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A PRE-CLINICAL EFFICACY AND RANDOMIZED CONTROLLED CLINICAL TRIAL OF *NEELKANTHI CHURNA* IN THE MANAGEMENT OF TYPE 2 DIABETES MELLITUS

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ABSTRACT

Ethnopharmacological relevance: *Neelkanthi (Ajuga bracteosa)* is a folklore medicine occurring in Western Himalayas. This plant has also been used traditionally in local areas for curing a number of diseases including Diabetes Mellitus. No antidiabetic action of *Neelkanthi* has been mentioned in *Ayurvedic* texts, but by virtue of its properties it acts as *Madhumehahara Aushada.* Hence the present study has been planned to assess the efficacy of *Neelkanthi Churna* in the management of *Madhumeha* w.s.r. to Type 2 Diabetes Mellitus on scientific lines. **Aim of the study**: The present study was carried out to investigate the effect of *Neelkanthi Churna* (NC) in a mice model of Diabetes Mellitus. Further, its clinical safety and efficacy was assessed in a randomized active-controlled clinical study. **Materials and methods**: In pre-clinical study, the mice in different groups were kept on high-fat diet for 16 weeks. Thereafter, the mice were treated with 610 and 1220 mg/kg of the NC preparation for subsequent 4 weeks. At the end, different biochemical and histopathological parameters of the NC treated groups were compared with disease control groups. In the clinical trial, 45 patients were randomly divided into following three groups: GROUP I: 15 Patients were managed with NC 3gm twice/day for 12 weeks. GROUP II: 15 Patients were managed with Tab. Metformin 1gm twice/day for 12 weeks. GROUP III: 15 Patients were given both Tab. Metformin 1gm twice/day and NC 3gm twice/day for 12 weeks. At the baseline and endpoint, serum biochemical and haematological parameters were studied. **Results**: In pre-clinical experiment, a significant dose-dependent decrease in the Fasting Blood Sugar, total serum cholesterol, triglycerides, low-density lipoprotein (LDL), and increase in high-density lipoprotein (HDL) was observed in NC-treated group compared to disease control. In clinical study, NC significantly decreased the levels of FBS, PPBS, HbA1c and urine sugar following treatment compared to the baseline. However, insignificant change was observed in the studied parameters among NC and Tab. Metformin at the end of the treatment. The other studied biochemical and haematological parameters remained unchanged in all groups following the treatment compared to the baseline. **Conclusions**: The results of pre-clinical and clinical studies supported the efficacy of NC in Type 2 Diabetes Mellitus. The study also suggested the clinical safety of the NC preparation.

KEYWORDS: Ayurveda; Diabetes Mellitus; High-fat diet; Metformin; *Neelkanthi Churna.*

1. INTRODUCTION

Diabetes Mellitus is a clinical syndrome characterized by hyperglycaemia due to absolute or relative deficiency of insulin.[1] Over a period of time, it can damage the blood vessels and nerves as well as many other body systems which consequently cause life threatening complications.[2-4] Diabetes Mellitus is becoming an epidemic health problem in developing countries. As the International Diabetes Federation indicates, the number of adults living with diabetes globally has been increasing from time to time. India leads the world with maximum number of diabetic patients hence it is termed as Diabetes capital of the world. The first WHO global report on Diabetes 2016 demonstrates that number of

adults living with diabetes has almost quadrupled since 1980 to 422 million adults and project the Diabetes as 7th leading cause of death in 2030. The real burden of the disease is however due to its associated complications which lead to increased morbidity and mortality. Much of the heart disease and stroke in these estimates was linked to diabetes.

In Ayurveda, Diabetes Mellitus can be understood as *Madhumeha* which is one among the *Vataja Prameha* and is a *Santarpanjanya Vikara* and grouped under *Ashatamahagada*. A comprehensive description regarding the etiology, pathology, prognosis and treatment of *Madhumeha* is available in classical literatures of *Ayurveda*. *Ayurveda* through its armamentarium can become a potential source of hypoglycemic drugs that may be relatively safe, significantly potent with negligible side effects and can improve quality of life.

