SUMMARY AND CONCLUSION
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Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years and continue to provide mankind with new remedies. Medicinal plant therapy is based on the empirical findings of hundreds and thousands of years.

Synthetic medicine is being restricted due to their side effect and humans are now demanding medicines that are more natural. As an important natural resource, traditional medicine and its pharmacological effects are receiving a serious attention worldwide during this decade. *Ziziphus jujuba Mill.* is a thorny Rhamnaceae plant with multiple medicinal properties and scavenging effect on free radicals. A literature survey shows that this plant is rich in biological active compounds, such as alkaloids, polyphenols, flavonoids, sterols, tannins, saponins, and fatty acids. Besides, Chinese dates have significant levels of antioxidant activity, reducing power, scavenging effect on free radicals. *Ziziphus* species are commonly used in folk medicine for the treatment of some diseases in the world such as digestive disorders, weakness, liver complaints, obesity, urinary troubles, diabetes, skin infections, fever, diarrhea, insomnia, tumors and cardiovascular disease (CVD).

Cardiovascular diseases are the most prevalent cause of death and disability in both developed as well as developing countries. Dyslipidemia has been found to be one of the most important contributing factors in heart disease. The higher coronary heart disease (CHD) risk in Asian Indians may be related to a higher prevalence of the metabolic syndrome, insulin resistance, and diabetes. Metabolic syndrome is found to predict development of type 2 diabetes mellitus and cardiovascular disease in the future. Due to the increased risk of diabetes and cardiovascular disease in people with the metabolic syndrome, there is an urgent need for strategies to prevent the emerging global epidemic of this condition.

Various herbal preparations are used in the management of dyslipidemia and diabetes mellitus in all over the world. Data on the efficacy of the herbal preparation from randomised controlled clinical trials is not sufficient. To date, only one reported
study is available on the lipid lowering potential of *Ziziphus jujuba* Mill. in dyslipidemic patients. Hence, the present study was planned to assess the effect of *Ziziphus jujuba* Mill. on the blood lipid profile and other parameters such as blood glucose, HbA1C, renal and liver functions in subjects with dyslipidemia.

The study was conducted in two sections.

**In vitro study**- Various parts of the sample (pulp, seed and peel) were analyzed for nutritional and functional properties such as proximate composition, phytochemicals, antioxidant components. The extracts of samples were also evaluated for their antioxidant activity using two techniques (RSA and RPA) and compared with the standards BHT and ascorbic acid. The analyzed nutrient composition of the dehydrated sample showed seed had the highest protein and iron content. Pulp contained higher amounts of total ash and phosphorus content. Three samples contained high amount of magnesium especially in pulp and seed. The nutritionally significant element manganese was not detected in Chinese jujuba. Potassium was the most predominant mineral. The Chinese jujuba is poor in contents of sodium, zinc and copper. The highest insoluble dietary fiber contents was found in the pulp, whereas, soluble dietary fiber in the PS was higher than pulp. Extract of pulp showed positive results for terpenoids in benzene and aqueous extracts. Steroids, alkaloids and flavonoids were present in methanol and aqueous solvents. Tannins and saponins were present only in the aqueous extract of the samples. Reducing sugars showed positive results for all solvents, especially in methanol and aqueous extracts. Overall, maximum phytochemical constituents were identified in the aqueous extract of pulp and seed. Highest polyphenol content was found in the pulp and raw peel. Pulp was a good source of glutathione whereas lowest value was shown in the seed. Maximum tannin content demonstrated in the raw and cooked peel. Pulp contained significantly higher amounts of ascorbic acid. Raw peel had the highest flavonoid content and maximum total saponin in methanolic extract. Overall, the methanol extracts had the strongest radical scavenging activity especially in seed and raw peel extracts. All extracts were found significantly greater than ascorbic acid. On the whole, the results
obtained from different extracts demonstrated that maximum reducing potency was found in the cold and hot aqueous extracts of raw peel.

