ORIGINAL ARTICLE

Comparative and Combined Synergetic Effects of Black Coffee and Metformin in Treatment of Type 2 Diabetes Mellitus

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ABSTRACT

Objective: To determine the comparative and combined synergetic effect of Black Coffee and Metformin in treatment of type 2 diabetes mellitus in mice model.

Study Design: Experimental, Randomized control study.

Place and Duration of Study: This study was conducted over a period of one year from May 2017 to April 2018 at Pharmacology laboratory and Multidisciplinary laboratory of Islamic International Medical College in collaboration with National Institute of Health, Islamabad.

Materials and Methods: A total of 50 male Balb/c albino mice were taken, group 1 was non-diabetic normal control (n=10) and diabetes was induced in experimental group (n=40) by using low dose streptozotocin (40mg/kg). After confirmation, diabetic mice were further divided into four groups (10 mice/group). Group 2 was diabetic control and remaining 3 groups were treated with black coffee, metformin and combination of both, respectively for 45 days. Blood samples were taken by intracardiac puncture for HbA_{1C}, which shows the long-term control. Statistical analysis was done applying SPSS 21. Comparisons of means of HbA_{1C} between the groups were analyzed using one way ANOVA (post hoc tuckey test). P value of <0.05 was considered significant.

Results: Black coffee treated (Group 3), metformin treated (Group 4) and combination of black coffee and metformin treated (Group 5) had significantly decreased serum HbA_{1C} levels in comparison with those found in diabetic control (Group 2) (P<0.05).

Conclusion: Combination of black coffee and metformin significantly decreases serum HbA_{1C} levels in diabetic mice as compared to metformin or black coffee treated diabetic mice separately.

Key Words: *Diabetes Mellitus, HbA_{1C}, Metformin, Pancreatic Islets Cells*

Introduction

Diabetes mellitus is spreading worldwide pandemic that is a raising wellbeing concern with numerous difficulties and an expanding ubiquity.[1] Despite stunning change over both essential and clinical therapeutic sciences, diabetes mellitus is at present a hopeless life-long sickness, rapidly affecting both genders.[2] Diabetes mellitus may be not a single illness rather an aggregation of metabolic issue connected with secondary harm on various organ framework that incorporate cardiovascular diseases, stroke, nephropathy, retinopathy, neuropathy, gangrene and even amputations.[3,4] Diabetes mellitus affects 382 million people globally.[5,6] The current prevalence of this disease in Pakistan is 11.77% according to a study conducted in 2015.[7] The pandemic of type 2 diabetes mellitus has been met by emerging approach and clinical tactics, including the generally-accepted commendation to institute drug therapy concomitant with lifestyle changes.[8,9] Metformin an older and broadly acknowledged prime agent, has antihyperglycemic properties and also other important functions such as enhancement in endothelial dysfunction, hemostasis and oxidative stress, insulin resistance, lipid profiles, and fat redistribution.[10] Metformin's efficacy, security profile, beneficial cardiovascular and metabolic effects and its capacity to be associated with other antidiabetic agents makes this drug the first glucose lowering agent of choice when treating patients with type 2 diabetes.[11,12] Even the most explored out oral antidiabetic drugs sometimes fail as monotherapy and eventually different drug combinations are to be

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considered.[10,13] Many new trends in management of diabetes are therefore being considered nowadays and the approach for diabetes treatment is moving towards the incorporation of more organic natural products which tend to counter the root causes of the disease.[14,15] Black coffee is the most commonly used energy boosting beverage worldwide. Recent studies have shown many potential health benefits of black coffee in humans.[16] Besides keeping you alarm and wakeful, coffee is the richest and intense source of antioxidants that work as meager warriors battling and securing against free radicals inside human body.[17]

Caffeine in black coffee regulates the hyperglycemic effect in diabetic patients by increasing insulin release from pancreatic beta cells by the sensitization of the ryanodine receptor and activation of 5'-adenosine monophosphate–activated protein kinase (AMPK). Caffeine also up regulates the insulin-like growth factor 1 signaling, which is responsible for enhanced insulin sensitivity as well as insulin secretion. Chlorogenic acid has an eloquent role in glucose metabolism by decreasing glucose output in the liver and promoting the synthesis of the "homeodomain transcription factor IDX-1", which directs beta cells to counter the increased glucose levels in plasma. Important antioxidants in coffee include hydrocinnamic acids and polyphenols, Hydrocinnamic acids are very effective at neutralizing free radicals and preventing oxidative stress. Polyphenols counter the increased insulin resistance and escalates the insulin sensitivity.[18,19,20,21]

The cumulative body of suggestion about lower frequency of diabetes among coffee users is conclusive enough to prove a positive impact of coffee consumption on the development of type 2 diabetes mellitus. Different studies have been conducted to evaluate the preventive role of black coffee on type 2 diabetes.[22]

To the best of our knowledge, no study has been conducted to explore the effect of black coffee as adjunct to metformin in treatment of diabetes patients. If this agent works to improve insulin sensitivity and decreases the insulin resistance, then this cost effective and easy administered agent with overall beneficial effects on health can be used in place of other antidiabetic agents who are usually given with metformin, when metformin monotherapy fails. This experimental study was done to determine the comparative and combined synergistic effect of Black Coffee and Metformin in treatment of type 2 diabetes mellitus in mice model.

