SKM VAIDHYA AMIRTHAM

News Letter of SKM in Siddha, Ayurveda and Unani

Vol : 2 **Issue : 3** **OCTOBER - DECEMBER 2023**

NEWLY LAUNCHED

Effective remedy for CERVICAL SPONDYLOSIS

650mg

- Cervical Myelopathy & Cervical Radiculopathy
- Cervical Degenerative disc disease
- Spasm of the muscles in the cervical region

"नित्यं हिताहारविहारसेवी समीक्ष्यकारी विषयेष्वसक्तः
दाता समः सत्यपरः क्षमावानाप्तोपसेवी च भवत्यरोगः"

He, who indulges daily in healthy foods and activities, who discriminates the good and bad of everything and then acts wisely.

Ref :Ashtānga Hridayam

Who is not attached too much to the objects of the senses, who develops the habit of charity, of considering all as equal, of truthfulness, of pardoning and keeping company of good persons only, becomes free from all diseases.

Articles are invited in Siddha, Ayurveda and Unani fields about clinical experience, rare medicinal preparations, successful treatments, Herbal informations and AYUSH Foods for our "SKM Vaidhya Amirtham" News letter which has around 10000 copies of circulation.

Please send your Articles/Suggestions to:
SKM Center for Ayush System Research and Education
Saminathapuram (Post), Modakkurichi,
Erode - 638 104. Tamilnadu, India.
email:techsupport@skmsiddha.org

Kadukkai maathirai (Siddha herbal formulation) reverses liver pathology associated with metabolic dysfunction in high fat diet-induced fatty liver disease a preclinical study

ABSTRACT:

The study explores the hepatoprotective effect of Kadukkai maathirai (KM) in high fat diet (HFD) induced nonalcoholic fatty liver disease (NAFLD) in rats. Total 54 Sprague Dawley rats were used in the study, 9 groups: Group I – IV kept as normal and test drug control and group V - NAFLD disease model- received HFD for 40 weeks. Group VI – IX received HFD for 40 weeks and then test drugs: Group VI – VIII received KM in three different doses for 45 days. Metformin (standard) was administered to Group IX for 45 days. On day 46, the blood and liver tissue were collected for analysis. KM at 36, 144mg/kg and metformin showed a significant decrease in ALP level, all three doses of KM and metformin showed a significant reduction in direct bilirubin levels. A significant improvement in HDL was observed in all doses of KM and metformin-treated groups. Oral glucose tolerance test (OGTT) findings in KM treated test groups showed significantly reduced plasma glucose levels. The KM treated groups and metformin-treated groups showed a reduction in body weight at 47th week, and significantly reduced relative liver weight when compared with the HFD group.

Histopathological evaluation of KM treated groups showed normal architecture of central vein and hepatic cords. Portal triads were also generally normal in their location and pattern. No indication of fatty liver. This study confirms the ability of phytoconstituents present in KM in reversing the metabolic dysfunction and liver pathology seen in NAFLD. Further studies are required to evaluate KM as a therapeutic agent.

INTRODUCTION:

Nonalcoholic liver disease (NAFLD) is a chronic disease that affects people worldwide; non-alcoholic steatohepatitis (NASH) is an advanced stage with hepatic inflammation and fibrosis.

Cirrhosis, liver cancer and liver failure can result from NASH[1,2]. Globally, NAFLD is growing due to pandemic of obesity, mainly in western countries, and is presently estimated to be at 24%[3]. In Asian countries, it was found to be between 8%-19% in individuals who are lean and non-obese[4,5,6]. NAFLD has risen to become the 2nd most common reason for the transplantation of liver in the United States[5]. In India, the NAFLD prevalence rate is 9–32%[7,8,9].

The precise pathogenesis of NAFLD is still largely unknown. The initiation and progression of NAFLD and its pathological mechanisms were described in a pathological model of NAFLD called the“multihit”model10. According to this model, NAFLD is associated with a deranged metabolism of fatty acid, which will lead to insulin resistance then to altered signalling transductions, which makes hepatocytes more susceptible to subsequent hits. Fatty acid oxidation in mitochondria causes oxidative stress and expression of proinflammatory cytokines, profibrogenic factors, adipocytokines, all these factors contributing in inflammation and necrosis, along with fibrogenic cascade activation[10,11]

NAFLD therapies are currently divided into two types: (1) life style associated interventions for weight reduction, healthy diet implementation, and exercise (2) pharmacological approach. Several medicines including antioxidants, insulin secretagogues, statins, and drugs acting on the renin-angiotensin-aldosterone pathway have been tested in clinical trials12,13. To date, the Food and Drug Administration (USFDA) has not yet approved any medications for treating NAFLD. As a result, there is an unmet medical need for drugs to treat NAFLD.

