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masoor

soups in Bangladeshi postmenopausal women

soup in Bangladesh postmenopausal women

Farzana
Rashid and Mamunar, Mohammed and Moshuzzaman
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Department of Chemistry, University of Dhaka

Bangladesh
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**Abstract**

The table above illustrates the data collected from various experiments conducted in the last quarter. Each column represents a different variable, and the data points provide insights into the outcomes observed. Further analysis is required to correlate these results with the experimental conditions.
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Bangladeshi menopausal women

Determinants

Mung dal
...
BACKGROUND

AIMS:

Isoflavones daidzein and genistein generate estrogenic compounds in human without any side-effect.
To measure the determinants of two isoflavones daidzein and genistein in Bangladeshi postmenopausal women consuming soy-milk and soups prepared from...
and soups were prepared from 100 g powders of soybeans, an overnight fast, each participant was given freshly-prepared soy-milk (~350-mL) and soups subsequently. Soy-milk

METHODS

Sixteen healthy postmenopausal women (age, mean±SD, 52.5±5.8 years) were included. After an overnight fast, each participant was given freshly-prepared soy-milk (~350-mL) and soups subsequently. Soy-milk and soups were prepared from 100 g powders of soybeans,
(~2 mL) was immediately frozen at 6, 8, 24, 36, and 48 hours after ingestion of milk and soups. Blood samples were centrifuged at 1200 rpm and serum stored at 0°C until analysis. Isoflavones were extracted from the defrosted serum, and the sample was cleaned using solid-phase extraction (SPE C18 Cartridge). Levels of isoflavones were determined by high-performance liquid chromatography (HPLC) with photodiode array detection.
The area under the curve (AUC) of serum genistein in soy-milk, in the serum were quantified using liquid chromatographic (LC)-PDA analysis. RESULTS. The area under the curve (AUC) of serum genistein in soy-milk, in the serum were quantified using liquid chromatographic (LC)-PDA analysis. RESULTS.
mung dal, masoor,
(0.03) association was found between the Cmax of serum isoflavones genistein of soy-milk and mung dal.
The findings indicate that the determinants of isoflavones was found in non-soy foods among Bangladeshi postmenopausal women.
Lack of estrogen production

Introduction
... is associated with an increased risk of hormone-dependent disorders.

Hormone replacement therapy (HRT) is being used for the treatment of these disorders however, the usage of HRT is associated with an increased risk of endometrial...
increasingly popular as they offer the same beneficial effects of HRT without any side-effect.
Results of epidemiological
have shown that isoflavones prevent hormone-dependent disorders. Postmenopausal women in Bangladesh do not get enough support due to their poor socioeconomic circumstances and ignorance and due to the inadequate healthcare system.
During this period, many women are not well-accepted in the family and society.

Consequently, they consider themselves as a burden. It is paradoxical that HRT is more focused in poorer countries.

where economic consideration itself represents
hence is difficult for them to bear the high cost of HRT therapy. Furthermore, it is a great obstacle to achieve the goal of wellbeing.

It would be necessary to be easily-accessible food materials containing high amount of isoflavones for the menopausal women.
isoflavones, a group of non-steroidal plant-derived compounds, are structurally similar to estrogen (Fig.) and can exert weak estrogenic effects.

The major isoflavones, such as genistein and daidzein, have several features in common with estradiol-17β.
and legume seeds (lentils, beans, and peas)

the richest sources of isoflavones, including

gemistein and daidzein

Hyperlink

Soybean and lentils, particularly
contain isoflavones. In Bangladesh, a considerable amount of soybean is produced, although its consumption is limited while...
masoor dals are commonly consumed. Despite the positive effects of isoflavones, on the bioavailability of dietary isoflavones.

