Nonalcoholic Fatty Liver Disease: Is it a Significant Noncommunicable Disease in India

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Submission : 19.09.2022 Publication : 30.09.2022
https://www.doi.org/10.56136/BVMJ/2022_00086

Nonalcoholic Fatty Liver Disease (NAFLD) is one of the common liver pathologies and has been on the rise in recent years, largely due to an increase in the prevalence of obesity. It is one of the most frequent causes of abnormal Liver Function Tests (LFT) and the need for a liver transplant. It ranges from mild steatosis, Nonalcoholic Steatohepatitis (NASH), and fibrosis to severe cirrhosis and Hepatocellular Carcinoma (HCC). Even children with obesity also develop NAFLD. There are several studies on the prevalence of NAFLD in the Indian population in recent times. Many cases of cirrhosis earlier labeled as cryptogenic are now confirmed to be due to NAFLD.

For defining NAFLD, there must be (1) evidence of hepatic steatosis, either by imaging or histology, and (2) lack of secondary causes of hepatic fat accumulation such as significant alcohol consumption, long-term use of a steatogenic medication, or monogenic hereditary disorders[(1)].

Though common in the west, NAFLD is also evolving as a major cause of liver disease in low-income countries. Now that India has become the 5th largest economy in the world with a rise in per capita income, it is showing a significant occurrence of disease. Shalimar et al.[(2)] did the first systematic review and metanalysis for India, which included Sixty-two datasets (adults 54 and children 8) from 50 studies. The pooled prevalence of NAFLD was estimated from 23,581 adult participants and 2903 children. Among adults, the estimated pooled prevalence was 38.6%. The NAFLD prevalence in average risk and high-risk subgroups was estimated to be 28.1% and 52.8%, respectively. Among children, the estimated pooled prevalence was 35.4 percent. The prevalence among non-obese and obese children was 12.4% and 63.4%, respectively.

The age of more than 40 years, central obesity, high Body Mass Index (BMI ≥ 25 kg/m^2), male gender, high Aspartate Transaminase (AST) and Alanine Transaminase (ALT) levels, a raised fasting blood glucose >100 are important risk factors for NAFLD. Metabolic syndrome and insulin resistance are more common in Indian NAFLD patients. Metabolic syndrome comprises of raised waist circumference, hypertension, dyslipidemia, and raised fasting plasma glucose levels. It may even occur in lean people; these patients tend to have other components of metabolic syndrome, including high triglycerides, impaired fasting glucose, and low HDL-C for women.

The pathophysiology of NAFLD is linked to obesity being associated with increased insulin resistance, deposition of intrahepatic triglycerides, reduced adiponectin levels, and insufficient free fatty acid oxidation. About 70% of patients with NAFLD exhibit metabolic derangements defining the metabolic syndrome as per International Diabetes Federation 2006 Criteria[(3)].

In addition to metabolic risk factors, studies from India have also suggested the role of small intestinal bacterial overgrowth, endotoxemia, and toll-like receptor expression in the pathogenesis of NAFLD. Patients with no identified metabolic derangement may also develop NAFLD, which may be related to specific gene defects, including PNPLA3 and TM6SF2 mutations.

These cases usually are asymptomatic unless advanced hepatic fibrosis develops, although some patients describe vague discomfort over the right upper quadrant. Patients with significant fibrosis may have fatigue. Few patients can present with compensated or decompensated chronic liver disease or even hepatocellular carcinoma.

The non-invasive diagnosis for NAFLD is radiological imaging with ultrasound, Computed Tomography (CT) scans, and magnetic resonance elastography quantifying the liver fat. There is an emphasis on an effective non-invasive scoring system. Nonalcoholic fatty liver disease fibrosis score and AST to platelet ratio index are commonly used. Several other biomarker composite scores are also available for clinical use. The general indications for performing a liver biopsy in patients with NAFLD are to confirm or exclude the diagnosis of other liver diseases and to determine amounts of damage to the liver for treatment and prognosis. In India, it is largely restricted to tertiary care centers[(4)].

