# Clinical efficacy of the co-administration of Turmeric and Black seeds (Kalongi) in metabolic syndrome — A double blind randomized controlled trial — TAK-MetS trial

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**KEYWORDS**
Black seeds;
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Co-administration;
Clinical efficacy;
Synergism;
Metabolic syndrome

**Summary**
*Objective:* To compare the clinical efficacy of Black seeds and Turmeric alone and its co-administration in lower doses among patients with metabolic syndrome (MetS).
*Design:* Double-blind-randomized-controlled trial.
*Setting:* Hijrat colony, Karachi, Pakistan.
*Intervention:* Apparently healthy males ($n=250$), who screened positive for MetS, were randomized to either Black seeds (1.5 g/day), Turmeric (2.4 g/day), its combination (900 mg Black seeds and 1.5 g Turmeric/day) or placebo for 8 weeks. Main outcome measures: body-mass-index (BMI), body-fat-percent (BF%), waist-circumference (WC), hip-circumference (HC), blood pressure (BP), lipid-profile (cholesterol, HDL-cholesterol, LDL-cholesterol and TG), fasting blood glucose (FBG) and c-reactive protein (CRP).
*Results:* At 4 weeks, compared to baseline, Black seed and Turmeric alone showed improvement in BMI, WC and BF%. Combination improved all parameters except HDL-cholesterol with lower FBG and LDL-cholesterol as compared to placebo. At 8 weeks, compared to placebo, Black seeds reduced lipids and FBG, while Turmeric reduced LDL-cholesterol and CRP. Interestingly, combination group with 60% dose of the individual herbs showed an improvement in all parameters from baseline. When compared to placebo, it reduced BF%, FBG, cholesterol, TG, LDL-cholesterol, CRP and raised HDL-cholesterol.

*Conclusion:* Turmeric and Black seeds showed improvement in all parameters of metabolic syndrome, when co-administered at 60% of doses of individual herbs with enhanced efficacy and negligible adverse-effects. The combination of Black seeds and Turmeric can therefore, be recommended with lifestyle modification as a starting point for patients with MetS to halt its future complications and progression.

## Introduction

Among non-communicable diseases (NCD), metabolic syndrome (MetS) is a major concern globally, specifically in South Asia, as it leads to a 2-fold increase in cardiovascular diseases (CVD) and a 1.5 fold increase in all-cause mortality.[1] Individual components are treated according to different consensus and guidelines, but for prevention and where there is no absolute indication for pharmacological intervention (obesity, pre-hypertension, low HDL-cholesterol and pre-diabetes), the first line of treatment is non-pharmacological which includes a healthy lifestyle, shown to reduce the incidence of MetS by 41% compared with placebo.[2,3]

Introducing early pharmacological treatment is controversial[4,5] as various pharmacological means used to prevent progression of MetS may not be compatible with each other. Several studies have supported the fact that lifestyle modifications are equally if not more effective in primary prevention of MetS and CVD.[6] Therefore, it is important to approach the individual components as a syndrome, rather than targeting clinical risk factors individually with aggressive pharmacological therapy, hence a multidisciplinary approach needs to be employed especially in primary prevention before the development of diabetes, hypertension and hyperlipidemia requiring definitive pharmacological therapies. It is also known that MetS might be more than the sum of its components, therefore, there is a need to use MetS criteria as endpoints for clinical trials. Trials using combinations of therapeutic interventions specifically targeted toward metabolic syndrome need to be conducted.[7]

The concept of complementary medicine is getting strength as evident by the fact that around 80% of the world population relies on complementary therapies mainly the herbs for its healthcare.[8] When dietary modification is of proven benefit as in the management of MetS, medicinal plants may serve as an adjuvant in the treatment and prevention of MetS as they contain a wide range of bioactive phyto-chemicals with diverse metabolic effects. These innovative dietary supplements can be proposed as the safe adjuvant treatments to reduce the progression, morbidity as well as the cost of treating MetS.[9] Moreover, use of complementary therapies is popular among patients with CVD risk as compared to the general population and among the most common complementary modalities used by individuals with CVD risk factors are natural products.[10] Similarly, natural products have contributed immensely in development of the modern medicine for cardiovascular disorders.[11]

Turmeric (*Curcuma longa*) and Black seeds (*Nigella sativa*) are some of the medicinal herbs which have been used for centuries and are acceptable to the public. We recently reported that the co-administration of these herbs produced enhanced effect in animal model of metabolic syndrome compared to when used alone.[12] The hypolipidemic and anti-oxidant effects of both the herbs are known,[13] although the combination has not yet been studied in a clinical trial for MetS.

In a study in Iran, 2 g/day of Black seeds per day given to patients with hypercholesterolemia for 4 weeks significantly reduced total cholesterol (Chol), low density lipoprotein (LDL-cholesterol) and triglycerides (TG) with no beneficial effects on fasting blood glucose (FBG) and high density lipoprotein (HDL-cholesterol).[14] Similarly, a 500 mg/day dose of powdered Black seeds administered with statin in dyslipidemic patients, improved the lipid profile more than with statin alone.[15] Black seed is known to reduce appetite, glucose absorption, hepatic gluconeogenesis, blood glucose, lipids and body weight as well as it simulates secretion of insulin from pancreas. It has also shown to improve glucose tolerance as efficiently as metformin with no significant adverse effects.[16]

