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Evaluation of Pharmacological Profiling of *Albizia Odoratissima* Bark Extracts on Ethanol-Induced Hepatotoxicity in Albino Rats

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Abstract: Alcohol consumption is a common cause of acute and chronic hepatic toxicity in patients. Discovery of novel therapeutic agents from medicinal plants is most recommended because of the presence of diverse phytochemicals. The aim of the study is to evaluate the therapeutic effect of methanolic extract of bark of *Albizia odoratissima* on ethanol-induced hepatotoxicity in rats. The following objectives A. odoratissima bark extract was screened for phytochemical analysis, and tests on acute toxicity and hepatoprotection using an Albino rat model were conducted. Twenty-four male albino rats were selected and divided into four groups (n=6). Animals received treatments for a period of 21 days. Normal control (Group-I) received vehicles; toxic control (Group-II) received ethanol (2 ml/kg/*p.o.*). Treatment groups (Groups-III and IV) received Silymarin (50 mg/kg/*p.o.*) and plant extract (250 mg/kg/*p.o.*). Biochemical parameters such as hepatic enzymes, biomarkers and histopathology were estimated to assess the severity of hepatic injury and or disease. Phytochemical analysis of extract reported the presence of secondary metabolites. Ethanol significantly increased SGPT and SGOT, ALP, TB, urea, and creatinine and significantly decreased total protein and albumin levels, indicating hepatic damage. Silymarin ameliorates all hepatic enzymes and biomarkers as compared to hepatotoxic control. Meanwhile, methanolic extract significantly decreased only SGPT, ALP, and creatinine, compared to hepatotoxic control. Histopathological examinations of silymarin and *A. odoratissima* revealed reduced liver inflammation. This study concludes that a 250mg/kg dose of *A. odoratissima* showed partial protective effects on ethanol-induced hepatotoxicity in rats as compared with silymarin.

Keywords: *Albizia odoratissima,* Hepatoprotective, Ethanol, Hepatic damage, inflammation.

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1. INTRODUCTION

Liver plays an essential role in physiological functions and bio-transformations such as phase-1 (oxidation, reduction, and hydrolysis) and Phase-2 (conjugations, sulfation, and acetylation) reactions of various chemical substances and drugs. It is also involved in glucose, lipid, and protein metabolism. Hepatic injury can impair its biological functions and eventually cause structural damage. Pathogenic microorganisms and viruses, hepatotoxins, drug overdose, chronic use of drugs, obesity and malnutrition, alcohol, auto-immune disorders, diabetes, and genetic factors are the various risk factors for the development of hepatic diseases and or disorders. Hepatotoxicity is a common organ toxicity that can result in catastrophic consequences ranging from metabolic disorders to death[1]. Alcohol causes a number of deleterious effects on the liver's physiological and metabolic processes. Excess or chronic alcohol consumption may develop into hepatitis, steatosis, fatty liver, cirrhosis, and fibrosis[2, 3]. According to the reports, alcohol abusers develop 80% hepatic fatty liver, 10% alcoholic hepatitis, and 10% liver cirrhosis. Pre-clinical studies reveal that oxidative stress and inflammation are responsible for liver injury in chronic alcoholics[4]. Further, it may lead to hepatic dysfunction with increased apoptosis and fibrotic changes [5]. Hepatic disorders are the most serious and common diseases reported worldwide[6]. Despite the tremendous advancement in modern medicine, the prevention and treatment choices for hepatic diseases and or disorders remains limited[7]. Conventional therapies for liver diseases are sometimes ineffective and may lead to major adverse effects and organ failure. Since ancient times, plant-based medicine(s) has been used in the treatment of various hepatic diseases. Plants and their products are thus in huge demand for the development of new drug molecules for primary health care. Herbal drugs are considered to be efficacious, economic, and safe for the prevention and treatment of liver disorders. In India, various herbal medicines or preparations such as Liv-52, Legalon, Hepatomed, Stimuliv, Himoliv and Kamilari are commercially available[8]. Furthermore, the Indian Medicinal Practitioner's Co-operative Pharmacy and Stores approved various Siddha, Ayurvedic, and Unani medicinal formulations for the treatment of hepatic disorders[9]. Medicinal plants such as Silymarin *marianum*, *Glycyrrhiza glabra, Opilia celtidifolia*, *Terminalia chebula* fruit, *Actino scirpus grossus* tubers, *Phyllanthus amarus, Premna tomentosa,* and *Picrorhiza kurroa* are shown to have hepatoprotective effects in both pre-clinical and clinical studies. *A. odoratissima* Benth. (*Mimosaceae*) is commonly known as "Black Siris" and "Ceylon rose-wood". In India, it is also known as Bhusirisah (Sanskrit), Karmaru (Punjabi), Cinduga (Telugu), Karuvagai (Tamil). It is a tall tree and is found throughout the sub-Himalayan region, in Assam, West Bengal, and the Western ghats of South India. In folklore medicine, *A. odoratissima* is used to treat leprosy, liver diseases, ulcers, skin diseases, rheumatism, diabetes, bronchitis, and burning sensations. According to the literature review, there is a paucity of scientific evidence on the hepatoprotective activity of *A. odoratissima* bark. The purpose of this study is to evaluate the hepatoprotective activity of methanolic extract of bark of *A. odoratissima* (MEAO) against ethanol-induced hepatotoxicity in rats.