A lot of potent antidiabetic drugs are available today, but none of them is free from untoward effects. Many *Ayurvedic* herbal drugs are effective in the management of Diabetes Mellitus and *Neelkanthi* is one of them. *Neelkanthi (Ajuga bracteosa)* is a folklore medicine occurring in Western Himalayas. This plant has also been used traditionally in local areas for curing a number of diseases including Diabetes Mellitus. No antidiabetic action of *Neelkanthi* has been mentioned in *Ayurvedic* texts, but by virtue of its properties it acts as *Madhumehahara Aushada.* Chopra et. al (1969) mentioned that *Neelkanthi* yields ceryl alcohol beta – sitosterol, cerotic and palmitic acids along with a glucosidic constituent. According to Karnick and Pathak 1982, it contains glucoside and tannin.

Bakuni et. al.have reported presence of hexacosanol, beta – sitosterol, tetracosanoic acid and a new compound characterised as tricontanyl docosanoate.

Sharma N.K. et.al (2002*)* reported presence of ceryl alcohol, beta- sitosterol, gamma sitosterol, a crystalline compound, cerotic and palmitic acids and a glucosidic constituent.

In order to rationalize its medicinal applications and establish biogeochemical link, the mineral elements (Na, K, Ca, Mg, Zn, Mn, Cu, Fe and Cr) of leaves and roots of *Ajuga bracteosa* and the nearby soil were studied. The herb contains comparatively larger amounts of chromium (leaves. 25 mg and roots 20 mg per 100 g) which may be correlated to its use as remedy for Diabetes as per Ahmed et. al. Chromium is another essential mineral that human require in trace amounts. It is an important component of many body building strategies to and in the development of lean muscle mass, as well as in the treatment of Diabetes and for weight loss. Chromium increases the metabolism of proteins, fats and carbohydrates. It significantly enhances the efficiency of insulin to regulate blood sugar levels.[5-7]

Neelkanthi is a folklore medicine widely used in this area for the treatment of *Madhumeha*. Keeping this in mind and its *Tikta* and *Kashaya Rasa* and *Katu Vipaka*, it was selected for present research work in order to prove its efficacy on scientific lines.

2. MATERIAL AND METHODS

2.1. Drugs and chemicals

The entire plant of *Neelkanthi Ajuga bracteosa* (Wall. Ex. Benth) (Family – Lamiaceae) was collected from areas nearby Palampur District Kangra, Himachal Pradesh, India. Self procurement of the drug has been done and *Neelkanthi Churna* has been prepared by taking the herb and prepared in RGGPG Ayurvedic College Pharmacy Paprola.

2.2. Preclinical study

2.2.1. Animals and ethics

Adult C57BL6/J strain male mice, weighing 20-25 gm of 6-8 weeks old were obtained from the animal house of CSIR-IHBT, Himachal Pradesh, India. The animals were kept in a well-ventilated room with a 12 hours light/dark circle in standard polypropylene cages under controlled room temperature (25 ± 3.3℃) and humidity (30-40%). They were fed with chow diet obtained from Golden Feeds Pvt. Limited, India. Water filtered through Euro guards was supplied to the animals ad libitum. Experimental protocol was approved by Institutional Animal Ethics Committee with reference number IAEC/IHBTP-8/March 2021. All the animals used in this study received human care in compliance with CPCSEA guidelines.

2.2.2. Preclinical experimental protocol

Adult C57BL6/J strain male mice, weighing 20-25 gm of 6-8 weeks old were selected and divided into three groups of six animals (n = 6) in each group as follows

Experimental groups of animals

GROUPS	TREATMENT	SPECIES	NO. OF ANIMALS
Group 1	Disease control group (high fat diet)	C57BL6/J mice	06
Group 2	ABEP - 1220 mg/kg body weight	C57BL6/J mice	06
Group 3	ABEP - 610 mg/kg body weight	C57BL6/J mice	06

The test drug was administered by oral route with the help of oral gavage. Test drug was administered for 4 weeks after induction of Diabetes Mellitus for 16 weeks with high-fat diet. Group 1 animals were kept on high-fat

diet for disease control. While Group 2 and Group 3 animals were given *Ajuga bracteosa* Wall. Ex. Benth entire plant powder (ABEP) in medium and low dose (1220 mg/kg and 610 mg/ kg BW) respectively after making homogeneous suspension in Tween 80.