Although Turmeric has been found to have some medicinal value in various diseases, but it is not well studied in its natural form; rather curcumin, an active principal of Turmeric has been widely studied.[17,18] Turmeric extract when given to hyperlipidemic obese patients, resulted in favorable effect.[19] Another trial reported that 3 months of Turmeric supplementation can decrease proteinuria, hematuria, and systolic blood pressure in patients suffering lupus nephritis[20] whereas another clinical study reported that ingestion of one dose of 6 g Turmeric increased postprandial serum insulin levels, but did not affect plasma glucose levels or glycemic index, in healthy subjects.[21]

In view of an emerging concept that herbs constituting combination of bioactive compounds possess ''effect enhancing and/or side-effects neutralizing'' properties,[22] we used the Black seeds and Turmeric in their natural form (powder) rather than their active constituents, shown in our lab recently to have synergistic interaction when used in combination.[12] On the other hand, animal studies have their impact on human health only when efficacy is proved in clinical studies.[23] Therefore, this study was aimed translating the beneficial effect of this animal study through a double blind randomized controlled clinical trial. Moreover, we aimed to see the effect of the combination among patients at risk of developing diabetes, hypertension and hyperlipidemia with no definitive indication for pharmacological management.

## Methodology

### Study setting and sample selection

Patients were recruited from a small community, Hijrat Colony, an urban—slum located at Mai-Kolachi, Karachi,
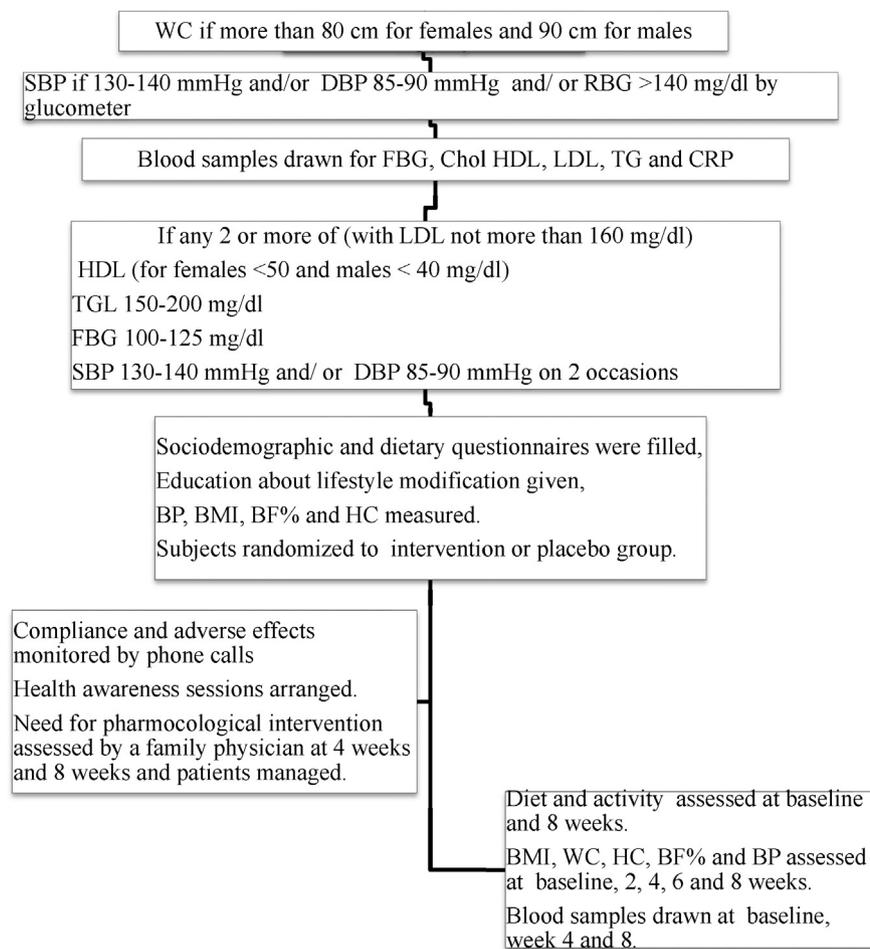
Figure 1 Study participants. Body mass index (BMI), waist circumference (WC), hip circumference (HC), body fat percentage (BF%), systolic blood pressure (SBP), diastolic blood pressure (DBP), random blood glucose (RBS), fasting blood glucose (FBG), low density lipoprotein (LDL), high density lipoprotein (HDL), cholesterol (Chol), triglycerides (TG), c-reactive protein (CRP).

Pakistan. After taking consent, male participants with central obesity as assessed by waist circumference screened positive for MetS (based on 3/5 criteria) were randomized to receive either intervention or placebo (Fig. 1).

## Sample size

The sample size was calculated for a 5% level of significance and a power of 80%. Assuming the population standard deviation of 3.62 from a previous study, considering systolic blood pressure as outcome,[20] a sample of 52 in each group was required to determine an effect size of 40%. Assuming a 20% non-participation rate, at least 62 subjects in each group were approached.

## Inclusion and exclusion criteria

Male residents of Hijrat colony having a waist circumference of >90 cm along with a total of three or more features of MetS who were not on any regular medications and who consented for a complementary therapy intervention were recruited. All subjects were with pre-diabetes (FBG with minimum 8–10 h of fasting between 100 and 125 mg/dl[24]),

dyslipidemia (LDL-cholesterol 130 mg/dl—160 mg/dl and/or triglyceride level of 150—200 mg/dl[24]) or pre-hypertension (systolic BP of 130—139 mmHg and a diastolic BP of 85—89 mmHg based on the average of two or more properly measured seated BP readings on each of two visits).[25]

Patients with known diabetes, hypertension or coronary heart disease or who required immediate pharmacological treatment were excluded. Patients already taking herbal supplements and patients on medications for hyperlipidemia or obesity were also excluded. Debilitated patients or patients on regular medications for chronic diseases like steroids, immune-suppressants and warfarin were excluded as well.