The benefits of herbal products on the prevention of NAFLD progression have got consideration in recent years because they are abundantly available throughout the world, especially in Asian nations and have few or no adverse effects14,15. Herbal medicines (HM) from various traditions, which are basis for herbal natural medicines and products, are critical in the development of hepatoprotective medications. As described by Siddha literature KM is a traditional polyherbal formulation of Indian medicine. KM has been used to treat anemia, generalized edema and ascites secondary to liver disease. Formulations of KM consist Piper nigrum (P.nigrum), Citrus lemon, Terminalia chebula (T.chebula), Eclipta alba, ferrous sulfate soaked in citrus lemon.

Each of the constituents present in KM known to have a hepatoprotective effect[16]. T. chebula consists of gallic acid and chebulic acid; gallic acid has anti-inflammatory and also antioxidant properties, and chebulic acid has antioxidant with hepatoprotective property. P nigruminhibits lipid peroxidation and superoxide free radical generation due to its antioxidant effect facilitated by the presence of phenols and flavonoids. Coumestans, present in E. alba, exerts a protective effect on liver cells in various disease conditions and stimulates regeneration of liver cells[17,18,19].

Even though there is evidence of KM's use in treating liver disorders by practitioners of Siddha form of medicine, its usefulness in NAFLD has not been explored. Hence, the protective activity of KM was explored by feeding a high fat diet to rats in this study.

High fat diet (HFD) can be given as pellet food or as liquid as they contain 40-70 percent fat calories. Feeding HFD for upto three weeks results in abundance of mononuclear inflammatory cells and severe steatosis in histology. And also HFD causes degenerative changes such as rarefied matrix and cristae loss, oxidative stress, and abnormal mitochondria. An elevated serum insulin was also noted20. Inflammation, hepatic steatosis, and mitochondrial lesions were also noted in HFD fed animals. Several important aspects of metabolic syndrome, for example hyperlipidemia, hypercholesterolemia, obesity, hyperinsulinemia were successfully replicated in the rat mode[121].

MATERIALS AND METHODS:

Materials:

Cholesterol was procured from Juniper Life Sciences, Bangalore, and rest of the compounds were bought from Spain based SPINREACT. Metformin was obtained from local pharmacy. Chemicals used for histology were purchased from Sigma Aldrich. KM - bought from India based SKM Siddha (GMP certified) Company Ltd. All remaining solvents, chemicals, reagents were from good laboratory grade.

Composition of high fat diet:

Lard - 4 kg, Normal pellet (powder) - 3 kg, Casein -1kg, Sucrose – 1 kg, Cholesterol – 500 g, Vitamin – 200mL, D-l methionine - 100 g.

Study design:

After obtaining approval from Institutional Animal Ethics Committee (IAEC), healthy adult female Sprague Dawley rats, 12 – 13 weeks old were used in the study. The females selected were nulliparous and nonpregnant. All the animals were housed individually in polypropylene cages and maintained at a temperature of 27 ± 3°C, relative humidity of 60 ± 10% and 12 hours light / dark cycle with paddy husk as the bedding material. Animals were maintained on standard laboratory diet, rat pellet food and water ad libitum. Animal care and handling were done according to the CCSEA (Committee for Control and Supervision of Experiments on Animals) guidelines. A total 54 rats were used in a study and they were divided into control (n = 24) and test (n = 30) rats.The groups were as follows: normal control and test drug control group consisted of total 24 rats, which were divided into 4 groups (n = 6 / group): Group 1- Gum acacia 2% 1mL/kg for 45 days, Group 2, 3, 4- test drug control, KM 36, 72, 144 mg/kg body weight for 45 days respectively.Toxic control group (HFD) consisted of total 30 rats. All these rats received HFD for 40 weeks to induce NAFLD. Following this, they were divided into 5 groups (n=6) and treated orally as follows. Group 5- toxicant control and received gum acacia 2% 1mL/kg orally for 45 days. Group 6, 7, 8- test drug KM36, 72, 144mg/kg body weight orally for 45 days respectively. Group 9- received metformin (30mg/kg body weight) orally for 45 days.