All groups received High fat diet (20 gm/day) and normal drinking water for 16 weeks. The treatment with test drug [*Ajuga bracteosa* Wall. Ex. Benth entire plant powder (ABEP] was started after 16 weeks and test drug was administered for 4 weeks. The high fat diet of treatment groups (Group 2 and Group 3) was withdrawn and they were shifted on normal chow diet and normal drinking water. On 30th day blood was collected for serum biochemical parameters. After that the animals were sacrificed by carbon monoxide asphyxia. The abdominal cavity of all the animals was dissected and liver, kidney, heart, adipose tissue and pancreas were removed immediately. The organs were cleaned with saline water, dried with blotting paper; they were weighed and recorded, and then preserved in 10% formalin for histopathology analysis.

2.2.4. Serum biochemistry

The blood samples were collected and serum was separated. The biochemical parameters, Fasting blood sugar, serum lipid profile (total cholesterol, triglycerides, LDL and HDL), Blood urea and serum creatinine were estimated using biochemical kits (ERBA, Transasia, India) using an automated ERBA-EM 200 Biochemical Analyzer as per manufacturer's instruction.

2.2.5. Histopathological analysis

The animals were sacrificed by using carbon dioxide asphyxia after one day interval of the end of treatment protocol of study. The abdominal cavity of all the animals was dissected and liver, kidney, heart, adipose tissue and pancreas were removed immediately. The organs were cleaned with saline water, dried with blotting paper; they were weighed and recorded, and then preserved in 10% formalin for histopathology analysis.

2.3. Clinical study

2.3.1. Participants

In the present study, total 45 patients were registered and all the patients from each group completed the trial. A written informed consent of the patients was taken before inclusion in the trial in the language he/she was well versed.

2.3.2. Diagnostic criteria

1. Fasting plasma glucose ≥126mg/dl and ≤ 200mg/dl
2. PPBS (2-hour plasma glucose level) ≥200mg/dl
3. HbA1C ≥ 6.5%

2.3.3. Inclusion criteria

Patients aged between 20 to 70 years, willing to participate in the trial giving written consent and fulfilling the diagnostic criteria, having Fasting Blood Sugar between 126 mg/dl - 200 mg/dl and Newly diagnosed patients of Type 2 Diabetes Mellitus having glycosylated Hb (HbA1C) between 6.5% to 10 %

2.3.4. Exclusion criteria

Patients with FBS < 126 mg/dl and > 200 mg/dl, Patients of Type-1 DM or Type-2 DM or insulin/OHA's other than Metformin/ any other medication for glucose control in the last 3 months, patients suffering from the complications of Diabetes Mellitus viz., diabetic neuropathy, diabetic nephropathy, patients with uncontrolled Hypertension, patients having concomitant disorders like ischemic heart disease, congestive heart failure, acute or chronic renal failure, patients suffering from major systemic illnesses requiring long duration of treatment (like, autoimmune disorders, tuberculosis, malignancy, *etc.*). The individuals who had participated in any other clinical study during last 3 months.

2.3.5. Clinical study protocol

The study was a randomized active-controlled clinical study comprising 3 parallel groups. The clinical study was performed at the Rajiv Gandhi Government Post-Graduate Ayurvedic College and Hospital, Paprola, H.P., India. The trial was conducted from 1st July, 2021 to 16th April, 2022. The CONSORT guidelines were followed in reporting the outcomes of the study. Random number table was used for the subject randomization. All the recruited patients completed the study. The patients in Group I (n = 15) were given 3 g of NC twice a day for 12 weeks, while the patients in Group II (n = 15) were given a tab. Metformin 1g twice a day for 12 weeks and the patients in group III (n = 15) were given both Tab. Metformin 1 g and NC 3 g twice a day. The patients were also advised to follow the same dietary plan and activity level as it was before the trial. The patients' serum levels of FBS, PPBS, HbA1c, total cholesterol, LDL, HDL, triglycerides, SGOT, SGPT, blood urea and creatinine were determined by ERBA-EM 200 Biochemical Analyzer. Similarly, the total leucocyte count, differential leucocyte count and haemoglobin level in the patients' blood were analyzed using H360 ERBA Hematology Analyzer (Ebra Mannheim, Germany). The erythrocyte sedimentation rate (ESR) was determined by the Westergren method. Primary outcome variables were the FBS, PPBS and HbA1c. Secondary outcome variables were BMI, total leucocyte count, differential leucocyte count, haemoglobin, ESR, blood urea nitrogen and serum creatinine, serum levels of total cholesterol, LDL, HDL and triglycerides. Follow up was done after every 14th day till the completion of trial. FBS, PPBS and Urine analysis was done every 14th day to assess the sustained effect and untoward effect of the drug, however HbA1C was done only at time of commencement and completion of the trial. Patients' compliance to the interventions was assessed by counting the number of packets/strips returned and asking if the patient skipped any doses. The clinical trial was approved by the Institutional Ethical Committee of the Rajiv Gandhi Government Post-Graduate Ayurvedic College and Hospital, Paprola, H.P., India vide approval