Autoimmune Hepatitis Can cause steatosis and may be confused with NAFLD/NASH, owing to the similar clinical presentation, including fatigue, abdominal discomfort, and hepatomegaly with elevated ALT levels. It is not infrequently associated with other autoimmune diseases, antinuclear and anti-smooth muscle antibodies are usually detectable in serum. A smaller subset of patients may have anti-LKM-1 or CYP2D6 antibodies.

A variety of drugs have either a true cause-and-effect relationship with hepatic steatosis or are known to accelerate pre-existing steatosis.

Lifestyle interventions are the primary modality for the management of NAFLD and have been shown to improve biochemical and histological outcomes in Indian patients[4]. Sustained weight loss of 5% body weight improves hepatic steatosis on imaging, histology, and associated metabolic parameters. Higher degrees of weight loss in the range of 7%-10% of body weight is needed to improve steatohepatitis and overall disease activity. Both aerobic exercise and resistance training reduce fat in the liver; physical activity of more than 150 minutes/week results in significant decreases in aminotransferases.

An antioxidant like vitamin E (α-tocopherol) 800 IUs/day in adults and children without diabetes and with biopsy-proven nonalcoholic steatohepatitis is reported to be useful. Some studies suggest improvement even in liver histology. British guidelines by National Institute for Health and Care Excellence (NICE) recommend vitamin E as an option regardless of diabetes status. Pioglitazone may be considered for patients with biopsy-proven nonalcoholic steatohepatitis with or without type 2 diabetes. Metformin has no significant effect on hepatic histology and is not recommended as a specific treatment for steatohepatitis. Statins are not recommended specifically for the treatment of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis, but they improve comorbid dyslipidemia and thereby result in decreased hepatic transaminase levels. For children, a randomized controlled trial showed that Docosahexaenoic Acid (DHA) supplementation improves liver steatosis at doses of 250 to 500 mg/day. GLP1 analogues & SGLT 2 inhibitors are unproven value.

In a 2015 prospective study of patients with biopsy-proven nonalcoholic steatohepatitis, bariatric surgery resulted in the disappearance of nonalcoholic steatohepatitis from almost 85% of patients after one year of follow-up. This could be a therapeutic option for appropriate morbidly obese patients with nonalcoholic steatohepatitis, whose condition does not respond to lifestyle modifications[5].

An increasing number of associated metabolic disorders (e.g., insulin resistance, type 2 diabetes, hypertension, dyslipidemia, visceral obesity) increase the risk of progressive liver disease. In patients with hepatic steatosis without fibrosis (nonalcoholic fatty liver) at initial evaluation, progression to nonalcoholic steatohepatitis and fibrosis is uncommon and occurs very slowly; fibrosis classification will increase by 1 level every 14 years. Progression to nonalcoholic steatohepatitis and advanced fibrosis is influenced by concomitant metabolic risk factors. Among patients with nonalcoholic steatohepatitis, 20% develop progressive fibrosis leading to cirrhosis. Over 15 years, all-cause mortality increased by 34% to 69% after the development of NAFLD[2].

NASH-related decompensated cirrhosis and HCC are now becoming leading indications for liver transplant in India, particularly in urban areas. However, there is a paucity of Indian data on transplant outcomes in patients with NASH.

With NAFLD being a lifestyle disease, efforts for prevention and control are required not only at the individual and family level but also at the government and administrative levels. The recent integration of NAFLD into the National Program on Prevention and Control of Cancer, Diabetes, Cardiovascular disease, and Stroke (NPCDCS) by the Ministry of Health and Family Welfare of India is an encouraging step in this direction[6].

In conclusion, the incidence of NAFLD is increasing & expected to be an important noncommunicable disease in India.