## Intervention

A total of 250 participants (accounting for 20% drop outs) (Fig. 1) were randomized to receive either powdered seeds of *Nigella sativa* (Black seed/Kalongi), powdered rhizome of *Curcuma longa* (Turmeric), combination of Turmeric powder and powdered Black seeds, or placebo (in a look-alike capsule of ispaghul husk) in a capsule taken twice daily for 8 weeks. All participants were given advice on lifestyle modifications.

## Randomization and drug management

A total of 60,000 look alike size 0 capsules (approximate filling capacity up to 1 g) were purchased from the market. Filling of Turmeric powder and powdered Black seeds as per

with curcumin doses ranging from 20 mg to 1000 mg. Considering the fact that Turmeric constitutes up to 5% curcumin,[34] the approximate doses of Turmeric would range from 400 mg to 20 g.

Considering the above findings the intervention groups were made as follows:

| Group | n | Intervention | Each capsule | Dose distribution | Dose/day |
|-------|---|--------------|--------------|-------------------|----------|
| Group 1 | 62 | Black seeds alone | 500 mg | 1 + 1 + 1 | 1.5 g/day |
| Group 2 | 63 | Turmeric alone | 800 mg | 1 + 1 + 1 | 2.4 g/day |
| Group 3 | 62 | Combination | 300 mg Black seeds + 500 mg Turmeric | 1 + 1 + 1 | 900 mg Black seeds/day + 1.5 g Turmeric |
| Group 4 | 63 | Placebo | 500 mg Ispaghula | 1 + 1 + 1 | 1.5 g/day |

protocol was done through professional drug filling machine which fills 12,000 capsules in one run. Blister packs of 10 were made and stored in refrigerator till it was dispensed to the participant. Sealed envelopes for randomization were made and randomization was done in blocks of 8 by the principal investigator. The patients and research officers were blinded to the intervention. Compliance was measured by weekly phone calls and drug diary. Participants were requested to visit the research site weekly for refilling, to monitor compliance or for monitoring adverse effects. In case, participants were unable to visit, the research officers arranged for a home visit. Emergency numbers were given to the participants for any possible adverse effects and a family physician was on call.

## Dose consideration

The dose of intervention was calculated based on the data from previous studies, where a dose of 3 g/day Black seeds for 3 months significantly reduced body weight, waist circumference and systolic BP[26] whereas, 2 g/day of Black seeds had a favorable but insignificant effect on cholesterol, LDL-cholesterol, TG and FBG in 6 weeks[27] although the same dose of Black seeds was significantly effective in reducing FBG when given for 3 months.[28] Another study showed that 1 g Black seeds taken before breakfast for 8 weeks resulted in significant reduction in Chol, LDL-cholesterol, TG and an increase in HDL-cholesterol.[29] The combination of 500 mg Black seeds and statin for two and a half months compared to statin alone caused a significant reduction in total cholesterol, LDL-cholesterol and TG with increase in HDL-cholesterol.[15]

With the availability of curcumin, the main constituent of Turmeric, most of the studies have used curcumin instead of whole Turmeric. Only one study was found on Turmeric, which reported an increase in post-prandial serum insulin secretion in healthy subjects with a single dose of 6 g.[21] Other studies on curcumin showed that curcumin at 500 mg for one week reduced cholesterol and triglycerides as compared to vitamin E,[30] while Turmeric extract (20 mg curcumin) improved lipids in healthy subjects in 30 days.[31] Curcumin 150 mg twice daily for 8 weeks compared to atorvastatin 10 mg or placebo had a favorable effect on endothelial dysfunction and inflammatory cytokines.[32] In another study on patients suffering from coronary artery disease, 45 mg/day of curcumin lead to an increase in HDL-cholesterol in 2 months.[33] Therapeutic effect has been seen

## Data collection

Two research officers were trained by the principal investigator. Socio-demographic data and data on dietary intake and physical activity were collected by a diet intake[35] and physical activity[36] questionnaire administered by the research officer. The total scores on the diet questionnaire (MEDFICTS) and international physical activity questionnaire (IPAQ) were calculated at baseline and at 8 weeks for a change in diet or physical activity score.

Blood sample was drawn at baseline and then at 4 and 8 weeks of intervention. Blood pressure, waist circumference (WC), Hip circumference (HC) and body mass index (BMI) were monitored every 2 weeks. FBG, c-reactive protein (CRP), Chol, LDL-cholesterol, HDL-cholesterol and TG were tested on Cobas c111.[37]

The patients as well as the research officers and principal investigator were blinded to intervention. Compliance and adverse effects were monitored weekly by drug diary, phone calls and fortnightly visits by research officers in case of no contact. Emergency contact number of a doctor was given to every participant to report any adverse symptom.

All patients (control and intervention group) were given education regarding lifestyle modifications (diet and exercise) for prevention and management of MetS at baseline, 4 weeks and at the end of study (8 weeks).

## Ethical consideration

Approval was obtained from the Ethics Review Committee (ERC) of the Aga Khan University (AKU) (2042-BBS-ERC-11). The trial was registered with Australian New-Zealand Clinical Trials Registry (ANZCTR) (Registration number, ACTRN12613001053718).