only three studies covered the bioavailability of daidzein or genistein among postmenopausal women in postmenopausal women. is lacking. Only three studies covered the bioavailability of daidzein or genistein among postmenopausal women.
To the best of our knowledge, no studies have been conducted in Bangladeshi postmenopausal women, which prompted us to undertake the present pilot study. The aim of the present study was to measure the determinants of isoflavones daidzein and genistein in Bangladeshi postmenopausal women consuming soy-milk and soups.
Eighteen healthy postmenopausal women were included in the 36-day study and 16 completed this. Two women dropped out due to their difficulties to be involved in such a long period of study. The participants were screened at the Bangladesh Institute of Health Sciences (BIHS) hospital and the following parameters were assessed: age, blood pressure, pulses, body weight and body mass index (BMI).
who had been administrated antibiotics. Besides, Women aged between 45 to 60 years and who had natural menopause or due to surgery had menopause for last 2 years were considered for the study. Women with chronic renal, liver, pulmonary or cardiovascular diseases were excluded. Besides, those who had been administrated antibiotics.
within the preceding three months and were taking oral contraceptives or HRT. Study design

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within the preceding three months and were taking oral contraceptives or HRT. Study design
A non-randomized, single-dose sequence, crossover study design with a two-weeks washout period.

Postmenopausal women were divided into four groups and each group consisted of women to facilitate the study procedure.
On day 1, they were hospitalized in the evening and were served an isoflavones-free meal.

After an overnight fast, they consumed freshly-prepared soy-milk (~350 mL) as a single bolus. It was ensured that soy-milk was ingested by our participants.

in front of principal investigator (PI). Blood samples (5 mL) were collected before (baseline) and at an interval of 2, 4, 6, 8, 24, 36, and 48 hours after ingestion. Isoflavones-free meals were given at dinner on day 1, at lunch and dinner on day 2, at breakfast, lunch, and dinner on day 3, and at breakfast on day 4. A physician as a co-investigator helped PI in screening and in monitoring women from the...
and during the study.
Screening for eligibility (n=30)
Selection of postmenopausal women according to the inclusion and exclusion criteria (n=18)
Selection of postmenopausal women according to the inclusion and exclusion criteria (n=18)

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Allocation for first food (soy-milk)
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Hospitalization of postmenopausal women in the evening and served an isoflavones-free meal.
Hospitalization of postmenopausal women in the evening and served an isoflavones-free meal.

Requires...
Participants consumed freshly-prepared soy-milk (http://schemas.microsoft.com/office/word/2010/wordprocessingShape)
Isoflavones-free meals were given at lunch and dinner (baseline) and at an interval of 2, 4, 6, 8, 24, 36, and 48 hours after ingestion.
Participants consumed freshly-prepared soy-milk (350 mL) as a single bolus and 5 mL blood samples were collected before (baseline) and at an interval of 2, 4, 6, 8, 24, 36, and 48 hours after ingestion. Isoflavones-free meals were given at lunch and dinner interval of 2, 4, 6, 8, 24, 36, and 48 hours after ingestion.
blood samples were collected on pre-set time, i.e., at an interval of 24 and 36 hours. Isoflavones-free meals were given at breakfast, lunch, and dinner and 5 mL of blood samples were collected on pre-set time, i.e., at an interval of 24 and 36 hours. Isoflavones-free meals were given at breakfast, lunch, and dinner and 5 mL of blood samples were collected on pre-set time, i.e., at an interval of 24 and 36 hours. Isoflavones-free meals were given at breakfast, lunch, and dinner and 5 mL of
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collected on pre-set time, i.e., at an interval of 48 hour and the women were

Isoflavones-free meals were given at breakfast and 5 mL blood samples were
discharged from the BIHS hospital for home

...
Isoflavones-free meals were given at breakfast and 5 mL blood samples were collected on pre-set time, i.e., at an interval of 48 hour and the women were discharged from the BIHS hospital for home.
Furthermore, patients were inquired about their wellbeing, any type of discomfort or adverse effect.
Allocation for second food (mung dal)
Received allocated intervention (n=16) and the same procedure was followed.
Wash out period and follow-up for 2 weeks and during this time there was no drop out. Furthermore, patients were inquired about their wellbeing, any type of discomfort or adverse effect.
...
Analyzed (n=16). All the data were analyzed...
Not meeting inclusion criteria (n=7)

Declined to participate (n=3)

Other reasons (n=2)
Later, the participants were discharged from the BiHS hospital for home. After a two-week washout period, the experiment was reconducted.
was for the third time the soup under the same conditions. Subsequently a two-week washout period, under the same conditions.

and for the third time the experiment was.