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Efficacy of 'Anuradha Oil 11' on incision wound healing

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Submission: 03.08.2022 Acceptance: 27.09.2022 Publication: 30.09.2022

https://www.doi.org/10.56136/BVMJ/2022_00076

Abstract

Background: The herbs/plants are used in traditional and folk medicines worldwide for treating wounds and burns. Animal models help to understand the tissue repair process and corroborate various approaches to formulate therapy for wound healing. **Objective:** To determine the efficacy of 'Anuradha Oil 11' (AO11) on incision wound healing in Wistar rats. **Methods:** There were four groups formed based on the treatment received as Test group (AO11), Control group, Standard I Hydroheal group, and Standard II Curcumin group. Six female rats were used per group in this study. Linear-length paravertebral incisions were made through the shaven skin. Three surgical sutures were placed, each one cm apart. A 0.1 mL of test material AO11, 0.1 mL of standard I hydroheal, and 0.1 mL of 1% solution of standard II curcumin respectively were applied on the wound once daily for nine days. The control group wound remained untreated. On day nine (post-wound), all the sutures were removed. On day 11, all the animals were anesthetized, and the breaking strength of previously wound-treated skin was measured using a tensiometer. **Results:** All the animals appeared normal and showed no clinical signs of intoxication till the end of the study. The test group showed a higher tensile strength (48.3 g/mm^2) required to open the wound when compared to the control group (38.14 g/mm^2). The increase in tensile strength was statistically significant between the test ($p<0.01$) and both the standard groups ($p<0.05$). There was no statistically significant change in the body weight of all the animals. No mortality was observed in any of the groups. **Conclusion:** The study concluded that the test material AO11 showed an increase in efficacy compared to control and is safe for use.

Keywords: Curcumin, hydroheal, incision, tensiometer, wound

Introduction

Wounds may be defined as a cut or breaks in the continuity of any tissue. Wound healing helps restore anatomically and functionally disrupted skin[(1)]. It is a complex and dynamic process that restores damaged tissues using several sequential events[(2)]. Some diseases like diabetes, immune-compromised conditions, ischemia, and conditions like malnourishment, aging, local infection, and local tissue damage due to burning or gunshot often delay wound healing[(2)]. Wound healing involves continuous cell-cell and cell-matrix interactions that allow the process to proceed in three overlapping phases, viz. inflammation (0–3 days), cellular proliferation (3–12 days), and remodeling (3–6 months)[(1)]. The typical treatment includes honey, silver nano-particles, copper complexes, stem cells, broad-spectrum antibiotics, etc. Newer techniques like VAC (Vacuum-Assisted-Closure) and Hyperbaric Oxygen Therapy (HBOT) have been attempted for wound healing with encouraging results but are costly along with some limitations[(3)].

The herbs/plants are used in traditional and folk medicines worldwide for treating wounds and burns. These plants produce secondary metabolites like alkaloids, flavonoids, glycosides, and tannins that possess various bioactivities. These phytochemicals can induce and promote the healing and regeneration of lost tissue by multiple mechanisms[(4)]. These phytomedicines are affordable and safe, although many are yet to be explored for their wound healing properties[(5)].

Different types of models have been developed and used for studying various mechanisms of wound healing activities[(6)]. Animal models help to understand the tissue repair process and corroborate various approaches to formulate therapy for wound healing[(7)]. The incision wound model is a virtuous animal due to its accuracy and reproducibility and facilitates multiple investigations. The wound(s) can be made anywhere on the body surface as well in most of the body organs. The shape of the wound depends on the purpose of the study. This model can investigate the wound healing process and influence different systemic and local compounds as well as dressing. This model has been extensively used in skin and every other organ available for surgery in the last century. The outcome measurement is primarily wound strength, collagen content, and histology[(8)].

The present study was conducted to establish the efficacy of the herbal formulation 'Anuradha Oil 11' (AO11) on incision wound healing when applied by the dermal route to female Wistar rats once daily for nine days following the Drug Discovery and Evaluation, Pharmacological Assay, H. Gerhard Vogel (Ed.), 2002, Protocol[(9)].