2. MATERIALS AND METHODS

2.1. Plant material collection and Identification

During the months of June and July, fresh bark of *A. odoratissima* was collected from the Seshachalam hills of Tirumala, Chittoor district, Andhra Pradesh, India. The medicinal plant bark was identified and authenticated by Dr. K Madhava Chetty of Sri Venkateswara University, Tirupati, Andhra Pradesh, India. (Voucher number: 0807).

2.2. Chemicals and Reagents

All the chemicals and reagents of high quality were employed and obtained from the standard companies and suppliers. Silymarin was procured from Microlabs, India. Ethanol (99% v/v), Methanol (99% v/v), and Ether were obtained from National Scientific Products, Guntur, Andhra Pradesh. For estimation of serum samples, biochemical kits from Agappe, Ernakulam, Kerala were used.

2.3. Extraction procedure

A weighed quantity of finely grounded bark powder was placed in filter bag and the extraction was initiated with methanol (solvent) by continuous hot percolation process by using Soxhlet apparatus at 60-70° c temperature. The obtained extract was collected by filtration with a muslin cloth. Thus, obtained filtrate was treated to solvent evaporation by rotavapor to obtain a crude extract (gummy or semisolid form), which was weighed and stored in a desiccator[10].

2.4. Qualitative phytochemical screening

The methanolic bark extract was screened for the presence of various phytoconstituents such as flavonoids, tannins, alkaloids, and steroids, saponins, and sterols[11].

2.5. Acute toxicity study

This study was conducted in accordance with Organization for Economic Co-operation and Development (OECD-425) guidelines. Selected five rats were fasted but had free access to the water. The first animal was administered a single dose of 2000 mg/kg extract by intragastric tube and kept for continuous observation for 24 hrs to record mortality if any. If the previous animals survived in the limit test and the remaining animals received the same dose sequentially and continuously monitored according to the guidelines. Initially, all the animals were closely observed every 30 min for 4 hrs, followed by routine observation throughout the day. During 14 days of study, general behavioral (tremors, convulsions, salivation, diarrhea, lethargy, sleep & coma), neurological and autonomic changes in the animals were recorded daily[12].

2.6. Experimental animals

Male Wistar albino rats (180-200 g) were procured from Raghavendra Enterprises in Bangalore, Karnataka, India. All the experimental animals were acclimatized one week prior to the experimentation and housed according to the standard guidelines: controlled conditions of 12 h light and dark cycle, relative humidity 50±5% approximately, and temperature of 22±2^{0}C. Animals were fed a standard pellet diet and water *ad-libitum* throughout the study.