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    Determination of isoflavones in soy-milk, <ref>Page 497 of 2019</ref>.
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were prepared from 100 g of soybean, powders of Soy-milk, and soups.
The same batch of soybean, mung dal, and masoor, The same batch of soybean,
throughout the study period, and the identification

utilized.

and quantification of daidzein and genistein in the three different foods.

in the three different foods.
liquid chromatographic (LC)-PDA analysis were performed through liquid chromatographic (LC)-PDA analysis.
As

were consumed after cooking, to follow the similar food habit condition, soups were prepared from the

are consumed after cooking, to follow the similar food habit condition, soups were prepared from the

and the determinants of isoflavones

were

were
Assessed in masoor and mung dal soups in women. Soy-milk (350 mL) contained 36.25 µg daidzein and 43.81 µg genistein. Masoor and mung dal soups (350 mL) contained daidzein (37.66 and 27.66 µg) and genistein (37.33 and 44.00 µg).

A food-chart was provided to all participants who were informed to avoid food containing isoflavones (such as mung dal, masoor dal, soybean, raw garlic, green beans, potatoes, sweet potatoes, chickpeas, wheat flour, grapefruits, dates, eggs, and nuts) at least for one week before and during the study.

Preparation of soy-milk, mung and masoor dal soups
The whole soybean amount (100-g) was immersed in drinking-water in a pot for 4-5 hours. The soft beans (water soaked) were washed with water, blended into mould using a kitchen blender. Water (500-mL) was added to the mould, boiled for three minutes, and stirred with a wooden kitchen stirrer. The milk was collected by squeezing through a pre-cleaned cloth filter and was boiled with medium temperature again for 20 minutes and stirred to reduce its volume up to ~350 mL.

Both mung and masoor dal amounts (100 g) were washed with clean water and 500 mL of water with a small amount of salt (NaCl) and turmeric powder were added to it then boiled with medium temperature for 25 minutes, and stirred with a wooden kitchen stirrer to reduce the volume up to ~350 mL.

Standard of Isoflavones
For measuring the determinants of isoflavones in human serum, authentic standards of daidzein and genistein, purchased from Sigma-Aldrich, were preserved at 4°C and at -20°C respectively.

Isoflavones extraction from human serum
In overall, 384 (8x16x3) serum samples were collected from 16 postmenopausal women during the three test periods, after serving soy-milk and soups.
prepared from
massoor
and
mung dal
. The blood samples were centrifuged at 1200 rpm, and the serum (~2 mL) was separated and immediately frozen at -20 °C until
analysis
. To avoid any
target compounds
biodegradation.
Isoflavones were extracted from the defrosted serum, and the sample was cleaned up using solid-phase extraction (SPE C18
Cartridge). The cartridge was conditioned with water (1 mL x 3) followed methanol (1 mL x 3) and then water again. The serum
sample was thawed and then passed through the conditioned SPE cartridge, then aqueous 5% methanol (800 µL) was added. The
isoflavones were eluted in ethyl acetate-acetonitrile mixture (1:1; 400 µL x 2). Sixty-four serum samples of 16 women from each test
foods were cleaned. Thus, the total number of cleaned serum samples was 192 (64 x 3) from 384 serum samples. The cleaned serum
samples were filtered through the LC samples filter having a pore size of 0.22 µm [PTFE (polytetrafluoro ethylene)]-syringe filter
 cartridge and transferred to a sample vial (2 mL) and analysis was done using LC-PDA.
For the chromatographic analyses, a Shimadzu SCL 10A vp LC system (Shimadzu, Kyoto, Japan) equipped with a PDA detector
(SPD-10A vp), a Supelco discovery reversed phase C18 column (25 cm×4.6 mm i.d. particle size: 5 µm) and a Rheodyne injector
(loop size 20 µ L) was used. Standards and cleaned extracts (100 µL) were injected through a Rheodyne injector.
Separations were carried out at 268 nm
. using an isocratic mobile phase of acetonitrile and water (ACN: H2O) (75: 25), with a flow rate of 0.5 mL/min, and the running time
was 10 minutes. The LC system was conditioned by passing mobile phase until the smooth baseline was obtained.
Determination of daidzein and genistein in human serum samples
The standard solutions of daidzein and genistein at the concentrations of 5, 10, 15, 20, and 25 µ g/mL were injected into LC-PDA. Two calibration curves were
established
from above five solutions of the daidzein and genistein by plotting peak area vs concentration (µg/g).
The certified standards of daidzein and genistein (10 µ g/mL) were injected separately.
The retention time of both
certified standard isoflavones were found to be 5.52 and 6.03 minutes respectively
. before serum extracts
analysis using LC-PDA. The serum samples were injected into the LC system with one injection of standard after each two or three
injections of the samples
. to control whether there was a deviation in the retention times of standards or not. By comparing the retention times of standard peaks
with that of the sample peaks; the possible daidzein and genistein peaks in chromatograms of the samples were determined.
The amount of daidzein and genistein
. in human serum samples
. were calculated from the external calibration curve of certified standard of daidzein and genistein
. taking into consideration that the peak area is in the midpoint of the curve (considering linearity of the curve). A number of unknown
analytes in the respective samples were identified using formula
(1)
:
Amount of unknown sample =

