WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 10, 242-249. Research Article ISSN 2277– 7105

ESTIMATION OF OXALATE CONTENTS IN MACROTYLOMA UNIFLORUM (LAM.) VERDC., PHASEOLUS LUNATUS LINN., AND PHASEOLUS VULGARIS LINN.

Salman Ahmed[1], Muhammad Mohtasheemul Hasan[1*] and Zafar Alam Mahmood[2]

[1]Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi, Karachi-75270, Pakistan.

[2]Colorcon Limited – UK, Flagship House, Victory Way, Crossways, Dartford, Kent, DA2 6 QD- England.

Article Received on
8 Aug 2015,

Revised on 28 Aug 2015,
Accepted on 17 Sep 2015

***Correspondence for Author**
Prof. Muhammad Mohtasheemul Hasan
Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi, Karachi-75270, Pakistan.

ABSTRACT

The seed flours of *Macrotyloma uniflorum* (Lam.) Verdc., *Phaseolus lunatus* Linn., and *Phaseolus vulgaris* Linn., were investigated for their total, soluble and insoluble oxalate contents by HPLC. The total oxalate content of *Macrotyloma uniflorum*, *Phaseolus lunatus* and *Phaseolus vulgaris* were found to be 1.24, 1.77 and 1.71 mg/ g respectively. The % age of soluble oxalate were *Macrotyloma uniflorum* (19.50), *Phaseolus lunatus* (15.08) and *Phaseolus vulgaris* (15.88). The calculated soluble oxalate: calcium ratio of all tested legume flours are lesser than one and hence will not create any resistance in calcium bioavailability.

KEYWORDS: *Macrotyloma uniflorum* (Lam.) Verdc., *Phaseolus lunatus* Linn., *Phaseolus vulgaris* Linn., oxalate.

INTRODUCTION

Oxalate is widely distributed in plant foods as potassium, sodium and ammonium oxalates (water-soluble form) and as insoluble calcium oxalates. Oxalate forms strong chelates with dietary calcium, thus rendering the complex unavailable for absorption and assimilation. It precipitates as insoluble salts accumulating in the renal glomeruli and contributes to the development of renal disorders.[1] Urinary oxalate originates from a combination of absorbed dietary oxalate and endogenous formation from oxalate precursors such as ascorbic acid and glyoxylate.[2] While other factors have to be considered in the development of renal disorder.

It is being recommended to limit the intake of oxalate-rich foods, specifically for individuals at risk for kidney stone formation.[1] Oxalate has been shown to be toxic to renal epithelial cells of cortical origin. It has been observed that exposure of renal epithelial cells to oxalate, leads to a disruption of the normal activities of the renal epithelial cells such as altered membrane surface properties and cellular lipids, changes in gene expression, disruption of mitochondrial function, formation of reactive oxygen species and decreased cell viability.[3] Membrane injury is considered to be the prime candidate for the binding of oxalate or calcium oxalate crystal and subsequent growth into kidney stones. Oxalate-induced membrane injury is mediated by lipid and protein peroxidation through the generation of oxygen free radicals with altered biochemical reactions, including depletion of the antioxidant defensive system and failure of the calcium pump. Accumulated calcium and oxalate precipitated and causing stone formation.[4]

Soluble and insoluble oxalate are separately measurable components in foods. Reliable methods for extracted oxalate analysis are ion electrophoresis, capillary electrophoresis, high performance liquid chromatography, gas chromatography and enzymatic assays using oxalate oxidase.[5] The high-performance liquid chromatography (HPLC) method is an accurate and reliable for oxalate determinations in plant materials.[1] In the present study the total, soluble and insoluble oxalate contents of *Macrotyloma uniflorum* (Lam.) Verdc., *Phaseolus lunatus* Linn., and *Phaseolus vulgaris* Linn. seed flours were estimated by HPLC.

MATERIALS AND METHODS

Apparatus and Instruments

Centrifuge (Hunan, China); HPLC (Shimadzu Prominence LC-20AT) coupled with a HPLC reverse phase column, Hibar® 250-4 Purospher® STAR RP-18 (Merck, Germany).

Chemicals and Reagents used

Hydrochloric acid (BDH, England); tetra methyl ammonium chloride; potassium di hydrogen phosphate (Sigma-Aldrich Chemie, Switzerland); oxalic acid dihydrate and orthophosphoric acid (Merck, Germany).