2.7. Ethical approval

The study protocol was approved (Reference No: IAEC/VMKVMC/2020) by the Institutional Animal Ethics Committee (IAEC) vinayaka medical cllege in accordance with

the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, Government of India.

2.8. Experimental design

A total of twenty-four male albino rats were selected randomly and divided into four groups, six rats each (n=6). The animals received respective treatments orally through oral gavage for a period of 21 days[13].
Group-I (Normal control): Received only the CMC as vehicle.
Group-II (Hepatotoxic control): Ethanol (40% v/v) at a dose of 2 ml/kg/orally
Group-III (Standard control): Ethanol-treated rats received Silymarin 50 mg/kg/orally
Group-IV Ethanol-treated rats received MEAO 250 mg/kg/orally
Groups-III and IV are considered as treatment groups which receive either standard drug or plant extract.

2.9. Biochemical estimation

Overnight-fasted rats (12 hr) were made more comfortable in a re-strainer and the blood collection procedure was carried out at room temperature. The blood samples were collected from tail vein puncture after dipping the tail in warm water, and serum was separated by centrifuge at 3000 RPM at 40^{0}C for 15 min and stored at a cold temperature (-80^{0}C) for further biochemical analysis. After sample collection, silver nitrate ointment is topically applied over the bleeding site. The levels of Serum-Glutamate Pyruvate Transaminase (SGPT), Serum-Glutamic Oxaloacetic Transaminase (SGOT), Alkaline Phosphatase (ALP), Total Protein (TP), Total bilirubin, urea, creatinine, and albumin were estimated by using a Semiauto analyzer with help of enzyme kit methods.

2.10. Histopathological studies

At the end of the treatment period, the animals were anesthetized under ether and sacrificed by cervical dislocation. The anterior abdominal wall was dissected out and the liver was identified and isolated. The liver specimen was cut into small pieces (3-5 µ thickness) with a microtome. These tissue slices were fixed in 10% of buffer formalin solution and followed by embedding in paraffin solution. Liver tissue slides were stained with haematoxylin and eosin and histopathological analysis was performed [14].

2.11. Statistical analysis

Data of each group (n=6) were expressed as Mean ± Standard Error of mean (SEM). The results were analyzed by using one-way Analysis of variance (ANOVA) with Tukey post-hoc test with a Graph pad prism (V.5). A confidence interval (95%) and $p \leq 0.05$ was set and considered to be statistical significance with comparative control groups.

3. RESULTS

3.1. Phytochemical Screening

The preliminary screening of MEAO revealed the presence of various compounds like flavonoids, tannins, steroids, alkaloids, phenols, saponins, and sterols were shown in Table 1.

Table 1: Preliminary phytochemical screening of methanolic extract of *A. Odoratissima*.

Classes of compound	MEAO
Flavonoids	+
Tannins	+
Steroids	+
Alkaloids	+
Phenols	+
Saponins	+
Terpenoids	-
Sterols	+
Anthocyanins	-
Carbohydrates	-

MEAO-Methanolic extract of A. Odoratissima
Presence (+), Absence (-)

3.2. Ethanol-induced hepatotoxicity

Ethanol (2ml/kg/*p.o.*/21 days) treated rats (Group-2) showed significant ($p<0.05$) increase in the levels of hepatic markers, namely SGPT, SGOT, ALP, total bilirubin, as compared to normal control (Group-1). In contrast to normal control, ethanol significantly ($p<0.05$) decreased TP and albumin levels. Therefore, at the end of the experimentation, ethanol effectively induced hepatotoxicity in normal rats, and thus Group-2 is referred to as the hepatotoxic control (Table 2 and 3).

Table 2: Effect of methanolic extract of *A. Odoratissima* on the hepatic enzymes and Total protein in ethanol-induced hepatotoxicity in rats.