Parameters assessed:

After overnight fasting, blood was collected by retroorbital puncture in vacutainers (for biochemical analysis). Aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), bilirubin (D-BIL), total protein, total cholesterol, triglycerides (TG), and HDLwere analyzed using a fully automatic analyzer.At the end of the study duration, the animals were sacrificed on 47th week by giving high dose intraperitoneal ketamine; then livers were dissected, weighed and subjected to histological examination[22].

Statistical analysis:

The results of control, test drug control, experimental groups were presented as mean±SD, and statistical analysis was performed.For bodyweight, relative liver weight, GSH: One-way ANOVA followed by post hoc Tukey's multiple comparison tests"SPSS version 16 Software. A $p<0.05$ was considered to be statistically significant.

Other biochemical parameters: One-way ANOVA followed by post hoc Dunnet's test (SPSS version 16 Software). A $p<0.05$ was considered to be statistically significant. For OGTT: values were expressed as Mean±SD 2-way ANOVA followed by post hoc Tukey test was used. AUC: (Mean±SEM) One-way ANOVA and then post hoc Tukey's multiple comparison tests (SPSS V-16 Software). A $p<0.05$ was considered as statistically significant.

Table 1: Effect of KM on liver function test and lipid profile in HFD induced NAFLD in rats

Groups	Serum AST (U/L)	Serum ALP (U/L)	Serum D-BIL (mg/dL)	Serum Cholesterol (mg/dL)	Serum HDL (mg/dL)	Serum TG (mg/dL)
Normal		169.5±54.5	0.05±0.01	83.5±8.9	53.5±6.1	85.36±65
HFD	157.6±33.4*	432.5±113.6*	0.156±0.069*	128.3±32.1	29.5±9.5*	172±52*
HFD+ KM36	150.4±24.2	281.0±120.4#	0.056±0.016#	78.0±13.4	49.2±4.8#	131±59
HFD+ KM72	178.3±23.4	311.3±56.6	0.075±0.06#	84.83±7.3	51.3±3.3#	101±45
HFD+ KM144	158.05±38.2	276.5±51.6#	0.058±0.013#	80.8±9.2	51.5±7.6#	129±93.24
HFD+ Metformin	151.9±35.7	280.1±91.7#	0.083±0.034#	87.1±11.7	54.8±6.3#	85±25

RESULTS:

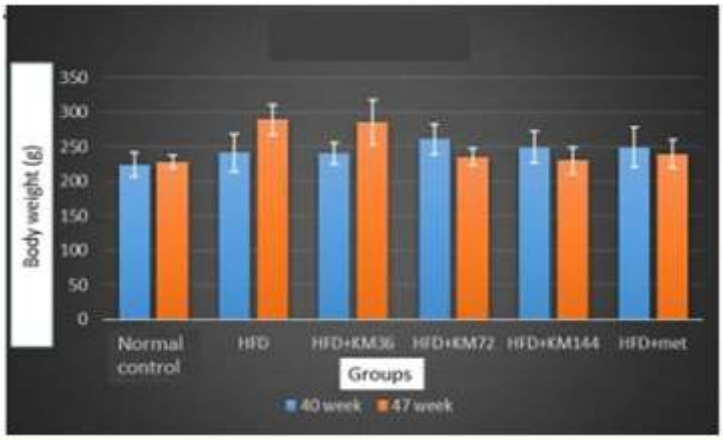

Figure 1: Effect of KM on body weight in HFD induced NAFLD in rats at 40th and 47th week

One-way ANOVA followed by post hoc Tukey test was performed. Values are expressed as mean±SD

The KM 72, KM144 groups and metformin-treated groups showed a reduction in body weight when compared to HFD group of animals, but no statistical significance was shown (Figure1).

Liver function test:

The present study data showed a significant increase ($p<0.05$) in the serum AST, ALP and direct bilirubin in HFD treated rats compared with normal rats. No statistical changes observed in AST values. The KM 36, 144mg/kg and metformin groups showed a significant decrease in ALP level, and all three doses of KM and metformin showed a significant reduction in direct bilirubin levels (Table 1).

Lipid profile:

There was a significant ($p<0.05$) increase in serum triglyceride and a significant ($p<0.05$) decrease in HDL levels in HFD fed rats as compared to normal control.A significant improvement in HDL was observed in all doses of KM and metformin-treated groups (Table 1). There was significant reduction in relative liver weight in KM36 group (0.02±0.003) when compared to HFD group (0.05±0.001). In HFD treated rats, the level of GSH, µmol/g (31.11±9.3) in liver homogenate was significantly reduced ($p<0.05$) compared to normal control (70.26±8.8). It was not significantly altered in test drugs and metformin-treated rats.