Plant material identification and sample preparation

Beans of *Macrotyloma uniflorum* (Lam.) Verdc., *Phaseolus lunatus* Linn., and *Phaseolus vulgaris* Linn., were purchased and identified by a taxonomist Department of Botany, University of Karachi. The voucher specimen number of *Macrotyloma uniflorum* (Lam.)

Verdc., (G.H.No.86483), *Phaseolus lunatus* Linn., (G.H.No. 86451) and *Phaseolus vulgaris* Linn., (G.H.No. 86536) were deposited in the Herbarium of University of Karachi. The seeds were separately grinded and powdered then passed through 600µm sieve and kept in an amber bottle at room temperature before commencing the experiment.

Standard curve preparation

The standards of pure oxalic acid were prepared in 1, 2.5, 5, 10, 20, 30, 40, 50 and 100µg/ml concentrations. The standards were prepared from 40 mg/ml of stock solution of pure oxalic acid. The samples and standards were filtered through a 0.45 µm acrylic disc into 1 ml HPLC auto-sample vials. The standard curve of oxalate concentrations (1.0-100µg/ml) was plotted against Peak area (response) (Figure-1). The selected HPLC conditions show strong positive relationship (R^2= 0.997) between oxalate concentrations and peak area.

Extraction of total and soluble oxalates

Total and soluble oxalates were extracted according to the method as described by Akhtar *et al.*,.[6] with some modification. 1gm of each sample was mixed with 30 ml of distilled water to make slurry. It was then homogenized with either 50 ml of 2N HCl or 50 ml of distilled water for the extraction of total and soluble oxalates respectively. The mixtures were centrifuged at 4000 rpm for 30 min and the supernatants were transferred to 100 ml volumetric flasks and made up to volume with distilled water.

Procedure of total, soluble and insoluble oxalates determination

HPLC of extracted oxalic acid was carried out with a RP-18 HPLC column. Mobile phase was 0.25% Potassium Di Hydrogen Phosphate and 0.002M Tetra methyl ammonium chloride buffered at pH 2.0 with pure ortho-phosphoric acid. The equipment consisted of dual piston HPLC pump with a UV detector set at 210 nm. The samples (5 μL) were injected in duplicate in the column and eluted at a flow rate of 1 ml/min. The oxalic acid peaks were eluted at 10 min. Each extract was analyzed for oxalate in triplicate. Total, soluble and insoluble oxalate contents was calculated by using following formula:

$y = 2727.2x$

$x = y / 2727.2$

T_{OX} or $S_{OX} = y / 2727.2$

$InS_{OX} = T_{OX} - S_{OX}$

where,

y= values on y-axis = Peak area (response) = 2727.2x

x= values on x-axis = concentration (µg/ ml)

T_{OX} = total oxalate

S_{OX} = soluble oxalate

InS_{OX} = insoluble oxalate = T_{OX} - S_{OX}

Statistical Analysis

Total, soluble and insoluble oxalate contents (mg/g) were subjected to unpaired student's *t*-test. All statistical calculations were performed with SPSS-20.

Table 1: Total, soluble and insoluble oxalate contents (mg/g) of *M. uniflorum, P. lunatus* and *P. vulgaris* seed flour.

Tested samples	T_{OX}	S_{OX}	InS_{OX}
M. uniflorum	1.24±3.74*	0.24±2.44*	1.00±1.82*
P. lunatus	1.77±0.00*	0.26±0.00	1.51±0.00*
P. vulgaris	1.71±0.00*	0.27±0.00*	1.44±0.00*

T_{OX} = total oxalate; S_{OX} = soluble oxalate; InS_{OX} = insoluble oxalate; N=3 extractions were done in triplicate for samples. Values are converted from µg/ml to mg/g; Results are mean of values ± S.E.M.); S.E.M.=Standard Error of Mean; **P*<0.01 showing significant values using unpaired student's *t*-test.