Confidentiality of patients was maintained by taking interview and blood samples in a private room. Data was preserved in lock and key and data entry was done in password protected computers. The questionnaires included only identity codes. Participants were allowed to leave the study at any point during the study period.

## Statistical analysis

Data was entered and analyzed on SPSS version 20. Means and standard deviation (SD) was computed. ANOVA was used to compare multiple independent groups if the data was normally distributed and for un-paired groups with
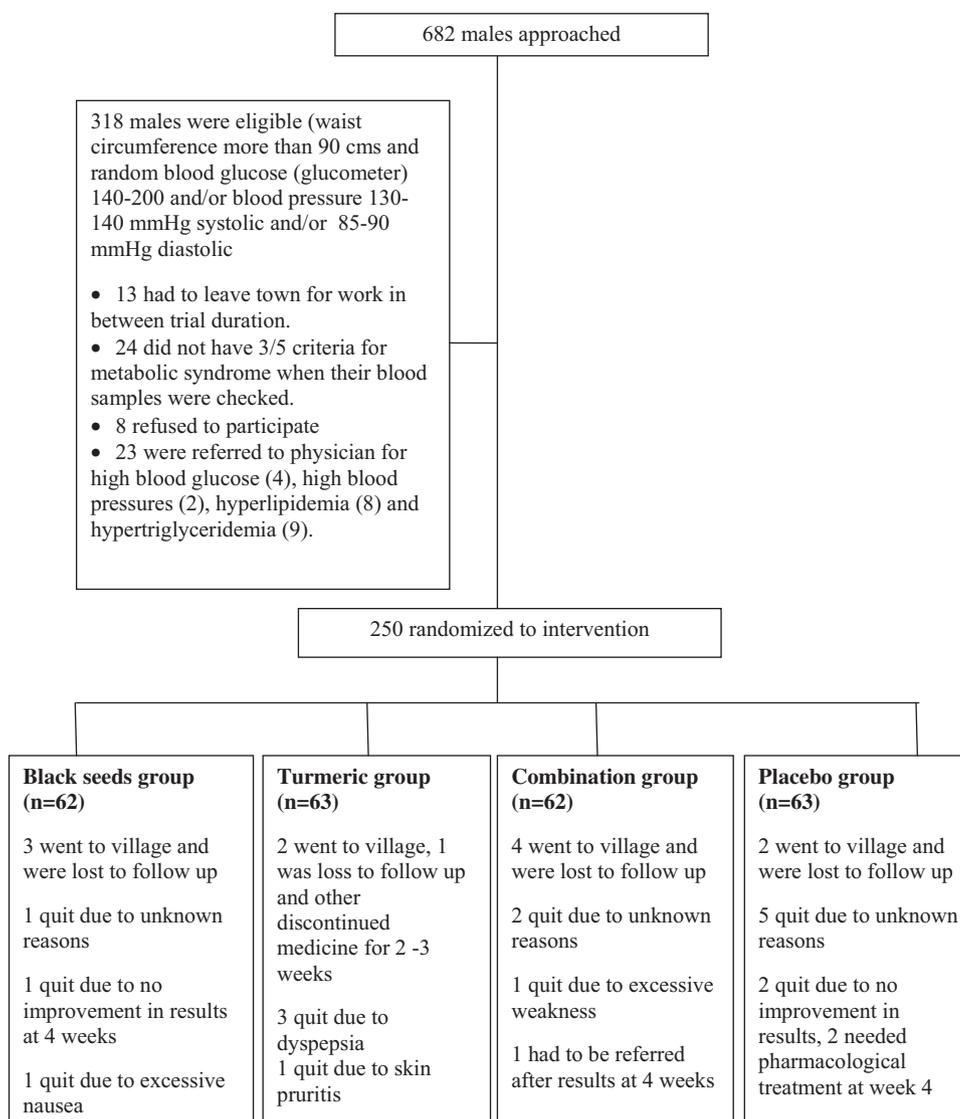
682 males approached

318 males were eligible (waist circumference more than 90 cms and random blood glucose (glucometer) 140-200 and/or blood pressure 130-140 mmHg systolic and/or 85-90 mmHg diastolic

- 13 had to leave town for work in between trial duration.
- 24 did not have 3/5 criteria for metabolic syndrome when their blood samples were checked.
- 8 refused to participate
- 23 were referred to physician for high blood glucose (4), high blood pressures (2), hyperlipidemia (8) and hypertriglyceridemia (9).

250 randomized to intervention

| Black seeds group (n=62) | Turmeric group (n=63) | Combination group (n=62) | Placebo group (n=63) |
|---|---|---|---|
| 3 went to village and were lost to follow up | 2 went to village, 1 was loss to follow up and other discontinued medicine for 2-3 weeks | 4 went to village and were lost to follow up | 2 went to village and were lost to follow up |
| 1 quit due to unknown reasons | | 2 quit due to unknown reasons | 5 quit due to unknown reasons |
| 1 quit due to no improvement in results at 4 weeks | 3 quit due to dyspepsia | 1 quit due to excessive weakness | 2 quit due to no improvement in results, 2 needed pharmacological treatment at week 4 |
| 1 quit due to excessive nausea | 1 quit due to skin pruritis | 1 had to be referred after results at 4 weeks | |

**Figure 2**    Follow up of study participants.

non-parametric data, Kruskalwallis test was used. For two unpaired groups student t-test was used for normally distributed data and Mann—Whitney $U$ test was used for un-paired non-parametric data. Wilcoxin rank sum was used for paired non-parametric data. For paired parametric data repeated measures of ANOVA was used. Multiple groups were compared with Bonferroni post hoc analysis. $p$ values < 0.05 were considered statistically significant, except when repeated measures of ANOVA was used, where a $p < 0.0014$ was considered significant. For each ANOVA that was significant at that level, a Bonferroni-corrected $p < 0.008$ was considered significant to adjust for multiplicity.