Materials and Method

Animals

Wistar female rats, weighing 162-255 gm, aged 10 to 12 weeks, procured from APT Testing and Research Private Limited, Pune, were used for the proposed study. They were housed three per cage under a controlled temperature of 20-24°C with relative humidity between 30-70% and 12 hours light and dark cycle.

They were fed with pellets of balanced animal food of Nav Maharashtra Chakan Oil Mills, Pune and water ad libitum.

Test material AO11 - The formulation AO was prepared using Sesame oil and pig fat combination in a certain proportion as a base. This formulation was prepared after giving repeated treatment of hot water extract of the following plant materials: *Curcuma longa* (Rhizome), *Glycyrrhiza glabara* (Rhizome), *Hamiltonia suaveolens* (Stem bark), *Typha angustifolia* (Flowers), *Azadirachta indica* (Leaf).

Method

The study was conducted in 2011-2012 at National Toxicology Institute (NTC), Pune. Four groups of animals as Test, control, Standard I Hydroheal, and Standard II Curcumin were formed; each containing six female rats. The female rats were shaved on the dorsal area of the trunk with electric clippers [WAHL animal clipper KM10 (blades made in the USA)]. A five cm length linear paravertebral incision was made through the shaven skin. One skin clip was made with the help of a scissor on the back of each animal. Three surgical sutures were placed, each one cm apart. A 0.1 mL of test material AO11 (AMAI Charitable Trust's, ACE Hospital and Research Centre, Pune), 0.1 mL of standard I hydroheal, and a 0.1 mL of 1% solution of standard II curcumin were applied daily once for a period of nine days. The control wound remained untreated. On day nine, post wound, all the sutures were removed.

On day 11, all the animals were anesthetized [ketamine (Unijules Life Sciences Ltd.) and xylazine (Indian Immunologicals Limited)], and the breaking strength of previously wound treated skin was measured by using a tensiometer (Make: Veekay Test Lab, Model Veekay Sr. No. 11421). This breaking strength was converted to the tensile strength using the formula[10]:

$$\text{Total strength} = \frac{\text{Maximum Breaking Strength in grams}}{\text{Area of wound}}$$

Length = 50.0 mm and breadth = 1.0 mm

Clinical signs of intoxication and at the site of application were observed in all the groups. Animals were weighed individually on days 0, 7, and 11 of the study. To measure the food consumption, 100g of feed was given to the cage, and at 24 hours, the food remaining was measured. This quantity of feed consumed was divided by the number of animals in each cage, and the amount consumed per animal was calculated. Reduced feed consumption is one of the early signs of toxicity.

Statistical analysis

The values were expressed in terms of mean±SD. Statistical analysis performed was a one-way analysis of variance method (Dunnett's Multiple Comparison Test) using GraphPad prism 5 software[11].

The Institutional Animal Ethics Committee of the NTC, Pune (IPU-0152.07) approved the experimental protocols.

Results

Clinical Findings

All the animals appeared normal and showed no clinical signs of intoxication at the site of application till the end of the study in all the groups; control, test, standard I (hydroheal), and standard II (curcumin). There was no statistically significant change in the body weight and the amount of food consumed by all the animals, as depicted in Tables 1 and 2. No mortality was observed in any of the groups. The animals did not show any other clinical signs like lachrymation, salivation, urination, diarrhea, tremors, and many other signs of toxicity.

Table 1: Mean and SD of body weight with days of follow-up

Groups	Days		
	Day 0	Day 7	Day 11
Control	210.1 (28.22)	213.9 (25.30)	217.3(23.27)
Test	189.0 (8.967)	200.3 (9.416)	205.8 (11.47)
Standard I Hydroheal	179.1 (5.748)	190.7 (5.279)	194.8 (7.367)
Standard II Curcumin	195.3 (24.26)	211.5 (27.71)	216.1 (25.87)

*Data shown as mean (SD).