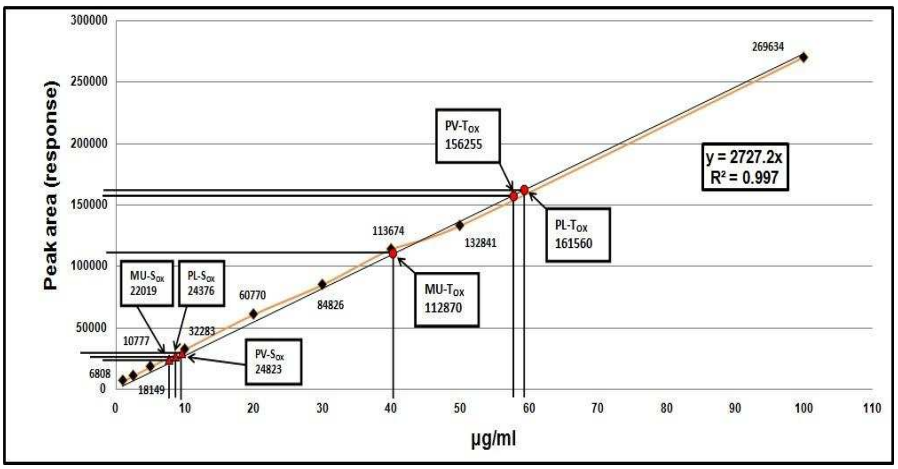

Fig 1: Standard curve for total and soluble oxalic acid contents.

RESULT AND DISCUSSION

Total oxalate estimated in *Macrotyloma uniflorum* as 1.24mg/g, less than previously reported 5.07 mg/g.[7] In present study, the total, soluble and insoluble oxalate of *Phaseolus lunatus* were estimated as 1.77, 0.26 and 1.51 mg/g respectively. Whereas, reported total oxalate contents are 0.04mg/g.[8] The total, soluble and insoluble oxalate of *Phaseolus vulgaris* were

estimated as 1.71, 0.27 and 1.44 mg/g respectively. Earlier studies ascribed the total (0.91-1.13), soluble (0.26-0.37) and insoluble (0.65-0.76) contents (mg/g) of *P. vulgaris* (red variety).[1,6] No doubt, total estimated oxalate contents of *P. vulgaris* are higher (1.71mg/g) than earlier reported values.

But the soluble oxalate content of tested sample is very less (15.88%) than reported values (28.57 and 32.74%).[1,6] The presented study suggest low %age soluble oxalate i.e. *M. uniflorum* (19.50), *P. lunatus* (15.08) and *P. vulgaris* (15.88). Differences in oxalate values for a single food may be due to analytical methods, and/or biological variation from several sources including cultivar, time of harvest, and growing conditions. Bioavailability of food oxalate and, thus, urine oxalate, will also be affected by salt forms of oxalate, food processing and cooking methods, meal composition, and the presence of *Oxalobacter formigenes* in the patient's gut.[5] As described earlier soluble oxalates are responsible for oxalate bioavailability and excretion. Hence responsible to form calcium oxalate complex in blood stream. So, presented study provides good agreement to provide no / less participation in any oxalate producing risk.

High oxalate foods have been known to exert a negative effect on calcium and iron absorption. This adverse effect on bioavailability of minerals is greater if the oxalate: calcium ratio in food exceeds 9:4. This ratio can be classified into three groups. Plants with an oxalate to calcium ratio greater than two means excess oxalate can bind calcium present in other foods eaten at the same time (non utilizable calcium). Plants with a ratio approximately one do not encroach on the utilization of calcium provided by other products and, therefore do not exert any demineralizing effects. However these foods are not good source of calcium. Foods with a ratio of one do not reduce the availability of calcium as far as other calcium sources are concerned. Plants with a ratio of less than one. Oxalate appears only to interfere slightly with zinc absorption. It has been reported that increasing the proportion of Mg^{2+} in solution inhibits the precipitation of calcium and zinc oxalates. This observation explains the minor effect of oxalates on zinc absorption from leafy vegetables, such as spinach, which has high levels of calcium and zinc, as well as relatively high levels of magnesium. Oxalic acid may cause greater decrease (temporary and transient) in mineral availability, if consumed with a high fiber diet.[9] Hyperoxaluria increases calcium oxalate super saturation and contributes to calcium stone formation. Normally, 90% of dietary oxalate binds to dietary calcium in the small intestine and passes into the stool as calcium oxalate; 10% of dietary oxalate remains