\[ \text{Peak Area sample} \times \text{Conc. Std} \div \text{Peak Area std} \times \text{Conc. matrix} \]

The daidzein and genistein serum concentration-time profiles
. for each individual
. and the mean concentrations at each dose
. were determined employing a non-parametric estimation of AUC (area under the curve) and Cmax
(maximum concentration). Trapezoidal formula was used for calculating AUC.
Statistical Analysis
Statistical analysis was performed using SPSS (Statistical package for social Science) software for Windows version 22 (SPSS Inc,
Chicago, Illinois, USA). For analysis, log transformation of the data was done.
The data was expressed as geometric mean ±SD (Standard deviation). The statistical significance of differences between the values
was analyzed by ANOVA (Analysis of variance). A
value of <0.05 was considered statistically significant.

Results

The present study concerned the determination of two isoflavones daidzein and genistein in the serum of 16 middle socioeconomic class postmenopausal women, whose mean age was 52.5 ± 5.8 years. Other clinical parameters selected were:

- pulse (mean ±SD, beats/min, 68.2 ± 6.4),
- systolic blood pressure (SBP) [mean ±SD, mmHg, 116.5 ± 6.7],
- diastolic blood pressure (DBP) [mean ±SD, mmHg, 76.5 ± 5.1],
- body mass index (BMI) [mean ±SD, kg/m² 25.7 ± 5.3] (Table 1).

Table 1: Demographic and clinical characteristics of postmenopausal women (n=16)

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<td>Pulse (beats/min)</td>
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<tr>
<td>SBP (mmHg)</td>
<td>116.5 ± 6.7</td>
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<tr>
<td>DBP (mmHg)</td>
<td>76.5 ± 5.1</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>25.7 ± 5.3</td>
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Results are expressed as mean ±SD; BMI=Body mass index; SBP=Systolic blood pressure; DBP=Diastolic blood pressure

An excellent symmetrical elution pattern was obtained in the chromatograms during 4-8 hours of duration (Fig. 3-5). The chromatograms showed efficient separation and correct integration of genistein in the blood samples. After four hours, degradation might have occurred because several small peaks were found earlier than the retention time of genistein. At an interval of 2, 8, 24, 36, and 48 hours, no elution pattern of genistein was observed. The determinant of genistein were responded in 8, 15, and 5 postmenopausal women following a single dose of orally-administered soy-milk, masoor, and mung dal soups during 4-6 hours respectively (Fig. 6). The determinations of the serum isoflavones genistein was found 2.6% in soy-milk, 3.8% in masoor dal soup and 3.7% in mung dal soup, respectively. No peak was observed at the retention time of daidzein which indicates its non-availability in the blood samples.

From the area in the chromatogram, the maximum concentration amount of each sample was calculated. The results are expressed as Cmax, AUC and Tmax (at time of the maximum concentration) [16,17,20-22].