Table 2: Food consumption (g/cage) with days of follow-up

Groups		Days		
		Day 0	Day 7	Day 11
Control	Cage 1	50.5	45.0	44.0
	Cage 2	47.5	44.5	42.0
Test	Cage 1	39.0	51.5	50.5
	Cage 2	48.0	54.5	47.5
Standard I	Cage 1	42.5	43.5	39.0
Hydroheal	Cage 2	49.0	49.5	48.0
Standard II	Cage 1	44.0	42.5	48.5
Curcumin	Cage 2	42.0	45.5	50.0

Tensile Strength Data

The mean tensile strength data of all the groups are depicted in Table 3. There was a statistically significant increase in tensile strength required to open the wound in the standard I (hydroheal) group and standard II (curcumin) group ($p<0.05$), when compared with the control group. The test group showed a comparable increase in the mean tensile strength ($p< 0.01$) required to open a wound compared to the standard I and II.

Table 3: Mean tensile strength measured on day 11

Groups	Breaking strength load in Kg	Tensile strength gm/mm^2
Control	1.907 (0.207)	38.14 (4.15)
Standard II Curcumin	2.066 (0.1883)	46.32 (4.17)
Standard I Hydroheal	2.267 (0.186)	45.30* (3.72)*
Test	2.417 (0.445)	48.30** (8.89)

Data shown as mean (SD). $^*p<0.05$, $^{**}p<0.01$. Three readings were taken to evaluate the mean.

The test group (0.1 mL) showed 41.14%, the standard I hydroheal group (0.1 mL) showed 30.56%, and the standard II curcumin group (0.1mL of 1% solution) showed a 0.642% increase in tensile strength required to open the wound when compared to the control group as depicted in Table 4.

Table 4: Tensile strength compared to control measured on day 11

Group	Increase in % Breaking Strength	Increase in % Tensile Strength
Standard II Curcumin	0.760	0.642
Standard I Hydroheal	29.54	30.56
Test	38.11	41.14

Figure 1 shows the comparison between the test and control groups.

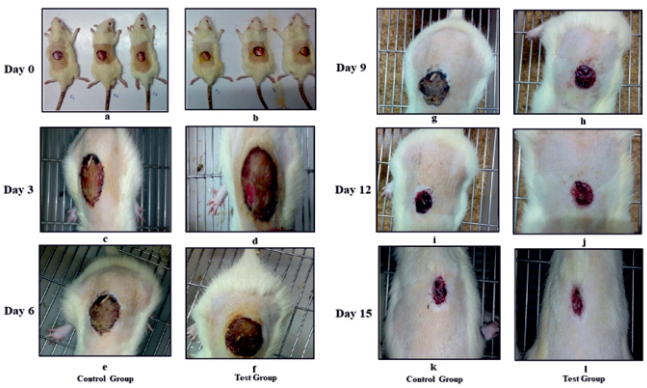

Figure 1: Comparison of control versus test group

Discussion

Wounds are common health concerns involving an intricate healing process comprising three imperative phases; inflammatory, proliferative, and remodeling. Wounds, if ignored, may turn infectious; hence need to be treated carefully and immediately. At times, chronic wounds develop free radicals and consequently result a delay in the wound healing process. Farfetched advancements in medicine have made numerous wound healing drugs available in the market, but these remedies are expensive with undesired side effects. These agents lack the potential to facilitate the wound healing process. However, many natural products have the ability to repair wounds by exerting anti-oxidant and anti-inflammatory effects and promoting tissue and collagen generation to reinstate the skin. Many phyto compounds are reported to contain cell growth factors and play a key role in fibroblast proliferation, and this property is deficient in synthetic compounds[12-14]. Plants and herbs have been reported to possess wound healing properties due to the presence of various active constituents like alkaloids, flavonoids, glycosides, terpenoids, etc.