number Ayu/IEC/2019/1217 dated 10th November, 2020. The clinical trial was performed as per the revised Declaration of Helsinki 2013 (8). A written informed consent of the patients was taken before inducting them for the trial. A detailed proforma was prepared to note down all the details of the patients and disease incorporating demographic profile, detailed history followed by general physical and systemic examination, anthropometric measurements and blood investigations. The trial was registered at the Clinical Trial Registry of India (https://ctri.icmr.org.in/) vide CTRI Registration Number: CTRI/2021/06/034030.

2.4. Statistical analysis

All the values were expressed as mean ± standard error of the mean, unless otherwise specified. The inter-group difference was analysed by one-way analysis of variance (ANOVA) with Tukey's *post hoc* test for the preclinical study parameters. The Student's t-test was used to compare clinical parameters before and after the treatment within the group. The clinical results between the groups after the treatment were also compared using Student's t-test. Statistical significance was considered at $p < 0.05$. SigmaStat® statistical software was used for statistical analysis.

3. RESULTS

3.1. Effect of NC on FBS levels of mice

The results showed that in all HFD induced diabetic mice ; the mean score of fasting blood sugar in disease control group (without any treatment) before trial was 159.167 and after trial it was 183.66, had increment of 15% fasting blood sugar which results significant status($p<0.05$) and in group 2 (NC 1220mg/kg bw) the mean score before treatment was 166.167 and after treatment it reduced to 121.167 ,had a 27% reduction in mean score , which was statically significant($p<0.05$) . In group 3 (NC 610mg/kg bw) the mean score before treatment was 161.167 and after treatment it reduced to 138.33, had a 27% reduction in mean score, which was statically significant($p<0.05$).

3.2. Effect of NC on other biochemical parameters

The results showed that disease control group was found significantly higher serum triglycerides, Cholesterol, LDL, urea and creatinine level when compared to oral administration of ABEP 1220 mg/kg body weight ($p<0.001$). and ABEP 610 mg/kg body weight ($p < 0.05$) in high fat diet induced diabetic mice. It also showed that disease control group was found significantly lower serum HDL levels when compared to oral administration of ABEP 1220 mg/kg body weight ($p<0.001$) and ABEP 610 mg/kg body weight ($p<0.001$) in high fat diet induced diabetic mice.

3.3. Effect on histological changes of adipose tissue in mice

The histopathological sections of pancreas, liver, kidney and adipose tissue of disease control group showed deranged pattern while treatment group showed mild derangement pattern and no derangement was found in Group 2 where test drug was administered in medium dose (1220 mg/kg body wt.).

3.3. Effect on clinical parameters of human subjects

The results of laboratory profile in the present clinical study showed statistically significant reduction in fasting blood sugar with 8.66%, 21.3% and 24.8% reduction in group I, II and III respectively which was statistically significant ($p<0.05$). In this series maximum FBS reduction was seen in Group III. The study also showed statistically significant reduction in post prandial blood sugar with 15.16%, 25.5% and 26.4% reduction in group I, II and III respectively which was significant statistically ($p<0.05$). In this series maximum PPBS reduction was seen in Group III. The study revealed reduction in mean score of glycosylated haemoglobin with 3.6%, 9.8% and 11.7% in group I, II and III respectively which was statistically significant ($p<0.05$). In this series maximum HbA1c reduction was seen in Group III. It also showed reduction in mean score of urine sugar with percentage reduction of 29.2%, 53% and 85.8% in group I, II and III respectively which was significant statistically ($p<0.05$). In this series maximum urine sugar reduction was seen in Group III. Overall effect of therapy was excellent in all three groups. All the groups have shown potent hypoglycaemic effect but Group III was perform better in all dimension of the study.

