6 ng/mL) were prepared as similar as calibration standards (Agrawal S. 2023)[24].

- **Sample preparation**

The clinical serum samples were stepwise thawed from -80 ^{o}C to -20 ^{o}C to room temperature.As reported in literature, 100 µL

of serum was withdrawn and loaded onto preconditioned HLB extraction cartridge. The preconditioning was performed by adding 1 mL methanol followed by 1 mL of ultrapure water. Next step was to wash the sample-loaded cartridge with2 mL of ultrapure water followed by methanol containing water (5 %, v/v). The collecting tubes were replaced and final drug elution was carried out with 500 µL of 0.5 % v/v formic acid in methanol (Bhatt J. et. al., 2007)[25] (Mishra et. al, 2022)[26] The final eluant was loaded into vials and subjected to LC-MS/MS analysis.

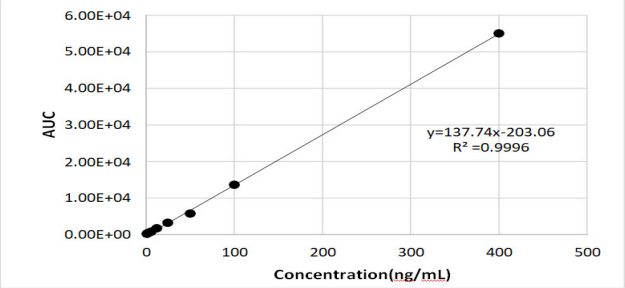

Figure 1. Calibration curve for AMD in serum matrix

- **LC-MS/MS conditions**

The clinical bioanalysis was performed using LC-MS/MS instrument. The reverse phase chromatographic separation of AMD was performed on Shimazdu Nexera XS Series Ultra High-Performance Liquid Chromatographic (UHPLC, Kyoto, Japan) system, equipped with LC-400XS pump, SIL-40C XS autosampler and CTO-40S column oven. The separation was executed using Waters Symmetry C18 analytical column (4.6 mm × 150 mm, 5 µm) with isocratic mobile phase composed of methanol (90 %) and 0.1 % v/v formic acid in ultrapure water at a flow rate of 0.7 mL/min. The column oven and auto-sampler temperature were pre- set to 25 ^{o}C and with injection volume of 20 µL. The run time for each sample was 5 minutes (Bisen *et al.,* 2023)[27]. The LC-MS system was hyphenated with Shimazdu LC-MS-8050 (Shimazdu Corporation, Kyoto, Japan) mass spectrometer. AMD was quantified in positive electrospray ionization mode (+ESI) using multiple reaction monitoring (MRM) operated at an unit resolution aimed at Q1 and Q3 quadrupoles. The instrument parameters like interface temperature, heat block temperature and desolvation line temperature were 300 ^{o}C, 400 ^{o}C and 250 ^{o}C, respectively. Nitrogen was used for providing inert gas atmosphere as a nebulizing gas, heating gas and drying gas. The respective flow of each gas was set at 3, 10 and 10 mL/min. The collision induced dissociation (CID) was done at 270 kPa by supplying continuous flow of inert argon gas. The Q1 and Q3 optimization, method development, MS/MS fragmentation were accomplished using LabSolutions software v5.99 (Shimazdu).

RESULTS AND DISCUSSION

This study compared the anthropometric, clinical, and biochemical changes in 63 cases and 63 controls of newly diagnosed stage 1 and 2 hypertension who were taken prescribed antihypertensives along with lifestyle modification. The subjects were supplemented with buckwheat flour for three months. The anthropometric and clinical profile detailed results were dicussed in another paper(Naqvi *et al.,* 2022)[28].

Bioanalytical method optimization for pharmacokinetics study.

- **Extraction method optimization**

To develop a robust and versatile bioanalytical method, precise analyte extraction is very essential. AMD extraction optimization was initiated using simple deproteination method using ACN, MeOH and acidified MeOH solution but it resulted into perplexing matrix effect at the retention time of analyte. Hence, we proceeded with liquid-liquid extraction (LLE) method. In LLE matrix effects are retracted, but analyte recovery was very poor, therefore this method was also rejected and chose solid-phase extraction (SPE) method for analyte extraction. SPE offered matrix interference-free, clean with excellent analyte recovery from serum samples.

- **LC-MS/MS optimization**

Post-SPE extraction samples were subjected to LC-MS/MS analysis of AMD. The chromatographic retention time and peak shape were optimized using different range of mobile phase and bonded stationary phase. The elution on Waters C18 column using methanol as a organic modifier along with 0.1% FA containing water yielded good peak shape and minimum retention time of 2.4 minutes. To identify the abundant Q1 and Q3 ion pair, various precursor-product ion optimization trials were performed. The +ESI mode Q1 and Q3 MRM pair for quantification was found to be 409.39 *m/z* and 238.1 *m/z*. The analyte dwell time between the quadrupoles was set to 200 msec. Q1 prebias, collision energy and

Q3 prebias for the analyte were set at -10, -17 and -27 respectively. The fragmentation pattern of AMD is depicted below in Figure 1,

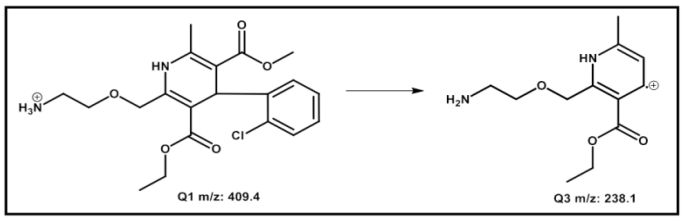

Figure 1. Schematic fragmentation pattern of Amlodipine

whereas the +ESI mass spectra for respective precursor ion and product ion is displayed in Figure 2.