**In vivo study**- This section of the study was conducted based on inclusion criteria in 2 phases as described below.

2. **PHASE 1:**

Dyslipidemic subjects were recruited from various health care centers in Mysore city, India with the help of the attending physicians. A randomized, cross sectional study was conducted. From among 305 subjects, a total of 217 subjects aged between 25-69 years met the inclusion criteria include type 2 diabetes mellitus with dyslipidemia and dyslipidemic subjects. A structured questionnaire was used to elicit information on their personal information, family and disease history, anthropometric measures, body composition, blood parameters, health habits and dietary pattern. Both males (129) and females (88) who were dyslipidemic participated in this study. The majority of respondents had basic education. Majority of subjects were in the first order of birth. More than 50 percent of them were in the low economic category. Out of 217 subjects with dyslipidemia, only 8.8% were non-diabetic while 91.2% were diabetic. A significant difference (P< 0.05) were seen between gender in somatic parameters such as weight, height, BMI, HC, WC, WHR, WHtR, TSF, TBF and BMR. The mean values of WC and WHR in male and female were higher than standard. According to WHR classification, 98% of males and 99% of females were found with different degree of obesity. The TSF were significantly (p<0.05) higher in females when compared to males. In males 91% and in females 89% were higher than Indian normal BMI value. Dyslipidemia of most subjects were detected by screening. Multiple regression of analysis (MRA) with Enter method was shown weight value was a significant predictor of total cholesterol followed by its combination with WHR. The results also suggest that WHR, BMI and MUAMC values were the best predictors of VLDL respectively. The results showed that no significant statistics in anthropometric measurements was found to change the level of FBS. Prevalence of metabolic syndrome was found to be 24 % among the subjects (55.7% males and 44.2% females). In the present study, anthropometric data demonstrated obesity,
especially upper body adiposity in majority of the subjects, which might increase CVD in them. Among all subjects 74% were non vegetarian, frequency and consumption of food items among patients showed 96.4% used rice daily followed by wheat and ragi in equal percentage (72.7%).

1. PHASE 2:

The effect of “Hypolip” the Ziziphus jujuba Mill. supplementation (capsule form, 500 mg, 4 capsules/d) on blood lipid profile was evaluated. From among 217 subjects in the phase 1, 45 subjects who expressed their willingness to co-operate were randomly divided into three groups for implementation of clinical trials. Participants were randomly assigned to receive experimental or placebo. Two different supplements namely PS (pulp and seed combined) and WF (whole fruit) were given to the subjects and were advised to consume it 2 capsules before the breakfast and 2 capsules before dinner for a period of 2 months. Based on the type of supplement administration, separate clinical trials were conducted as follows:

1. A randomized, single blind clinical trial on non diabetes high cholesterol and high triglyceride (Group 1) on PS supplement
2. A randomized, double blind clinical trial on diabetes high cholesterol and normal triglyceride (Group 2) on WF supplement
3. A randomized, double blind clinical trial on diabetes high cholesterol and high triglyceride (Group 3) on WF supplement

Anthropometric measures (Height, Weight, Waist and Hip circumference, MUAC and SFT), biochemical measures (lipid profile, Fasting blood sugar, HbA1C, ALT, AST, urea, uric acid, creatinine, total protein, Hb, GSH and LPO), dietary intake (24 hours dietary recall) and blood pressure were measured at base line and at the end of the intervention.

This is the first study to explore the hypolipidemic potential on dyslipidemic diabetic subjects and second study on dyslipidemic subjects and possible mechanisms of action of the Ziziphus jujuba Mill., systematically, using in vivo.
Results showed a significant reduction from before to after intervention in triglyceride (p<0.001), VLDL (p<0.001) and TG/HDL (p<0.05) in Group 1. On the basis of our results on the jujuba analysis the phytochemical components such as polyphenol, saponins and tannins were found on the PS supplement. It can be stated that these phytochemicals might be effect on decreasing lipid profile.

