Effect of *Kasni* seed preparations on serum glutamic pyruvic transaminase and glutamic oxaloacetic transaminase levels in newly diagnosed patients of type 2 diabetes mellitus

Praveen Katiyar¹,², Amod Kumar¹, Arvind K. Mishra³, Rakesh K. Dixit¹, Ajay K. Gupta⁴

¹Department of Pharmacology & Therapeutics, King George's Medical University, Lucknow, U.P., India
²University Institute of Health Sciences, C.S.J.M. University, Kanpur, U.P., India
³Department of Internal Medicine, King George's Medical University, Lucknow, U.P., India
⁴University Institute of Pharmacy, C.S.J.M. University, Kanpur-208024, U.P., India

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*Correspondence:*
Dr. Praveen Katiyar,
E-mail: drpraveenkatiyar@gmail.com

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ABSTRACT

**Background:** Kasni (*Chichorium intybus* L.) reported to play an important role in the effective management of serum liver enzymes SGPT & SGOT in various animal models and this study is extension to newly diagnosed patients of type 2 diabetes mellitus.

**Methods:** Newly diagnosed 90 patients of Type2 DM, age 35-65 years, of either sex were divided into 3 groups. In group I only Metformin sustained release once a day and in group II/III 6 grams crude seed powder or 50 ml decoction of crude seed powder was given twice a day for 90 days in combination with Metformin sustained release orally once in a day. Serum liver enzyme levels of SGPT & SGOT were measured at zero, 30th, 60th and 90th day.

**Results:** All the three groups showed a significant reduction in SGPT & SGOT across the four time periods. Post hoc Tukey HSD test shown that there was a significant difference between group I & II (p=0.011) and group I & III (p=0.000) for SGPT and group I & II (p=0.012) and group I & III (p=0.000) for SGOT.

**Conclusions:** The add on therapy with Kasni seed preparations is more effective for the management of altered SGPT and SGOT levels in Type2 diabetes mellitus patients than only oral hypoglycaemic agent in decreasing SGPT & SGOT of selected patients. Among Kasni seed preparation treated groups, decoction was found more effective than crude seed powder.

**Keywords:** Diabetes, Kasni, SGPT, SGOT, AST, ALT

INTRODUCTION

Escalating prevalence of diabetes mellitus is one of major concern of health care professionals because as per International diabetes federation, by 2030 the estimated population may reach by almost 600 million globally.¹ Type 2 Diabetes is associated with increased BMI (Body Mass Index) due to sedentary lifestyle and increased processed food intake. Type 2 diabetes is commonly found in middle aged individuals. Type 2 diabetic individuals are at higher risks of liver function test abnormalities than individuals who do not have diabetes.² Previous studies have indicated that circulating concentration of liver function tests like SGPT (ALT - Alanine aminotransferase, which stands for serum glutamic-pyruvic transaminase) and SGOT (AST - Aspartate aminotransferase, which stands for serum glutamic-oxaloacetic transaminase) are increased in individuals with insulin resistance and the metabolic disorder.³,⁴ In addition, these components of liver function tests are increased in type 2 diabetes mellitus patients.⁵ Hence, it is essential to look for safer and effective alternate treatment for these effects.
function tests have been shown to be positively correlated with the risk of future type 2 diabetes mellitus. \textsuperscript{4,5,2} SGPT is found in particularly large amounts in the liver and its role in the process that converts food into energy is very important. Normally, ALT (SGPT) is normally found inside the liver cells. But if the liver is injured or inflamed, ALT is released by the liver into the bloodstream. By estimation of blood levels of ALT doctors can may get important information about the liver and whether an inflammation, disease, drug, or any other problem is affecting it. While AST is found in many tissues throughout the body, including the liver, heart, muscles, kidney, and brain. If any of these organs or tissues is affected by disease or injury, AST is released into the bloodstream; i.e. AST isn’t as specific an indicator of liver damage as ALT and thus a good index of cellular injury to the liver parenchyma.