## Results

Among participants, 54% had attended a primary (22%) or a secondary school (32%) and 41% were illiterate or had attended informal schooling (madrassa); rest were at least graduates. Majority of the subjects were either from Khyber Paktoon Khwa (52%) or Punjab (27%); rest were from Sindh or Baluchistan provinces. Regarding family history, 26% had a family history of diabetes, 20% had hypertension in the family and 9% had a family history of ischemic heart disease. Among participants, majority (88%) was married and 38% of them were laborers. The frequency of smokers was 19% and 34% used chewable tobacco. Quantity of Black seeds and Turmeric taken per day as a part of cooked food was not different among the intervention groups.

Fig. 2 shows the algorithm of study participants, showing loss to follow up of 12.4% (31/250).

The mean age of participants was $44 \pm 13.3$ years, with a mean BMI of $27.4 \pm 3.9 \, kg/m^2$, WC of 98 cm and a body fat percentage (BF%) of $32 \pm 5.7$. The mean random blood glucose (RBG) was $158.4 \pm 66.6$ mg/dl with FBG of $120.4 \pm 14.4$ mg/dl. Mean systolic BP was $129.9$ mmHg $\pm 18.81$ and diastolic BP $79.9 \pm 12.5$ mmHg. Mean baseline cholesterol was $184 \pm 35$ mg/dl, LDL-cholesterol $115 \pm 26.6$ mg/dl, HDL-cholesterol $33.8 \pm 8.1$ mg/dl and TG $170.3 \pm 48.3$ mg/dl. There was a significant reduction in the dietary score which

**Table 1**  Baseline characteristics of participants in the TAK-MetS trial.

| Parameter | Black seeds, $n = 62$ | Turmeric, $n = 63$ | Combination, $n = 62$ | Placebo, $n = 63$ | p-value |
|---|---|---|---|---|---|
| Age (years) | 45.1 ± 11.7 | 42.4 ± 13.7 | 46.7 ± 14.8 | 41.57 ± 12.8 | 0.15 |
| Weight (kg) | 79.4 ± 9.6 | 77.0 ± 9.7 | 74.7 ± 10.5 | 76.7 ± 10.8 | 0.10 |
| BMI (kg/m$^2$) | 27.4 ± 3.1 | 28.1 ± 5.0 | 26.6 ± 3.6 | 27.5 ± 4.12 | 0.253 |
| WC (cm) | 100.7 ± 8.1 | 95.7 ± 12.4 | 99.1 ± 10.9 | 96.51 ± 13.1 | 0.09 |
| HC (cm) | 100.93 ± 12.1 | 99.4 ± 9.2 | 98.07 ± 8.2 | 99.28 ± 9.4 | 0.107 |
| BF% | 32.7 ± 4.8 | 32.5 ± 7.02 | 31.4 ± 4.05 | 31.2 ± 6.5 | 0.344 |
| SBP (mmHg) | 131.8 ± 20.2 | 129.7 ± 19.8 | 132.5 ± 17.8 | 125.5 ± 16.7 | 0.18 |
| RBG (mg/dl) | 150.1 ± 63.4 | 144.3 ± 76.4 | 157.6 ± 58.0 | 181.4 ± 11.0 | 0.11 |
| DBP (mmHg) | 82.5 ± 12.1 | 78.3 ± 14.0 | 81.5 ± 12.0 | 76.8 ± 11.5 | 0.058 |
| FBG (mg/dl) | 121.9 ± 14.1 | 117 ± 12.7 | 123.2 ± 15.5 | 119.1 ± 15.3 | 0.446 |
| LDL (mg/dl) | 110.2 ± 28.0 | 111.1 ± 22.2 | 119.1 ± 27.2 | 119.5 ± 27.3 | 0.12 |
| HDL (mg/dl) | 34.3 ± 7.8 | 33.9 ± 8.2 | 33.5 ± 9.1 | 33.7 ± 7.4 | 0.89 |
| Chol (mg/dl) | 184.3 ± 33.6 | 176.6 ± 29.6 | 193.9 ± 19.5 | 180.8 ± 23.3 | 0.06 |
| TG (mg/dl) | 169.5 ± 44.3 | 165.2 ± 40.1 | 182.7 ± 26.1 | 163.6 ± 42.7 | 0.09 |
| CRP (mg/dl) | 2.9 ± 2.4 | 2.12 ± 2.27 | 2.23 ± 2.04 | 2.10 ± 2.1 | 0.103 |

Values expressed as mean ± standard deviation (SD).
$p < 0.05$ considered significant, associations determined by ANOVA/Kruskalwallis.
BMI, body mass index; WC, waist circumference; HC, hip circumference; BF%, body fat percentage; RBG, random blood glucose; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; LDL, low density lipoprotein; HDL, high density lipoprotein; Chol, cholesterol; TG, triglycerides; CRP, c-reactive protein.

was lower at 4 weeks and 8 weeks in all the four groups but this difference was not significant when the treatment and control groups were compared to each other.

Table 1 shows the mean baseline clinical characteristics among the intervention and control groups, which was not significantly different.