The AO11 oil containing pig fat and without pig fat has been previously evaluated by the investigator, Patankar et al. for its safety profile[11]. The oil was assessed for its acute and sub-acute dermal toxicity and mucus membrane irritation potential. The formulation was non-toxic to the hemopoietic system and non-irritant to the mucus membrane. There was an absence of renal and hepatic toxicity.

In the present study, incision wound healing efficacy of AO11 was evaluated where all the animals appeared normal and showed no clinical signs of intoxication till the end of the study of nine days. This indicates that the herbal formulation AO11 is tolerable and safe to use without harm. Usually, alteration in body weight is considered as an important parameter for the assessment of the response of an individual to the drug and might indicate its side effects. There was no statistically significant change in the body weight of any of the animals. Similar results were reported by Patankar et al.[11], wherein there exists a minor difference in the relative weight of vital organs of rats from both groups, indicating an insignificant effect on the vital organs due to dermal application of test material for 28 days. The test group (48.30%) showed an increase in mean tensile strength when compared to the control group (38.14%) and both the standards, i.e., hydroheal (45.30%) and curcumin group (46.32%). Studies have reported that extracts of herbal drugs, *Bacopa monniera*, have shown antimicrobial activity and enhanced wound breaking strength; *Tridex procumbents* have shown a significant increase in tensile strength of incision wounds[12,13].

A study by Talekar et al. has been reported wherein polyherbal formulations have shown rapid skin regeneration and wound contraction[14]. This herbal formulation comprises rhizomes of *Curcuma longa* (turmeric) and *Glycyrrhiza glabra* (Licorice), the bark of *Hamiltonia suaveolens*, *Typha angustifolia* flowers, and *Azadirachta indica* (Neem) leaves incorporated in *Sesamum indicum* oil and pig fat used as a base. *Curcuma longa* containing active constituent curcumin is a well-known traditional medicine used widely and reported as a wound-healing agent. It possesses anti-inflammatory action and the ability to enhance granulation, tissue formation, collagen deposition, and wound contraction required for tissue repair[15]. Similarly, *Glycyrrhiza glabra* is reported to have wound healing properties which may be attributed to its anti-oxidant and anti-inflammatory activities[16]. *Hamiltonia suaveolens*, *Typha angustifolia*, and *Azadirachta indica* are traditionally used wound healing agents that are evaluated and reported[17,18]. Sesame oil is used as a healing oil in folklore medicine[19]. It has beneficial wound healing and antimicrobial properties due to its lignan-containing phytoconstituents. Various pharmacological activities like anti-oxidant, antimicrobial and anti-infective are exhibited by different components of the AO11 formulation that promote wound healing.

The literature search did not have any mention of such herbal formulation tested for external and internal wounds as well. In modern medicine, wound management consists of systemic corrections of any co-morbid condition with antibiotics, local use of cleansing agents including antiseptic/antibiotic solutions, etc. However, there is a lack of local agents available to promote the wound healing process. The present formulation shows its regenerative function in wound management. Despite the limitations of conducting the study in a small sample size and lesser days of follow-up, the study shows an increase in the efficacy of the AO11 as compared to the control.

Conclusion

In conclusion, AO11 showed an increase in tensile strength compared to the control group but a decrease in tensile strength compared to standard I and II groups. Thus, it can be concluded that the test material, AO11, may be effective compared to the control group and can be safe for use. It can be used as an internal wound healing agent, including surgical wounds, body incisions, or other internal organ injuries. This formulation could be the first of its type of herbal formulation which can be used in incision wounds confirming its safety and efficacy as well.

Patent: This drug formulation has been submitted for national and US patents and has been accepted in the name of the corresponding author.

1. Indian Patent (Number: 350253) dated 17th February 2011
2. United States patent (Number: US 8,709,509 B2) dated Apr 29, 2014
3. European patent (Number: 2675528) dated 03.05.17

Conflict of Interest: Nil

Source of Support: Nil

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