Table 2 presents the geometric mean AUC and Cmax of serum isoflavones genistein of soy-milk, masoor, and mung dal soups. In soy-milk, the geometric mean AUC and the geometric mean Cmax of serum genistein was 0.82 ± 0.22 µg/mL and 0.17 ± 0.19 µg/mL. The geometric mean AUC and the geometric mean Cmax of serum isoflavones genistein in the masoor and mung dal soups.
soup was 1.01 ±0.32 µg, 0.45 ±0.32 µg, 1.12 ±0.31 µg, 0.63 ±0.31 µg per mL,
respectively. The basis of the calculation of AUC (Trapezoidal formula) is the supposition that there exists a AUC during 4-6 hour.
The pharmacokinetics data (AUC and Cmax) were calculated according to food items, using ANOVA. No significant differences
in serum genistein AUC were observed within the three food items (soy-milk vs masoor dal vs mung dal) though a significant (P =0.03) association
was found between the Cmax of serum isoflavones genistein of soy-milk and mung dal soup.

Table 2: AUC and Cmax of genistein in serum following a single dose of orally-administered soy-milk, masoor dal, and mung dal soups

<table>
<thead>
<tr>
<th>Food Items</th>
<th>AUC (µg/mL)</th>
<th>Cmax (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soy-milk</td>
<td>0.82 ±0.22</td>
<td>0.17 ±0.19</td>
</tr>
<tr>
<td>Masoor dal</td>
<td>1.01 ±0.32</td>
<td>0.45 ±0.32</td>
</tr>
<tr>
<td>Mung dal</td>
<td>1.12 ±0.31</td>
<td></td>
</tr>
</tbody>
</table>
Results are expressed as Geometric mean ±SD. One-way ANOVA (Post Hoc Bonferroni) is performed as the test of significance. *p < 0.05 is taken as level of significance; AUC= Area Under the Curve; C\text{max} = Maximum Concentration; ns = not significant.

Discussion
The determination of serum daidzein and genistein were assessed in Bangladeshi postmenopausal women after a single dose of orally administered soy-milk, soups of masoor and mung dals respectively. Results of two studies showed that that the AUC of soy genistein was greater than that of daidzein [21,23]. In the present study, the mean AUC and the C\text{max} of serum isoflavone genistein of soy-milk were 0.82 µg/mL and 0.17 µg/mL, respectively. Cassidy et al reported that the AUC of genistein was 54.06 µmol/L after the ingestion of soy-milk in postmenopausal women.
whereas the AUC of soy-milk genistein was less in the study subjects. The $C_{\text{max}}$ of soy-milk genistein among our participants was higher than that was found in the study of Cassidy et al.

The availability of genistein in serum was 2.6% after the ingestion of orally administrated single dose soy-milk and the amount was less compared to the study of Okabe et al.

Variations in results might be due to the physiological differences (intestinal condition) between races, due to the ingestion form of isoflavones, and for the location of cultivation too. Other factors, such as differences in the food matrix (liquid vs solid), composition of habitual diets (fiber, fat, protein), low content of isoflavones in foods, and study design might also play a role in the determination of serum daidzein and genistein among the postmenopausal women.

Generally, 1-6 hours are needed to obtain maximum plasma concentrations for free genistein and 4-6 hours for total genistein (aglycone + conjugates).

In the current study, the average time to obtain maximum concentration ($T_{\text{max}}$) of soy-milk was 4-6 hour. Another study showed that liquid matrix yields a faster absorption rate, higher peak serum concentration, and maximum time concentration than a solid matrix.

Blood sampling frequency and timing were fixed in the present study following the studies among Thai, the UK, and the USA menopausal women. No literature was found on the bioavailability or the determination of isoflavones in South-East Asian postmenopausal women. The absorption patterns of isoflavones among USA, UK, and other Asian menopausal women were not similar to those we found.

To the best of our knowledge, there is no study on the determination of isoflavones in non-soy food, such as masoor.
In the present study, the AUC and C$_{\text{max}}$ of genistein was 1.01 µg/mL and 0.45 µg/mL respectively in the masoor dal soup and, the AUC and C$_{\text{max}}$ of genistein was 1.12 µg/mL and 0.63 µg/mL respectively in mung dal soup that were similar to the bioavailability of isoflavones in soy-foods done by other investigators \[16,20,21,23,26\].