At 4 weeks, there was a significant difference in the weight, HC and LDL-cholesterol levels among groups as shown in Table 2. Applying Mann—Whitney U test, FBG ($p = 0.04$) and LDL-cholesterol ($p < 0.001$) were significantly lower in the combination group when compared to placebo, while the weight ($p$, 0.001) and HC ($p$, 0.009) were

significantly lower in combination group when compared to Black seeds group. Adverse effects reported till the 4th weeks were mild, like nausea and dyspepsia (Fig. 2).

Table 3 shows comparison of parameters of MetS among treatment and control groups at 8th week using ANOVA/Kruskalwallis test. At the end of 8 weeks, TG ($p$, 0.001), cholesterol ($p$, 0.02), FBG ($p$, 0.02), LDL-cholesterol ($p < 0.001$) and HDL-cholesterol ($p$, 0.02) in the group receiving Black seeds alone were significantly improved compared to placebo group by Mann—Whitney U test, although only cholesterol ($p < 0.001$) and TG ($p < 0.001$) were significantly reduced when compared to baseline (Table 4). Comparison

**Table 2**  Comparison of parameters of MetS at 4 weeks of intervention among the treatment and control group in TAK-MetS trial.

| Parameter | Black seeds, $n = 62$ | Turmeric, $n = 63$ | Combination, $n = 62$ | Placebo, $n = 63$ | p |
|---|---|---|---|---|---|
| BMI (kg/m$^2$) | 27.2 ± 3.0 | 27.6 ± 4.8 | 26.2 ± 3.6 | 27.3 ± 3.5 | 0.119 |
| Weight (kg) | 78.8 ± 9.3 | 75.5 ± 9.5 | 73.7 ± 10.5 | 76.1 ± 10.4 | 0.04* |
| WC (cm) | 99.4 ± 7.8 | 95.1 ± 12.3 | 97.5 ± 9.9 | 95.6 ± 13.5 | 0.218 |
| HC (cm) | 101.6 ± 7.5 | 98.9 ± 9.1 | 97.25 ± 7.4 | 99.3 ± 8.9 | 0.04* |
| BF% | 31.4 ± 5.0 | 31.2 ± 6.6 | 29.6 ± 3.6 | 30.7 ± 6.3 | 0.25 |
| SBP (mmHg) | 127.3 ± 18.0 | 128.1 ± 17.7 | 127.6 ± 15.7 | 127.6 ± 14.9 | 0.92 |
| DBP (mmHg) | 80.0 ± 10.7 | 77.7 ± 11.5 | 78.9 ± 10.1 | 78.7 ± 10.3 | 0.61 |
| FBG (mg/dl) | 116 ± 34.2 | 114.4 ± 15.8 | 112.1 ± 18.1 | 118.7 ± 21.6 | 0.07 |
| LDL (mg/dl) | 111.0 ± 28.1 | 112.6 ± 21.4 | 106.8 ± 24.6 | 122.6 ± 19.2 | 0.001* |
| HDL (mg/dl) | 35.1 ± 6.8 | 35.1 ± 7.1 | 34.1 ± 8.8 | 33.7 ± 7.7 | 0.483 |
| Chol (mg/dl) | 180 ± 41.1 | 170.3 ± 27.8 | 180.1 ± 31.0 | 181.8 ± 34.7 | 0.25 |
| TG (mg/dl) | 167.8 ± 58.5 | 163.1 ± 27.1 | 162.5 ± 44.9 | 161.7 ± 36.3 | 0.97 |

Values expressed as mean ± standard deviation (SD).
* $p$ value < 0.05, associations determined by ANOVA/Kruskalwallis test
BMI, body mass index; WC, waist circumference; HC, hip circumference; BF%, body fat percentage; RBG, random blood glucose; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; LDL, low density lipoprotein; HDL, high density lipoprotein; Chol, cholesterol; TG, triglycerides.

Table 3 Comparison of parameters of MetS among treatment and control groups at 8th week in TAK-MetS trial.

| Parameter | Black seeds, $n = 61$ | Turmeric, $n = 63$ | Combination, $n = 62$ | Placebo, $n = 63$ | p-value |
|---|---|---|---|---|---|
| BMI (kg/m$^2$) | $26.9 \pm 2.9$ | $27.2 \pm 5.03$ | $25.9 \pm 3.4$ | $27.3 \pm 3.6$ | 0.09 |
| Weight (kg) | $78.1 \pm 9.2$ | $74.6 \pm 9.6$ | $72.9 \pm 10.0$ | $75.9 \pm 10.5$ | 0.04[*] |
| WC (cm) | $98.8 \pm 8.4$ | $94.7 \pm 12.2$ | $96.18 \pm 9.7$ | $95.9 \pm 12.7$ | 0.311 |
| HC (cm) | $100.9 \pm 7.5$ | $96.1 \pm 14.1$ | $96.5 \pm 7.2$ | $99.4 \pm 8.8$ | 0.008[*] |
| BF% | $30.2 \pm 4.8$ | $30.25 \pm 6.1$ | $28.1 \pm 3.6$ | $30.6 \pm 6.00$ | 0.04[*] |
| SBP (mmHg) | $126.5 \pm 18.4$ | $125.3 \pm 16.0$ | $124.5 \pm 14.6$ | $125.5 \pm 14.7$ | 0.95 |
| DBP (mmHg) | $78.8 \pm 11.4$ | $75.8 \pm 10.6$ | $76.07 \pm 9.9$ | $78.8 \pm 10.4$ | 0.37 |
| FBG (mg/dl) | $111.9 \pm 33.6$ | $116.1 \pm 19.6$ | $101.7 \pm 17.0$ | $116.1 \pm 24.5$ | <0.001[*] |
| LDL (mg/dl) | $103.3 \pm 29.9$ | $105.4 \pm 20.5$ | $93.6 \pm 18.1$ | $138.9 \pm 13.2$ | <0.001[*] |
| HDL (mg/dl) | $35.4 \pm 6.9$ | $35.5 \pm 7.0$ | $36.2 \pm 7.8$ | $33.4 \pm 7.7$ | 0.10 |
| Chol (mg/dl) | $162.8 \pm 39.1$ | $165.0 \pm 26.4$ | $165.1 \pm 29.6$ | $179.2 \pm 28.9$ | 0.03[*] |
| TG (mg/dl) | $141.8 \pm 64.3$ | $153.9 \pm 31.4$ | $145.7 \pm 43.5$ | $162.3 \pm 32.2$ | 0.003[*] |
| CRP (mg/dl) | $2.4 \pm 2.2$ | $1.4 \pm 1.01$ | $1.5 \pm 1.16$ | $2.8 \pm 2.5$ | <0.001[*] |