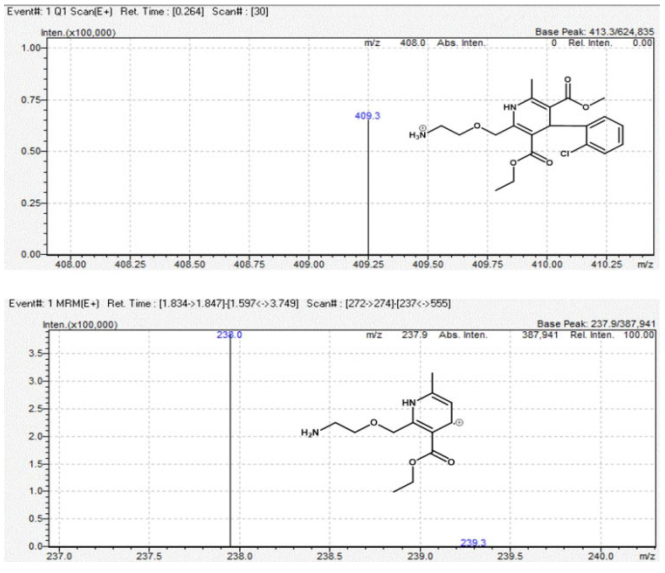

Figure 2. An ESI positive mass spectrum of AMD showing precursor ion (Q1: 409.39 m/z)and product ion (Q3: 238.1 m/z).

- **Clinical bioanalysis**

The bioanalytical method was extended for clinical bioanalysis of serum samples received. The sample extraction was performed using SPE and serum levels of AMD was estimated using LC-MS/MS. The analysis quality integrity was maintained by observing LQC, MQC and HQC between the sample run. The lower limit of quantitation was found to be 3.125 ng/mL.

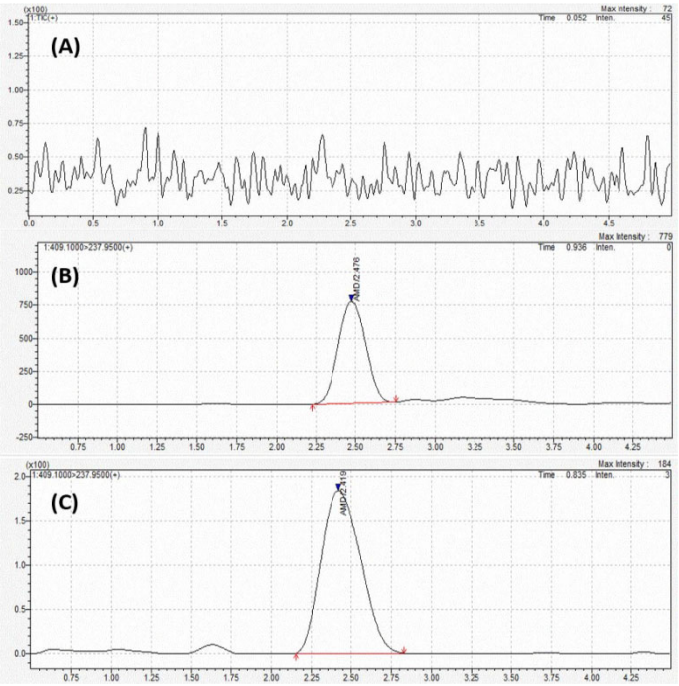

Figure 2. Representative LC-MS/MS chromatogram of Amlodipine (A) blank serum sample (B) serum extracted LLOQ and (C) clinical sample under analysis.

For pharmacokinetics study blood were taken at different time point against drug concentration. It has been found that the mean drug concentration was more in case group (Amlodipine +buckwheat) as comparison to control group (Amlodipine only).

Table 1. Clinical analysis of pharmacokinetic samples

Group	Control (ng/mL)		Case(ng/mL)		Unpaired t test	
	Mean	SD	Mean	SD	t-value	p-value
0 hr	13.701	0.380	13.552	0.402	1.20	**0.237**
8 hr	7.949	0.487	8.553	0.343	-4.53	**<0.001**
24 hr	3.759	0.395	4.592	0.443	-6.27	**<0.001**
48 hr	0.181	0.036	1.476	0.233	-24.59	**<0.001**
Intragroup (Repeated Measures ANOVA)	F=6218.8, p<0.001		F=6105.8 p<0.001			

Table 1 showed that from 0 hour to 48 hour, highly significant difference in mean concentration of drug was found between control and case group at 8 hour, 24 hour & 48 hour was observed (p<0.001). According to repeated measures ANOVA significant changes in mean concentration of drug were found in both group (p< 0.001), however this change was more in control as case group.

Conclusion

Present study shows that regular consumption of buckwheat has favorable impact on cardiovascular profile by reducing weight, BMI, systolic and diastolic blood pressure and improving lipid profile. Drug concentration was higher in the case group(with buckwheat) as compared to control group(without buckwheat), showing that buckwheat may increase drug residence time in blood more effectively reducing blood pressure and improving lipid profile.

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