According to World Health Organization (WHO) three fourth of world population cannot afford modern medicine\textsuperscript{6} and about 70% population of entire world depends upon traditional and folk medicines. In India, about 80% of the rural population depends upon traditional and folk medicines for their health care.\textsuperscript{7} The plant \textit{Cichorium intybus Linn} (Family: Compositae, Asteraceae) commonly known as Chicory or Kasni is used as liver tonic and as an alternative medicine in treating hepatotoxicity. Dealing with the biological study, most of the reports proved the different parts of the plant to have hepatoprotective and antihepatotoxic activity,\textsuperscript{6,8} and same has demonstrated in animal studies.\textsuperscript{9,10} In a recent study of Raiza Philip et al. an association between the level of liver enzymes, ALT and AST was found in type 2 diabetic patients when a significant increase in the level of these liver enzymes was observed in comparison to the normal persons. Kasni was also found easy to use and well tolerated in all patients.\textsuperscript{11} Thus, these liver enzymes can be used as a biomarker for the assessment of type 2 diabetes. Therefore, in the present study one of an initial attempt was made to investigate the possible beneficial effect of Kasni seed preparations in combination with oral hypoglycemic agents on the level of liver enzymes, SGPT and SGOT, in treatment of newly diagnosed patients of type 2 diabetes mellitus.

**METHODS**

The study protocol was approved by Institutional Ethics Committee, King George’s Medical University, Lucknow, U.P., India (Ref. Code: 58 E.C.M. IIB/P21, letter no.: 2649/R.Cell 12 dated 20.10.2012). All participants were provided with specific written information about the aims of the study and were informed about all possible expected outcomes from the study. Written consent was taken from the study subjects. The included ninety (90) patients were divided into 3 groups. Each group having 30 patients (19 male and 11 females) n=30 matched with each other in terms of age and sex. The Group - I patients on oral hypoglycemic agent were advised not to take any herbo-mineral preparation during the study duration, and this group served as standard. The patients of Group – II were advised with Kasni crude seed powder (6 gms in the morning in fasting condition and 6 gms in the evening) in combination to oral hypoglycemic agent. The patients of Group – III were advised with Kasni decoction (by instructing the patients to boil provided 6 grams crude seed powder in 100 ml water till 50 ml decoction remained) in combination to oral hypoglycemic agent. The oral hypoglycemic agent prescribed was Glycomet SR containing Metformin Sustained Release once in a day in every group for 90 days. Preparations of Kasni seeds were given twice everyday upto 90 days in group II and III. A supervisor cautiously ensured that the selected patients were taking preparations of Kasni seeds appropriately. Blood samples were collected from all subjects before starting oral hypoglycemic agent/combination of crude \textit{C. intybus} seed powder and hypoglycemic agent/combination of \textit{C. intybus} crude seed powder decoction and oral hypoglycemic agent. Final sample was collected 12 hours after the last dose of 90th day treatment with standard drug and in combination with preparations of Kasni seeds.

**Plant material**

Kasni seeds of indigenous variety were obtained from International Institute of Herbal Medicine, Lucknow, through Organic India Pvt. Ltd. from organic certified fields. It carries WHO standard for identification of herbs. Some of these seeds were cultivated in the herbal garden of C.S.I.M. University, Kanpur and then grown plant was supplied to National Botanical Research Institute (NBRI) Lucknow, India, there it was identified as \textit{Cichorium intybus} L. (Ref. No: NBRI/CIF/222/2011). The Kasni seeds were cleaned, desiccated and crushed to a powder with an electric microniser. The envelopes containing 6 gms of Kasni seed powder were prepared and provided to patients of group II and group III with respective instructions i.e. to take as such crude seed powder (preparation 1) for group II or by preparing infusion by boiling in group III (preparation 2), and patients were asked to use it regularly as per direction. Advices about dietary and lifestyle changes were given to both \textit{C. intybus} treated groups and standard group.

**Biochemical analysis of serum parameters**

Each patient's liver enzymes SGPT and SGOT was measured at the beginning of the trial, then at zero, 30th, 60th, and 90th days. Venous blood sample was collected in plain tube clot activator vacutainer from each subject for the SGPT and SGOT levels estimation. All biochemical serum analysis was performed with fully automatic random access analyzer Biosystem A-25 manufactured by Biosystem Diagnostics Pvt. Ltd. an ISO 9001:13485
standard and CE mark company. SGPT and SGOT were measured by the IFCC (International Federation of Clinical Chemistry) method and expressed in IU/L.