Materials and Methods

This randomized control trial was carried out at Pharmacology laboratory and Multidisciplinary research laboratory at Islamic International Medical College with the collaboration of National Institute of Health (NIH) Islamabad Pakistan. Before starting the study, a formal approval by the Ethics Review Committee of Islamic International Medical College, Riphah International University, was taken. The duration of this study was 12 months (May 2017 to April 2018). A total of 50 healthy, 6-8 weeks old male, weighing 30-50 g albino Balb/c mice were included in the study. All the mice were accommodated in standard cages which were made up of plastic and placed on metallic racks, at the Animal house of NIH, Islamabad. Room number 13 was allocated for the research procedure. The mice had free access to tap water through the inverted bottles of 250ml capacity fixed on top of the cages. These bottles were cleaned and filled on daily basis according to the protocol of the animal house. The normal standard diet was prepared at the NIH, which was served with standard food pellets. Animal house atmosphere was maintained at room temperature of 20 ± 2 oC with relative humidity of 50-70% with a light and dark cycle of 12 hours each. After acclimatization for 1 week, the mice were randomly divided into two groups; 10 mice were allocated to Group 1 and remaining 40 mice were allocated to the Experimental Group. Group 1 was labeled as Normal Control and was given normal diet for 5 days whereas the Experimental group was given normal diet plus streptozotocin, (STZ), (40mg/kg/day)[23] intraperitoneally for consecutive 5 days. After 5 days, confirmation of diabetes in experimental group was done by measuring and comparing fasting blood glucose levels (mg/dl) with Group 1. The blood sample was taken from lateral tail vein of all mice with 1 ml syringe and the blood glucose levels were measured by using EASY GLUCO Ultra Plus Auto Coding meter Iso tech Co. Ltd. Experimental group was then further divided into four groups i.e. 2

(Diabetic control), 3 (Black coffee treated), 4 (Metformin treated) and 5(Combination of Black coffee and Metformin treated).

Group 2 mice were given normal standard diet only. Group 3 mice were given normal diet mixed with Black Coffee (5g/kg/day)[24] orally for 45 days. Group 4 mice were given normal diet along with Metformin (200mg/kg/day)[25] orally mixed in drinking water for 45 days. Group 5 mice were given normal diet mixed with Black Coffee (5g/kg/day) orally and Metformin drug (200mg/kg/day) orally mixed in drinking water for 45 days. After 45 days of treatment, final sampling of the experiment was done from group 3, 4 and 5 which included HbA_{1C} (%) by cardiac puncture. Fixed time nephelometry certified by National Glycohemoglobin Standardization Program (NGSP) was employed for HbA_{1C} estimation (%). For this study, PA50 fully auto specific protein analyzer was used.

Statistical analysis was done by applying the Statistical Package for Social Sciences version 21 (SPSS 21). Results were documented as Mean ± Standard Error of Mean (SEM). Comparison of means of HbA_{1C} (%) among the five groups were analyzed by using the One way ANOVA and Post Hoc Tuckey tests. P value of <0.05 was considered significant.

Results: HbA_{1C} (%)

The result of Mean ± SEM of HbA_{IC} (%)in group 2 (7.82 ± 0.11) was significantly higher than group 1 (P value >0.05) as shown in Table I. While on comparison of Mean ± SEM of HbA_{IC} (%) in group 3 (6.02 ± 0.29), group 4 (5.76 ± 0.45) and group 5 (4.90 ± 0.28) were significantly lower than group 2 (P value <0.05). Table I shows the comparison of mean ± SEM of HbA_{IC} (%) of all the groups.