free and is absorbed in the colon and subsequently excreted in the urine.[10] The 24 - 53% of urinary oxalate originated from dietary oxalate at typical intakes of 10 to 250 mg per day. So, dietary oxalate makes a much greater contribution to urinary oxalate.[5] Small increases in oxalate excretion has pronounced effects on the production of calcium oxalate in the urine, implying that foods high in oxalate can promote hyperoxalurea and increase the risk of stone formation.[9] Hyperoxaluria may simply be a result of high dietary oxalate intake. However, increased enteric absorption of dietary oxalate can occur in those on a low-calcium diet and may partially explain why a low-calcium diet has been associated with increased frequency of calcium stone disease.[10] The American Dietetic Association's *Nutrition Care Manual* recommendation for patients with kidney stones is to restrict dietary oxalate to less than 40 to 50 mg per day. The next recommendation is to add calcium to each meal to bind oxalate. The total calcium intake for the day should be divided between as many eating occasions as possible. Calcium will bind oxalate in the gut, preventing it from being absorbed.[5] The amount of oxalate absorbed from a food is the critical aspect of dietary choice. This is influenced by three major factors: the amount and form of oxalate in the food as consumed, the amount of calcium and magnesium in the oxalate-containing food and the presence or absence of oxalate-degrading bacteria (*Oxalobacter formigenes*) in the gut.[5] The ability of various oxalate-containing foods to increase urinary oxalate excretion and pre-disposition to stone formation depends on both its oxalate content and bioavailability.[6] Oxalate is poorly absorbed under non-fasting conditions. It has been demonstrated that only 2-12% of oxalate (soluble form) is absorbed from foods but that once absorbed, free oxalate binds to calcium ions to form insoluble calcium oxalate.[9] The amount of soluble oxalates in taken food item is important because soluble oxalate, not total oxalate is responsible for oxalate absorption (bioavailability) and its excretion. Simply, soluble oxalate is directly proportional to the oxalate absorption/excretion.[5] Insoluble oxalates are not absorbed into the blood stream and remain largely undissolved within the digestive tract. The calculated calcium contents (mg/g) of *M. uniflorum, P. lunatus* and *P. vulgaris* are 0.04, 0.03 and 0.03 respectively (un published data). As described earlier about the oxalate: calcium ratio which affect the bioavailability of minerals and soluble oxalate are responsible for oxalate bio availability. So, the calculated soluble oxalate and calcium ratio will be *M. uniflorum* (0.48:0.08), *P. lunatus* (0.53:0.06) and *P. vulgaris* (0.54:0.06). The calculated ratios suggest that oxalates in all tested legume flours are not tend to cause any resistance in calcium bioavailability.

CONCLUSION

Overall total oxalate contents are high in legume flour of *M. uniflorum, P. lunatus* and *P. vulgaris* but soluble oxalates are very low. Therefore, all foods studied should not have any adverse effect of oxalate on minerals bioavailability. Their regular consumption no doubt would significantly increase the daily intake of oxalates but lower soluble oxalate would not pose any risk to people who are at risk of forming kidney stones. However, oxalate content and oxalate: calcium ratio in mixed plant foods which are commonly consumed as raw and cooked among vegetarians, should be investigated.

REFERENCES

1. Judprasong K, Charoenkiatkul S, Sungpuag P, Vasanachitt K, Nakjamanong Y. Total and soluble oxalate contents in Thai vegetables, cereal grains and legume seeds and their changes after cooking. J Food Comp & Anal, 2006; 19(4): 340-347.
2. Chai W, Liebman M. Oxalate content of legumes, nuts, and grain-based flours. J Food Comp & Anal, 2005; 18(7): 723-729.
3. Aggarwal A, Tandon S, Singla SK, Tandon C. Diminution of oxalate induced renal tubular epithelial cell injury and inhibition of calcium oxalate crystallization in vitro by aqueous extract of Tribulus terrestris. Int Braz J Urology, 2010; 36(4): 480-489.
4. Selvam R. Calcium oxalate stone disease: role of lipid peroxidation and antioxidants. Urological Res, 2002; 30(1): 35-47.
5. Massey L.K. Food oxalate: factors affecting measurement, biological variation, and bioavailability. J Am Dietetic Assoc, 2007; 107(7): 1191-1194.
6. Akhtar MS, Israr B, Bhatty N, Ali A. Effect of cooking on soluble and insoluble oxalate contents in selected Pakistani vegetables and beans. Int J Food Properties, 2011; 14(1): 241-249.
7. Sudha N, Mushtari Begum J, Shambulingappa KG, Babu CK. Nutrients and some anti-nutrients in horsegram (Macrotyloma uniflorum (Lam.) Verdc.). Food Nutr Bull, 1995; 16: 81-83.
8. Relfe S. High Oxalate Levels in Different Foods.2009 [cited 2015 28 June]; Available from:https://www.relfe.com/2009/oxalate_diet_low_high_levels_kidney_stones_cause.html#cereal.
9. Noonan S. Oxalate content of foods and its effect on humans. Asia Pacific J Clinical Nutr, 1999; 8(1): 64-74.

10. Hall PM. Nephrolithiasis: Treatment, causes, and prevention. Cleveland Clinic J Med, 2009; 76(10): 583-591.