Area under the curve (AUC):

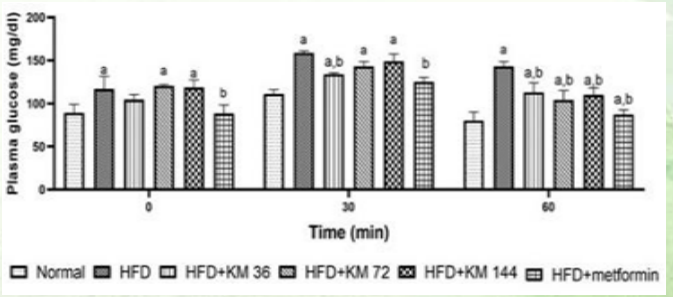

Figure 2a: Effect of KM on plasma glucose profile in OGTT study in HFD induced NAFLD in rats at 47th week.

Note: a-vs Normal control, b-vs HFD, $p<0.05$, 2- way ANOVA followed by post hoc Tukey's multiple comparisons test was performed.Values are expressed as mean ± SD.

At 0 min: Compared to normal control animals, HFD group, KM72 and KM 144 group showed a significantly increased plasma glucose levels. This showed these groups had impairment in insulin response. Compared to HFD group only metformin group showed significantly reduced plasma glucose levels. At 30 min:Compared to normal control animals, all the groups except metformin showed significantly increased levels of plasma glucose levels. Compared to HFD group, KM36 and metformin group showed a significantly reduced plasma glucose levels (Fig.2a).

At 60 min: Compared to normal control animals, all the groups except metformin showed significantly increased levels of plasma glucose levels. Compared to HFD group, all the groups showed significantly reduced plasma glucose levels (Fig.2a).

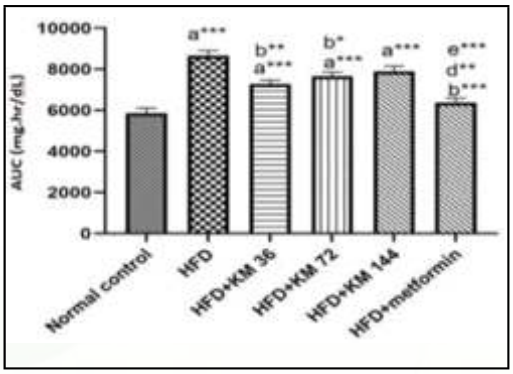

Figure 2b: Effect of KM on the area under the curve of OGTT study in HFD induced NAFLD in rats 47th week.

Note: a- vs Normal control, b- vs HFD, e-vs HFD+KM144. One-way ANOVA followed by post hoc Tukey test was performed. Values are expressed as Mean ± SEM
*p<0.05, **p<0.01, ***p<0.001

The AUC in HFD fed rats was significantly ($p<0.05$) higher than those in normal control. There was a reduction in AUC with all doses of KM but it was significant ($p<0.05$) at doses of KM 36 and 72mg/kg as compared to HFD alone treated rats. The AUC was significantly lower ($p<0.05$) in metformin treated group than HFD alone fed rats and was comparable to normal (Fig.2b).

Histopathology:

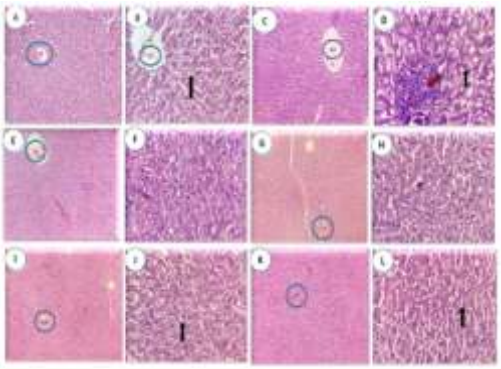

Figure 3: Effect of KM on histopathology of liver in HFD induced NAFLD in rats.