There was a significant (p<0.05) difference in cholesterol between before and after intervention in Group 2. The available literature suggests a strong role for use of saponins as medicine in particularly in disorders of cholesterol metabolism. Although, there was no significant reduction in LDL between two groups before and after intervention but 43% decreasing LDL in experimental group. Presence of saponins in Ziziphus was reported to have hypolipidemic effects such as decreasing total cholesterol, LDL-C and triglycerides levels. The present study suggests the pharmacological lowering effects of saponins which are present in Ziziphus jujuba Mill. on dyslipidemic subjects. A significant reduction (p<0.05) in systolic blood pressure were shown after intervention in the experimental group. A significant decreasing (p<0.001) in CHD risk was found from 7.66% before intervention to 5.33% after intervention.

There was a significant reduction in cholesterol (p<0.01), LDL (p<0.01), Triglyceride (p<0.01), VLDL (p<0.05), TG/HDL (p<0.01) and Non-HDL (p<0.05) and CHD risk factors (p<0.001). In this group moderate CHD risk factors significantly (p<0.001) converted to low risk factors.

The results obtained in this investigation indicate that the pulp, seeds, peel and PS of Ziziphus jujuba Mill. are potent sources of nutritional and antioxidant compounds especially magnesium, potassium, ascorbic acid, polyphenol, glutathione, tannin and saponins. However, few studies have reported concerning the antioxidant activity of tropical and subtropical fruit seeds and peels. Preliminary study suggests that food industry waste by-products could be a good source of cheap and natural antioxidants with significant amount of antioxidant with a beneficial health effect in human. Therefore, re-utilization and re-cycling of this waste could be used as natural antioxidant compounds in food processing and preservation. To achieve the complete
utilization of such fruits, it would be beneficial if the seeds and peels could be used as a source of natural food additives and ingredients. This study also suggests that *Ziziphus jujuba Mill.* therapy was a well-tolerated and effective treatment for dyslipidemic subjects. Orally administration of jujuba supplements did not show any side effects on liver and kidney as assessed by biochemical measurements. The antihyperlipidemic activity of *Ziziphus jujuba Mill.* was experimentally born out but it has to be standardized for common use. Our results suggest that *Ziziphus jujuba Mill.* decrease the serum lipid profile level and CHD risk factors in dyslipidemic type-II diabetic and non-diabetic subjects. Besides, *Ziziphus jujuba Mill.* may potentially be a new beneficial candidate to widen therapeutic options for treating dyslipidemia. It should be noted that dyslipidemia and diabetes are conditions in which herbs are used. *Ziziphus jujuba Mill.* could be used as anti-diabetic agent in the management of diabetes associated with abnormalities of lipid profiles. Doctors caring for such patients should be aware of this fact and should try to encourage their patients to talk regarding the use of herbs as it may affect the outcome and the management of their disease.

**Strength of the Study**

- This is a first prospective intervention study which has focused on the effects of *Ziziphus jujube Mill.* to evaluate somatic, biochemical and dietary variables in diabetic dyslipidemic subjects and it is second intervention study on dyslipidemic subjects.
- Applying randomized clinical trial double blind, placebo control group is a powerful clinical trial in an attempt to eliminate subjective bias on the part of both.
- Awareness of those patients who did not know the status of their blood lipids.
Limitations of the Study

- The main limitation was a small sample size in clinical trials particularly in the experimental subjects in Group 2.

- Lack of previous established data related to detection of effective dosage of *Ziziphus jujube* Mill. in dyslipidemic subjects.

- To speak local language for foreigner researchers who are not familiar to their language was a limitation to communicate with subjects.

- Whether these findings can be generalized to other dyslipidemic subjects requires further study.

- Although the researchers are aware of conducting long term intervention studies with cross-over design as part of clinical studies, due to the limited time for Ph.D research it was not possible to conduct such studies.