Table I: Comparison of Mean ± SEM of HbA_{IC} (%) in all five Groups

Groups	Group 1 Control	Group 2 Diabetes	Group 3 Black Coffee	Group 4 Metformin	Group 5 Comb:of Black Coffeeand Metformin
Mean ± SEM of HbA_{1C} (%)	4.75 ± 0.13	7.82 ± 0.11	6.02 ± 0.29	5.76 ± 0.45	4.90 ± 0.28
P value	0.000*				

P value <0.05*

Table II: Multiple Comparison of Mean Difference of Hba1c (%) of Control and Experimental Groups

Groups	Mean Difference	P value
1 vs 2	3.07	0.000*
1 vs 3	1.27	0.020
1 vs 4	1.01	0.102
1vs 5	0.15	0.995
2 vs 3	1.79	0.000*
2 vs 4	2.06	0.000*
2 vs 5	2.91	0.000*
3 vs 4	0.26	0.961
3 vs 5	1.12	0.053
4 vs 5	0.85	0.219

The graphical representation of HbA_{1C} (%) results shows a marked difference between the Black coffee, Metformin, Combination of Black coffee and Metformin as compared to the diabetic control mice. Metformin has a better role in lowering HbA_{1C} (%) than black coffee, yet the role of combination therapy is astonishing in aspect to lower HbA_{1C} (%) nearly to the normal control range.

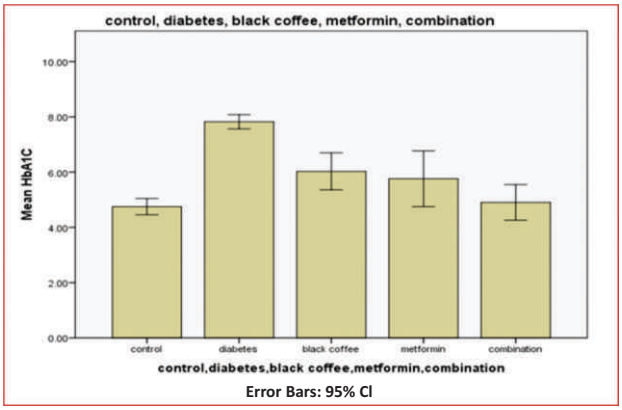

Fig 1: Graphical representation of HbA_{1C} (%) results

Discussion

The results of present study confirm that hyperglycemia induced by streptozotocin, is ameliorated by all the experimental agents to an appreciable extent, yet the result of combination therapy of Black coffee and Metformin is very impressive.

In present study, the antidiabetic effect of Metformin is seen in group 4 and in combination with Black coffee in group 5. Improvement of HbA_{1C} in Group 4 is supported by study of S.H Chung et al., who compared the antidiabetic effect of metformin and compound k in diabetic db/db mice and proposed that normalization of raised plasma glucose levels and improvement in insulin levels in metformin treated group.[26] In present study, improvement in

HbA_{1C} in Groups 3 and 5 along with better improvement in Group 5 which is given combination of black coffee and metformin is observed. Kobayashi M et al., also demonstrated similar results who used black coffee, caffeine extract, decaffeinated coffee against different sets of experiments to analyze the preventive part of black coffee on development of STZ induced diabetes and also the reversal of worsening offered by STZ induced hyperglycemia in male C57 BL/ 6J mice. He demonstrated that continuous Black coffee ingestion prevented the development of STZ induced diabetes mellitus and also revealed that the black coffee can recover the hyperglycemia induced metabolic changes by analyzing the biochemical and histopathological parameters.[27] Mukesh Doble et al., did a study to demonstrate comparative and combined effects of plant phenolic compounds, chlorogenic acid and ferulic acid with metformin and thiazolodinedione on the uptake of 2-deoxyglucose (2DG) by L6 myotubes of rats. He established that a combination of different concentrations of chlorogenic acid and metformin or THZ, has a synergistic effect in the uptake of 2DG with a maximum of 5.0 and 5.3 times respectively, when contrasted to the control. Ferulic acid in combination with metformin or THZ has likewise displayed a synergistic impact and the 2DG uptake increases by 4.98 and 5.11 fold when compared to the control.[28]

Hence when Metformin is given in combination with black coffee, HbA_{1C} which shows the long term control of diabetes is improved demonstrating that black coffee can be used as adjunct to metformin in the treatment of type 2 diabetes mellitus.

Conclusions

Black coffee significantly lowers HbA_{1C} levels in diabetic mice model. Combination of black coffee and metformin significantly decreases serum HbA_{1C} levels in diabetic mice as compared to metformin or black coffee treated diabetic mice separately.

Study limitations

Study should also have involved oral glucose tolerance test, serum insulin level estimation, morphological study of pancreas, and immune histochemistry of histology of pancreas but owing to the cost and availability issue, the above mentioned parameters could not be explored.

Recommendation

- Further explorations need to be directed on active constituents of black coffee and highlight their individual hypoglycemic role.
- The pharmacokinetic properties and interaction of black coffee with other drugs should be studied.
- The comparative and combination therapy of black coffee with modern glucose lowering drugs should be investigated.

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