Values expressed as mean ± standard deviation (SD).
[*] $p$ value < 0.05, associations determined by ANOVA/Kruskalwallis.
BMI, body mass index; WC, waist circumference; HC, hip circumference; BF%, body fat percentage; RBG, random blood glucose; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; LDL, low density lipoprotein; HDL, high density lipoprotein; Chol, cholesterol; TG, triglycerides; CRP, c-reactive protein.

of combination and placebo groups, showed that there was a significant improvement in CRP ($p$, 0.007), BF% ($p$, 0.04), Chol ($p$, 0.02), FBG ($p < 0.001$), TG (0.03), LDL-cholesterol ($p < 0.001$) and HDL-cholesterol ($p$, 0.04) between the two groups. Turmeric group showed improvement in cholesterol ($p$, 0.009), LDL-cholesterol ($p < 0.001$) and CRP ($p < 0.001$) when compared to placebo group. Combination group showed a significant reduction in HC ($p$, 0.001), BF% ($p$, 0.008), weight ($p < 0.001$), TG ($p$, 0.02) and CRP ($p < 0.001$) when compared to Black seeds group.

No new adverse effects were reported from 4th till 8 weeks except one patient who withdrew due to weakness and weight reduction after 6 weeks.

Improvement in the parameters at 4th week and 8th week from baseline is shown in Tables 4 and 5 respectively. At 8 weeks, there was a significant improvement in BMI and BF% among the placebo group compared to baseline.

## Discussion

This clinical trial was an attempt of translating efficacy of co-administration of the two herbs, which were found to improve parameters of MetS in fructose-fed rats at lower doses of individual herbs.[12]

Participants with MetS were recruited, with any 3 positive criteria for MetS with borderline dyslipidemia, hypertension and impaired fasting glucose. Although, this excluded the effect of pharmacological interventions, but due to a definitive indication of lifestyle modifications, including dietary modification and physical activity, all the participants irrespective of their assigned group were advised lifestyle modifications. Consequently, we found an improvement in BMI and BF% among the treatment as well as placebo group. A part of the improvement in parameters of MetS can be attributed to the reduction in body weight and body fat, but treatment was clearly an adjuvant to lifestyle modifications which were similar across all groups indicated by the

diet and activity scores at baseline and 8 weeks of intervention. Most of the improvement observed with intervention was evident at 4 weeks of intervention.

Turmeric alone (2.4 g/day), although was more effective than placebo in reducing the total cholesterol, LDL-cholesterol and CRP, but had no effect on the blood glucose. This effect on lipids was not significant when compared to baseline. Similar effects on lipids have been found in another study without significant effect on blood glucose with 2 g of Turmeric powder taken for 8 weeks.[38]

When given alone, Black seeds reduced cholesterol and triglycerides but had no effect on LDL-cholesterol and HDL-cholesterol as compared to baseline data. Previous studies also found insignificant increase in HDL-cholesterol when Black seeds powder was given for 2 month, though significant improvement was observed in 6 months.[15,39] There was also an improvement in fasting blood glucose when compared to placebo but the difference was not significant when compared to baseline in the Black seed alone group.

The Turmeric and Black seeds in combination improved all the components of lipids along with blood glucose and CRP at 8 weeks of treatment, though CRP was not altered even at a higher dose of Turmeric when given alone as compared to baseline.

Due to a marginal improvement in systolic and diastolic BP in all the treatment groups, the difference between groups was not significant at 4 weeks or 8 weeks. It is possible that improvement in BF% and BMI has probably resulted in an improvement in BP among all the treatment groups which is a known phenomenon,[40] but interestingly this effect was not observed in the placebo group despite a reduction in BMI indicating an independent effect on BP. The effect of Black seeds on BP is already known,[39] although when used alone Black seeds did not significantly reduce the BP when compared to baseline, yet the improvement of BP among combination group can be explained considering Black seeds as a component along with Turmeric. Constituents of Turmeric such as curcuminoids although have