Normal (A, B): Showed normal liver structure. HFD (C, D): Clear indication of NAFLD steatosis with macrovesicular hepatocytes with large intracytoplasmic fat droplets or smaller droplets pushing the nucleus to the periphery of the cell (indicated by arrows). (CV- Central vein) Aggregation of inflammatory cells indicating mild or moderate inflammation. HFD+KM36 mg/kg (E, F): Liver structure mostly looked normal. However, there were few steatotic cells (indicated by black arrow) and mild aggregation of inflammatory cells (indicated by red arrow). (CV-Central vein). HFD+KM72mg/kg (G, H):Showed normal liver structure. HFD+KM1 44mg/kg (I, J): Showed normal architecture of central vein (CV) and hepatic cords (indicated by arrow). Portal triads were also generally normal in their location and pattern. No indication of fatty liver. HFD+metformin (K,L): Showed normal architecture of central vein (CV) and hepatic cords (indicated by arrow). Portal triads were also generally normal in their location and pattern; no indication of fatty liver (Fig.3).

DISCUSSION:

Non-alcoholic fatty liver disease (NAFLD) is characterised by fat deposition in the liver, in the absence of alcohol consumption, viral infection, or other specific etiologies[24,25]. NAFLD is at present widely regarded as a manifestations of metabolic syndrome along with insulin resistance, as the major pathophysiology, due to its significant links to obesity, impaired glucose metabolism, T2DM, hypertension, with hypertriglyceridemia[25].

Obesity is an energy balance condition in which daily energy spending is not balanced with everyday energy consumption to maintain body weight[26]. Obesity has a complex aetiology, but dietary habits, notably a consumption of HFD are understood as an risk feature in its progress[27]. In rats, a high-fat diet causes steatohepatitis, periportal collagen buildup, stellate cell activation, raised ALT levels, and enhanced stellate cell- derived TGF beta and serum TNF-α levels[25]. HFD consumption leads to increased body and liver weight, hepatosteatosis, hepatocyte dysfunction, and increased adipose mass[28]. The present study showed elevated body weight (BW) which was significant in HFD treated animals compared to the normal animals, similar to findings of Xu et al[27].

Treatment with KM in doses of 72 and 144mg/kg in the HFD model showed a decreasing trend in body weight though it was not clinically significant. Probably, a longer duration of treatment with KM is required. The relative liver weight was comparable to normal control in HFD treated rats who received KM in varying doses. This could be due to a decrease in hepatosteatosis, as seen in histopathology of the liver[29]. Animal studies have demonstrated that eating a high-calorie, high-fat diet increases the amount of fatty acids in the blood while increasing de novo fat synthesis, resulting in fatty liver, raised bilirubin, and elevated liver enzymes. Serum ALT and AST levels increase, often indicate that a leak from damaged hepatic cells and reflect hepatocyte injury[30,31]. An increase in serum ALP indicates cholestasis. Though AST, and ALT were not significantly changed, a histopathological examination of the liver revealed improved hepatic structure with hepatocytes almost normal in KM treated groups. Treatment with KM in HFD rats significantly ($p<0.05$) reduced ALP, which reflects improvement in the bile flow of the liver.

In HFD-fed rats, elevated levels of malondialdehyde and protein carbonyls, as well as low levels of GSH, suggest enhanced lipid peroxidation and protein oxidation. There is a constant balance between intrahepatic antioxidants and reactive oxygen species (ROS) to prevent oxidative damage. ROS can build up when there is an imbalance and cause steatohepatitis by inducing lipid peroxidation, protein oxidation, cytokine production, and increased collagen synthesis and cell death[32]. Hepatic GSH levels were not significantly altered by KM in HFD treated rats indicating that it did not play an important role in the present NAFLD model.

High fat intake is also linked to hepatic lipid buildup and elevated triglycerides, leading to steatosis, insulin resistance and dyslipidemia[33,34]. There was a decline in triglyceride levels in HFD fed rats treated with KM, but it was insignificant. HDL improvement was noted in HFD fed rats who were treated with KM. Various mechanisms can increase HDL - an increased transport of cholesterol away from tissues or other type of lipoproteins to HDL; a decrease in the transfer in esters of cholesteryl from HDLs to liver; or other type of lipoproteins via the cholesteryl ester transfer protein (CETP), upregulation in the LCAT enzyme or ABCA1. Which of these contributed to the action of KM has to be elucidated[35,36].