**Statistical analysis**

Results are presented as mean ± SD. Statistical analyses were conducted on using IBM SPSS Version 20 software by using mixed between-within subjects ANOVA followed by post Hoc Tukey HSD test to make a comparison between groups. P-values < 0.05 were considered statistically significant.

### RESULTS

A mixed between- within subjects analysis of variance was conducted to compare the effects of three types of treatments (Group I, II & III) on SGPT (ALT) and SGOT (AST) levels across four time periods (zero day, thirty days, sixty days, and ninety days).

**SGPT (ALT)**

There was a significant interaction between time and type of treatments, Wilk’s Lambda=0.221, F(6, 170)=31.941, p<0.001, partial eta squared=0.530 (Table 1).

### Table 1: Interaction between time and type of treatments and main effect for time.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Value</th>
<th>F</th>
<th>Hypothesis df</th>
<th>Error df</th>
<th>Sig.</th>
<th>Partial Eta Squared</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TIME</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pillai’s Trace</td>
<td>.980</td>
<td>1412.820b</td>
<td>3.000</td>
<td>85.000</td>
<td>.000</td>
<td>.980</td>
</tr>
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<td>Wilks’ Lambda</td>
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<td>1412.820b</td>
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<td>85.000</td>
<td>.000</td>
<td>.980</td>
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<td>Hotelling’s Trace</td>
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<td>85.000</td>
<td>.000</td>
<td>.980</td>
</tr>
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<td>Roy’s Largest Root</td>
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<td>3.000</td>
<td>85.000</td>
<td>.000</td>
<td>.980</td>
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<td></td>
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<td></td>
</tr>
<tr>
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<td>.634</td>
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<td>Roy’s Largest Root</td>
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<td>99.005c</td>
<td>3.000</td>
<td>86.000</td>
<td>.000</td>
<td>.775</td>
</tr>
</tbody>
</table>

Measure: SGPT (ALT); a. Design: Intercept + GROUP
Within Subjects Design: TIME
b. Exact statistic
c. The statistic is an upper bound on F that yields a lower bound on the significance level.

### Table 2: Serum GPT (ALT) (IU/L) level for all the three groups across four time periods.

<table>
<thead>
<tr>
<th>Time Periods</th>
<th>Group I (Only oral Hypoglycemic agent used)</th>
<th>Group II (Kasni crude seed powder &amp; Oral Hypoglycemic agent used)</th>
<th>Group III (Decoction of Kasni seed powder &amp; Oral Hypoglycemic agent used)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
<td>N</td>
</tr>
<tr>
<td>Zero day</td>
<td>42.100</td>
<td>3.3564</td>
<td>30</td>
</tr>
<tr>
<td>Thirty days</td>
<td>39.900</td>
<td>3.38710</td>
<td>30</td>
</tr>
<tr>
<td>Sixty days</td>
<td>38.400</td>
<td>3.27583</td>
<td>30</td>
</tr>
<tr>
<td>Ninety days</td>
<td>37.0667</td>
<td>3.25823</td>
<td>30</td>
</tr>
</tbody>
</table>

There was a significant main effect for time, Wilk’s Lambda=0.020, F(3, 85)=1412.820, p<0.001, partial eta squared=0.980 (Table 1), with all groups showing a decrease in SGPT (ALT) level across the four time periods (Table 2).

The main effect comparing the three type of treatments was significant, F(2,87)=8.625, p=0.000 (p<.05), partial eta squared=0.165, suggesting large difference in the effectiveness of the three treatments (Table 3).

Post hoc Tukey HSD test is showing that there is a significant difference between group I & II (p=0.011) and group I & III (p=0.000) (Table 4). So, Kasni crude seed powder and with oral hypoglycaemic agent and Kasni seed powder decoction with oral hypoglycaemic agent is more effective than only oral hypoglycaemic agent in decreasing Serum SGPT (ALT) level of selected patients.

Profile plot is showing that Kasni seed powder decoction is more effective than Kasni crude seed powder in decreasing Serum SGPT (ALT) level (Figure 1).
SGOT (AST)

There was a significant interaction between time and type of treatments, Wilk’s Lambda=0.258, F(6, 170)=27.489, p<0.001, partial eta squared=0.492 (Table 5).