The patients in all groups took full course of the allocated treatment. Among 45 randomized patients, 25 (55.5%) were males, and rest were females. All the studied clinical parameters following respective treatment in all groups summarized in Table 1. The other biochemical parameters studied for the safety, including blood urea, serum creatinine, SGOT, SGPT, serum cholesterol, triglycerides, HDL, LDL and haematological parameters (total leucocyte count, differential leucocyte count, haemoglobin, and ESR) remained unchanged before and after the therapy, and an insignificant difference was observed in both the groups when compared at the end of the treatment.

4. DISCUSSION

In the pre-clinical study, High Fat Diet (HFD) induced Obesity model was selected to assess the hypoglycaemic effect of *Ajuga bracteosa*. Biochemical investigations like Fasting blood sugar, blood urea, serum creatinine, serum cholesterol, serum triglycerides, serum HDL etc. were done. Histopathological examination of pancreas, liver, kidney and adipose tissue sections from animals of each group was simultaneously undertaken to compliment the biochemical parameters assessment.

Test drug was administered for 4 weeks after induction of obesity for 16 weeks with high-fat diet. Group 1 animals were kept on high-fat diet for disease control. While Group 2 and Group 3 animals were given *Ajuga bracteosa* Wall. Ex. Benth entire plant powder (ABEP)

in medium and low dose (1220 mg/kg and 610 mg/ kg body wt.) respectively after making homogeneous suspension in Tween 80.

Changes in biochemical parameters, total body weight and histopathological studies in disease control group, test drug administered in medium and low dose were analyzed.

❖ **Biochemical Parameters:** There was significant decrease in Fasting blood sugar in treatment groups as compared to disease control group. Test drug given in medium dose reduced more Fasting blood sugar in comparison with test drug given in low dose. The herb contains larger amount of Chromium which may be correlated to its use as remedy for Diabetes as per Ahmed et. Al. Chromium is another essential mineral that human require in trace amounts. Chromium increases the metabolism of proteins, fats and carbohydrates. It significantly enhances the efficiency of insulin to regulate blood sugar levels.[4-6] The mechanism of action of *Ajuga bracteosa* might be attributed to its secretagogue effect by escalating the release of insulin from B-cells of pancreas, increasing the utilization of glucose by peripheral tissue, reducing hepatic gluconeogenesis, inhibiting metabolic degradations of carbohydrates, or by preventing oxidative stress.[7] There was significantly decrease in serum cholesterol levels, serum triglycerides level, serum LDL level in treatment groups as compared to disease control group. Test drug given in medium dose reduced more Fasting blood sugar in comparison with test drug given in low dose. The possible action may be by reducing lipid peroxidation in plasma and tissues (A Chenni et al. J Ethnopharmacol.2007)[8]

There was significant increase in serum HDL levels in treatment groups as compared to disease control group.

The total body weight was higher in disease control group while the groups administered with test drug had markedly reduced body weight.

❖ **Histopathology:** The histopathological sections of pancreas, liver, kidney and adipose tissue of disease control group showed deranged pattern while treatment group showed mild derangement pattern and no derangement was found in Group 2 where test drug was administered in medium dose (1220 mg/kg body wt.).

Thus, the analysis of body weight changes, serum biochemical parameters and histopathological studies shows that administered test drug markedly reduced the raised Fasting blood sugar and lipid levels caused by High Fat Diet. The mechanisms involved in hypoglycaemic activity of NC in humans may be similar to rodents as discussed earlier.

CONCLUSION

The present study investigated the effect of administration of NC in diabetic mice as well as humans. The assessment was done on various objective parameters. The results obtained from present study revealed a statistically significant reduction in FBS, PPBS, HbA1c levels and some of signs and symptoms of *Madhumeha* in all three groups but best results were observed in group III wherein *Neelkanthi Churna* was given as add on therapy to Metformin.

- The results of present study proved that *Neelkanthi Churna* is effective in the management of *Madhumeha.*
- The results of experimental study also endorsed the hypoglycaemic activity of *Neelkanthi*.
- No untoward effect of *Neelkanthi Churna* was observed during the entire trial period.
- Therefore, the present study confirms the efficacy and safety of NC as a promising hypoglycaemic agent.

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