Free fatty acids (FFA) found in HFD are important intermediaries of lipotoxicity, acting as cellular toxins thus causing lipid accumulation via insulin resistance. In the setting of insulin resistance, a cascade of molecular changes in insulin signalling has been demonstrated, eventually leading to triglyceride accumulation in the liver[37]. The OGTT has the benefit of being performed in physiological conditions, as glucose is administered orally, safeguarding the effect in enterohormone response and kinetics of physiological glucose absorption[38]. AUC analysis showed an improving trend with all doses of KM though it was significant with KM 36 and 72mg/kg. The beneficial action could be due to either enhanced insulin secretion or improved glucose uptake by tissues, thus lowering insulin resistance in HFD fed rats.

The benefits of KM could be due to its herbal ingredients (P nigrum, T chebula, E alba and C lemon) and phytoconstituents (piperine, D-allose, chebulic acid, etc.). In vitro beta cells were stimulated by T chebula extract, which resulted in enhanced insulin production[39]. According to Subramanium et al., the extract suppressed lipogenesis by lowering lipogenic enzyme expression, boosted fatty acid oxidation by activating proliferator- activated receptors (PPAR), and triggered anti-inflammatory responses[40]. These mechanisms could play a role in overcoming insulin resistance.

P nigrum has an antioxidant action that is mediated by phenols and flavonoids. It improved lipid profiles and raise HDL levels in the blood in others studies. The underlying mechanism for black pepper-mediated increase in blood serum HDL levels could be piperine- induced ABCA1 overexpression[41]. Brahma Naidu et al. demonstrated that P nigrum extract reversed metabolic changes seen due to gluconeogenesis and ketogenesis; and hepatic lesions that occur in metabolic syndrome[42].

CONCLUSION:

In High-fat diet treated rats, the dose of 36 mg/kg of KM improved glucose tolerance, HDL levels, decreased relative liver weight and bilirubin levels and cholesterol. Other doses of KM when administered to HFD fed rats affected parameters to a varying extent.

SKM Vaidhya Amirtham CME Programme - Nashik

On December 10, 2023, our company successfully organized the SKM Vaidhya Amirtham CME Programme in Nashik, Maharashtra. The event commenced with a warm welcome speech delivered by Dr. V.M. Ravichandran. Distinguished speakers, including Dr. L. Mahadevan, Dr. Ramdas Avhad, and Dr. Mahesh Birla, shared their insights on topics such as "Current Trends and Recent Advances in Clinical Rheumatology in Ayurveda", "Pain Management by Panchakarma" and "Klaibyam. an Ayurvedic Approach". The inauguration of the program and the launch of innovative products like Manyawin Forte Softgel Capsule, Katiwin Softgel Capsules, Rheumowin Forte Tablet, and Fungiwin Powder added significant value to the occasion. The event was concluded with a gracious vote of thanks extended by Shri. S.K. Sharath Ram, Executive Director of SKM Siddha and Ayurveda Company (India) Pvt. Ltd.









Katiwin

Softgel Capsules

650mg

NEWLY LAUNCHED

Effective remedy for

LUMBAR SPONDYLOSIS

- Sciatica / Lumbosacral radiculopathy
- Lumbago due to Muscle sprain and spasm
- Facet arthropathy in the Lumbar Spine
- Vertebral and skeleto-muscular problems with numbness
- Degeneration of the lumbar spine ligaments

Sahachara — A nerve-healing agent that aids in reversing nerve damage, alleviates symptoms associated with radiculopathy.

Devadaru — An anti-inflammatory agent that alleviates pain and inflammation and ease muscle spasms and strains.

Nirgundi — A potent analgesic, aiding in the reduction of intense pain and tenderness.

Punarnava — Mitigates symptoms of neuro--muscular disorders such as sciatica, addressing pain in calves and thighs along with numbness.

Eranda — Apana Vayu Anulomana helps to balance the Apana Vata.

Ashwagandha — Facilitates recovery post-injury and induces an anabolic effect on bones affected by osteoporosis.

Bala — Anti-inflammatory and analgesic characteristics, it alleviates joint and muscle pain, eases stiffness and improves the mobility of the lumbar spine.

Shunti — Supports healing of ligament injuries in the lumbar spine.

Jeevaniya panchamoola — Enhances blood circulation and diminishing inflammation of the lumbar vertebrae, offering nourishment to the bones.

Printed & Published By:

SKM Center for Ayush system Research and Education

Saminathapuram, Modakkurichi, Erode - 638 104. Tamilnadu, India

Tel Fax: +91 424 2500590, 2501238 Website URL: www.skmsiddha.com

Feedback your comments to the E-mail: techsupport@skmsiddha.org | designs@skmsiddha.org