There was a significant main effect for time, Wilk’s Lambda=0.019, F(3, 85)=1447.792, p<0.001, partial eta squared=0.981 (Table 5), with all groups showing a reduction in Serum SGOT(AST) level across the four time periods (Table 6).

The main effect comparing the three type of treatments was significant, F(2,87)=8.372, p=0.000 (p<.05), partial eta squared=0.161, suggesting large difference in the effectiveness of the three treatments (Table 7).

### Table 3: Analysis of between group effects.

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
<th>Partial Eta Squared</th>
</tr>
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<tr>
<td>Intercept</td>
<td>495433.403</td>
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<td>8613.349</td>
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<td>.990</td>
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<td>GROUP</td>
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<td>496.086</td>
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<td>.165</td>
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<tr>
<td>Error</td>
<td>5004.175</td>
<td>57.519</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

Measure: SGPT (ALT); Transformed Variable: Average

### Table 4: Multiple comparisons group I, II & III for SGPT (ALT).

<table>
<thead>
<tr>
<th>(I) GROUP</th>
<th>(J) GROUP</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tukey HSD</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>I</td>
<td>II</td>
<td>2.8833*</td>
<td>.97911</td>
<td>.011</td>
<td>5.2180</td>
</tr>
<tr>
<td>I</td>
<td>III</td>
<td>3.9250*</td>
<td>.97911</td>
<td>.000</td>
<td>6.2597</td>
</tr>
<tr>
<td>II</td>
<td>I</td>
<td>-2.8833*</td>
<td>.97911</td>
<td>.011</td>
<td>-5.2180</td>
</tr>
<tr>
<td>II</td>
<td>III</td>
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<td>.97911</td>
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<tr>
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<td>.97911</td>
<td>.539</td>
<td>1.2930</td>
</tr>
</tbody>
</table>

Measure: SGPT (ALT); Based on observed means. The error term is Mean Square (Error) = 14.380.

* The mean difference is significant at the .05 level.

### Table 5: Interaction between time and type of treatments and main effect for time.

**Multivariate Tests**

<table>
<thead>
<tr>
<th>Effect</th>
<th>Value</th>
<th>F</th>
<th>Hypothesis df</th>
<th>Error df</th>
<th>Sig.</th>
<th>Partial Eta Squared</th>
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<tr>
<td>Wilks' Lambda</td>
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<td>1447.792b</td>
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<td>85.000</td>
<td>.000</td>
<td>.981</td>
</tr>
<tr>
<td>Hotelling's Trace</td>
<td>.51.099</td>
<td>1447.792b</td>
<td>3.000</td>
<td>85.000</td>
<td>.000</td>
<td>.981</td>
</tr>
<tr>
<td>Roy's Largest Root</td>
<td>.51.099</td>
<td>1447.792b</td>
<td>3.000</td>
<td>85.000</td>
<td>.000</td>
<td>.981</td>
</tr>
<tr>
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<td>38.856</td>
<td>6.000</td>
<td>168.000</td>
<td>.000</td>
<td>.581</td>
</tr>
</tbody>
</table>

Measure - SGOT (AST); a. Design: Intercept + GROUP; Within Subjects Design: Time
b. Exact statistic; c. The statistic is an upper bound on F that yields a lower bound on the significance level.
Table 6: Serum GOT (AST) level (IU/L) for all the three groups across four time periods.

<table>
<thead>
<tr>
<th>Time Periods</th>
<th>Group I (Only oral Hypoglycemic agent used)</th>
<th>Group II (Kasni crude seed powder &amp; Oral Hypoglycemic agent used)</th>
<th>Group III (Decoction of Kasni seed powder &amp; Oral Hypoglycemic agent used)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
<td>N</td>
</tr>
<tr>
<td>2. Thirty days</td>
<td>37.9667</td>
<td>3.36804</td>
<td>30</td>
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<td>4. Ninety days</td>
<td>34.9667</td>
<td>3.23220</td>
<td>30</td>
</tr>
</tbody>
</table>

Table 7: Analysis of between group effects.