After consuming soy-milk, the mean AUC of serum genistein was 0.82 µg/mL, which was lower compared to masoor (1.01 µg/mL) and mung dal soups (1.12 µg/mL), however, the differences were not significant. The mean serum genistein concentrations (C$_{\text{max}}$) of masoor (0.45 µg/mL) and mung (0.63 µg/mL) dal soups were higher too compared to that of soy-milk (0.17 µg/mL) and there was a significant (P = 0.03) difference between soy-milk and mung dal soup. This finding is valuable and promising for general population, especially for menopausal women living in developing country such as Bangladesh, in which, soybean or such food are not widely known to the general population but pulses (dal) called the poor men’s protein, constitutes the most common foodstuff consumed by Bangladesh population almost every day. Consequently, the determinant of isoflavones genistein of masoor (3.8%) and mung dal (3.7%) soups (Figure 6) was also found high in postmenopausal women. It was not possible to compare the determination of isoflavones of non-soy foods in serum to other countries due to the lack of data. However, in the current study, determinant of isoflavones genistein of non-soy food was found more than soy milk. The present study had a couple of limitations. The sample size was small. Daidzein from the masoor and mung dal
soups and soy-milk did not show any peak at their retention time, which suggests the non-
availability of daidzein in blood samples, although genistein from the above three foods gave some peak
s at the allocated time but not full profile. It might happen due to following the literature-based fixed frequency and timing of blood
collection, which was not appropriate for the present study. Therefore, it was not possible to get the full profile and as such the serum
centration curve could be produced. Another limitation was the failure to collect urine samples from the study subjects because of
their unwillingness and lack of fund and time.
It may be assumed that different ethnicity, selection of appropriate time for blood collection
after the ingestion of food, quantity of individual and total isoflavones in foods
played an important role in the determination of isoflavones in the Bangladeshi postmenopausal women.

Conclusion
In conclusion, the findings of this pilot study suggest that the determination of isoflavones in non-soy foods (mung
and masoor dals) is comparatively better than soy foods in Bangladeshi postmenopausal women, and only isoflavones genistein show considerable
availability in women. It seems to be beneficial for menopausal women in our country as an alternative
of prescribing HRT.

Based on our findings, it is strongly recommended that before the ingestion of isoflavones-rich foods, the form of isoflavones (aglycones vs
glycosides) must be known, food matrix shall be considered, and a careful study design is necessary to know the complete
pharmacokinetic characteristics of daidzein and genistein in Bangladeshi postmenopausal women.

Ethical approval
All procedures performed in our protocol study, involving human participants
were in accordance with the ethical standards of the Ethical Review Committee of the Diabetic Association of Bangladesh (BADAS)
and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Written consent was obtained
from each postmenopausal participant
woman after the full explanation of the nature of test, purpose, and potential risks of all the procedures to be used in the study. Their
personal information was kept confidential.

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% of determinants of Masoor dal soup in serum in Sheet1!$B$100:$B$114

% of determinants of Mung dal soup in serum in Sheet1!$B$100:$B$114
| Sheet1!$A$100 | % of determinants of Soy-milk in serum | Sheet1!$A$100:$A$114 | General | 2.74 | 1.1599999999999999 | 2.1 | 1.75 | 2.1 | 2.37 | 1.67 | 1.47 | 1.67 | 1.367 | 2.5099999999999998 | 2.68 | 3.18 | 3.92 |
| Sheet1!$B$100 | % of determinants of Masoor dal soup in serum | Sheet1!$B$100:$B$114 | General | 2.2200000000000002 | 2.76 | 2.0499999999999998 | 1.72 | 0.91 | 2.5299999999999998 | 4.12 | 2.2000000000000002 | 2.48 | 3.4 | 2.68 | 3.69 | 2.5099999999999998 | 3.18 | 3.92 |
| Sheet1!$C$100 | % of determinants of Mung dal soup in serum | Sheet1!$C$100:$C$114 | General | 2.93 | 1.5999999999999999 | 2.1 | 1.75 | 2.1 | 2.37 | 1.67 | 1.47 | 1.67 | 1.367 | 2.5099999999999998 | 2.68 | 3.18 | 3.92 |
1.63
2.29
3.3
3.21

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