**Table 4** Parameters compared from baseline in treatment and control groups.

|  | BMI, kg/m$^2$ | WC, cm | HC, cm | BF% | LDL, mg/dl | HDL, mg/dl | Chol, mg/dl | TG, mg/dl | FBG, mg/dl | SBP, mmHg | DBP, mmHg | CRP, mg/dl |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B | <0.001* | <0.001* | 0.063 | <0.001* | 0.137 | 0.406 | <0.001* | <0.001* | 0.03 | 0.002 | 0.006 | 0.347 |
| B + T | <0.001* | <0.001* | <0.001* | <0.001* | <0.001* | <0.001* | <0.001* | <0.001* | <0.001* | <0.001* | <0.001* | 0.01** |
| T | <0.001* | <0.001* | <0.001* | <0.001* | 0.006 | 0.006 | 0.008 | 0.019 | 0.373 | 0.009 | 0.04 | 0.08 |
| P | <0.001* | 0.005 | 0.59 | 0.001* | 0.45 | 0.86 | 0.68 | 0.87 | 0.56 | 0.11 | 0.03 | 0.789 |

Repeated measures of ANOVA used to compare all parameters at 4 and 8 weeks from baseline.

* $p < 0.0014$ considered significant.

CRP compared at 8 weeks from baseline by Wilcoxin rank sum test.

** $p < 0.05$ considered significant.

B, Black seeds; T, Turmeric, P, placebo; BMI, body mass index; WC, waist circumference; HC, hip circumference; BF%, body fat percentage; RBG, random blood glucose; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; LDL, low density lipoprotein; HDL, high density lipoprotein; Chol, cholesterol; TG, triglycerides; CRP, c-reactive protein.

**Table 5** Parameters compared at 4 and 8 weeks from baseline in treatment and control groups.

| Group | Week | BMI, kg/m$^2$ | WC, cm | HC, cm | BF% | LDL, mg/dl | HDL, mg/dl | Chol, mg/dl | TG, mg/dl | FBG, mg/dl | SBP, mmHg | DBP, mmHg |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B | 4 | 0.004* | <0.001* | 0.063 | <0.001* | 0.137 | 0.406 | 0.65 | 1.00 | 0.03 | 0.006 | 0.006 |
|  | 8 | <0.001* | <0.001* |  | <0.001* |  |  | <0.001* | 0.001* |  |  |  |
| B + T | 4 | <0.001* | <0.001* | <0.001* | <0.001* | 0.002* | 0.88 | <0.001* | 0.001* | 0.002* | <0.001* | 0.003* |
|  | 8 | <0.001* | <0.001* | <0.001* | <0.001* | <0.001* | <0.001* | <0.001* | <0.001* | <0.001* | <0.001* | <0.001* |
| T | 4 | <0.001* | <0.001* | 0.001* | <0.001* | 0.006 | 0.006 | 0.008 | 0.019 | 0.373 | 0.009 | 0.04 |
|  | 8 | <0.001* | 0.001* | 0.28 | <0.001* |  |  |  |  |  |  |  |
| P | 4 | 0.005* | 0.005 | 0.59 | 0.01 | 0.45 | 0.86 | 0.68 | 0.87 | 0.56 | 0.11 | 0.03 |
|  | 8 | 0.001* |  |  | 0.001* |  |  |  |  |  |  |  |

Repeated measures of ANOVA used to compare all parameters at 4 and 8 weeks from baseline.

* $p < 0.008$ considered significant.

B, Black seeds; T, Turmeric, P, placebo; BMI, body mass index; WC, waist circumference; HC, hip circumference; BF%, body fat percentage; RBG, random blood glucose; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; LDL, low density lipoprotein; HDL, high density lipoprotein; Chol, cholesterol; TG, triglycerides; CRP, c-reactive protein.

been shown to have dose-dependent effects on vascular resistance in animals but there are no clinical trials validating this effect in patients with MetS[41] and even in our study Turmeric alone did not affect the BP significantly.

Frequency of adverse effects like dyspepsia was the highest when Turmeric was used alone while Black seeds alone and the combination of both herbs at the 60% of individual doses did not show any significant adverse effects, which indicates that either the lower dose of Turmeric in combination form is contributing factor in disappearing side effect of Turmeric or the Black seeds, has some role in neutralizing this side effect of Turmeric. Interestingly, the dropout rate was low, possibly because of enthusiasm among participants. Regular lifestyle modification advice motivated them to improve their diet although there was no change in activity scores at the end of the study. This might be due to the fact that most of the participants belonged to labor class working from dawn to dusk and they lived in small houses with no facilities for physical activity. Change in diet alone resulted in weight reduction in the placebo group as ispaghula husk in such a small quantity is unlikely to have any role in weight reduction.[42]

The interventions therefore were found to be efficacious when given with advise on lifestyle modification, and the co-administration of Black seeds and Turmeric, though given in lower doses was the most effective in improving all parameters of MetS when compared to effect of individual herbs. The acceptability of the population to these medicinal herbs was also commendable and their enthusiasm was evident by their efforts to adhere to lifestyle modifications, despite the fact that most of the participants were asymptomatic and hence unaware of their risk factors when the study started.

## Conclusion

Through this clinical trial, the efficacy of co-administration of Black seeds and Turmeric was proven among a high risk population with MetS. The results revealed significant beneficial effect of intervention on all parameters of MetS, with negligible adverse effects, when prescribed with advice on lifestyle modification. The combination of Black seeds and Turmeric can therefore be recommended for regular use along with advice on dietary modification and physical activity as a starting point for patients at risk of MetS to halt the future complications and progression of this syndrome. Future studies to validate the long term preventive role of these medicinal herbs in metabolic syndrome are warranted.

## Conflict of interest

The authors report no conflicts of interest to disclose.

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