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
<th>Partial Eta Squared</th>
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<td>.989</td>
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<td>GROUP</td>
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<td>.161</td>
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<td>Error</td>
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<td>87</td>
<td>57.202</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Measure: SGOT (AST); Transformed Variable: Average

Table 8: Multiple comparisons group I, II & III for SGOT (AST).

<table>
<thead>
<tr>
<th>Tukey HSD</th>
<th>(I) GROUP</th>
<th>(J) GROUP</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>95% Confidence Interval</th>
<th>Lower Bound</th>
<th>Upper Bound</th>
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<tbody>
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<td>II</td>
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<td></td>
<td></td>
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</tr>
<tr>
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<td>III</td>
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</table>

Measure: SGOT (AST); Based on observed means. The error term is Mean Square (Error) = 14.301. * The mean difference is significant at the .05 level.

DISCUSSION

This was evidenced in the previous studies and also noted in the present study, that there was an increase in the level of SGPT and SGOT in patients suffering from diabetes mellitus as compared to normal individuals. The increase in the liver enzymes recorded in the present study clearly indicates the onset of type 2 diabetes and could be used as a biomarker for the assessment of type 2 diabetes. Type 2 diabetes is a metabolic disorder with a more complex etiology and is commoner than type 1, but much less is known about its pathogenesis. Alternation in liver enzymes, SGPT and SGOT has been reported by Kasni crude seed powder with oral hypoglycaemic agent and Kasni seed powder decoction with oral hypoglycaemic agent is more effective than only oral hypoglycaemic agent in decreasing serum SGOT level of selected patients. Profile plot is showing Kasni seed powder decoction with oral hypoglycaemic agent is more effective than Kasni crude seed powder with oral hypoglycaemic agent in decreasing SGOT (AST) level (Figure 2).
Zafar R et al. Results of their studies showed that Kasni could afford a protection against hepatocellular damage. Ahmed B et al described that Kasni normalized the tissues as neither fatty accumulation nor necrosis was observed. Tabassum N et al. and Abdulrahman L et al. also reported that Kasni possesses both prophylactic and curative activity against acute and chronic hepatotoxic models. These above results are in agreement with present finding as Kasni (Cichorium intybus) seeds could decrease the levels of SGPT and GOT in newly diagnosed diabetes mellitus type 2 patients. Significant improvement in SGPT and SGOT level indicates that Kasni seeds can be used as add on therapy to those patients whose increased liver enzymes like SGPT, SGOT can not be controlled by conventional drugs. Combinations of oral hypoglycemic drugs are used to achieve liver enzymes control and to improve hepatotoxicity due to hyperglycemia. Hypoglycemic drug sulfonylurea Metformin (Glycomet SR) was used for this study. With the labeling of Tolbutamide by the U.S. Food and Drug Administration in 1962, sulfonylurea class of drugs quickly became the mainstay of treatment for type 2 diabetes mellitus. Although newer agents have recently entered the marketplace, sulfonylureas still play a primary role in pharmacologic management of type 2 diabetes mellitus. Glycetem SR (metformin) was the drug of choice because of certain advantages like it lowers blood glucose primarily by decreasing hepatic glucose output and reducing insulin resistance and when used as monotherapy, metformin does not cause hypoglycemia. The reported incidence of lactic acidosis during metformin treatment is less than 0.1 cases per thousand patient years and the mortality risk is even lower. Metformin does not promote weight gain and can reduce plasma triglycerides by 15–20% and is the only therapeutic agent that has been demonstrated to reduce macrovascular events in type 2 diabetes mellitus.

On the basis of present study authors suggest Kasni seed preparations as an adjuvant therapy to the newly diagnosed patients of uncomplicated type 2 diabetes mellitus. The use of Kasni seeds for primary control of SGPT, SGOT in patients with metabolic disorder could be promising after few more investigation of herb-drug interactions in vivo, specific ingredient responsible and to determine the precise molecular mechanism of action of Kasni (C. intybus).

CONCLUSIONS

The add on therapy with Kasni seed preparations is more effective for the management of altered serum SGPT and SGOT levels in Type 2 diabetes mellitus patients than only oral hypoglycaemic agent in decreasing SGPT & SGOT of selected patients. Among Kasni seed preparation treated groups, decoction was found more effective than crude seed powder.

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