Group	SGPT (U/l)	SGOT (U/l)	ALP (U/l)	TP (U/l)
Normal control	76.23±1.79	83.18±0.78	133.4±1.9	8.56±0.39
Ethanol (40% v/v, 2 ml/kg/p.o.)	209.35±1.6 ^{x}	201.07±2.65 ^{x}	279.9±8.6 ^{x}	2.71±0.13 ^{x}
Ethanol + Silymarin (50 mg/kg/p.o.)	93.3±1.23 $^{x, y}$	96.18±1.59 $^{x, y}$	153.6±3.76 ^{y}	7.53±0.33 ^{y}
Ethanol + MEAO (250 mg/kg/p.o.)	169.98±1.74 $^{x, y, z}$	193.42±2.19 $^{x, z}$	200.3±4.75 $^{x, y, z}$	3.89±0.32 $^{x, z}$

MEAO-Methanolic extract of *A.odoratissima*, SGPT-Serum Glutamic-pyruvic Transaminase Oxaloacetic Transaminase, SGOT-Serum Glutamic-Oxaloacetic Transaminase, ALP-Alkaline phosphatase, TP- Total protein. Data (n=6) are expressed as Mean±Standard error mean. x, y, z, symbol represents significant difference with x=Normal control, y=Ethanol (40 % v/v, 2 ml/kg), z=Silymarin (50 mg/kg/*p.o.*) and MEAO (250 mg/kg/*p.o.*) groups, respectively. p<0.05* is considered as statistical significance. Table 2 Treatment with Silymarin (50 mg/kg) significantly (p<0.05) lowered SGPT, SGOT, and ALP levels and increased TP levels as compared to the hepatotoxic control group. Treatment with MEAO (250 mg/kg) significantly (p<0.05) reduced only SGPT and ALP levels as compared to hepatotoxic control group. Treatment groups showed a significant difference (p<0.05) in SGPT and SGOT levels, while MEAO (250 mg/kg) showed significant difference in all hepatic enzymes as compared to normal control. Moreover, MEAO (250 mg/kg) demonstrated a significant difference (p<0.05) with standard control Silymarin. The results depict that, MEAO (250 mg/kg) exhibited partial hepatic protection in ethanol-treated rats as compared with silymarin.

Table 3: Effect of methanolic extract of *A. odoratissima* on Total bilirubin, Blood urea, Serum creatinine, Blood Albumin in ethanol-induced hepatotoxicity in rats.

Group	Total Bilirubin (mg/dl)	Blood Urea (mg/dl)	Serum Creatinine (mg/dl)	Blood Albumin (g/dl)
Normal control	0.51±0.05	13.98±0.69	0.31±0.09	5.3±0.25
Ethanol (40% v/v, 2 ml/kg/p.o.)	6.37±0.31 ^{x}	29.56±0.75 ^{x}	2.33±0.2 ^{x}	2.68±0.08 ^{x}
Ethanol+ Silymarin (50mg/kg/p.o.)	3.09±0.32 $^{x, y}$	15.07±0.67 ^{y}	0.55±0.03 ^{y}	4.14±0.3 $^{x, y}$
Ethanol + MEAO (250mg/kg/p.o.)	5.10±0.56 $^{x, z}$	26.61±0.94 $^{x, z}$	0.92±0.14 $^{x, y}$	2.94±0.37 $^{x, z}$

MEAO-Methanolic extract of A. odoratissima.
Data (n=6) are expressed as Mean±Standard error mean.
x, y, z, symbol represents significant difference with x=Normal control, y=Ethanol (40% v/v, 2 ml/kg), z=Silymarin (50mg/kg/p.o.) and MEAO (250mg/kg/p.o.) groups, respectively.
p<0.05* is considered as statistical significance.

Results (Table 3) demonstrates that, Silymarin (50 mg/kg) significantly (p<0.05) decreased total bilirubin, blood urea, and creatinine levels and simultaneously raised albumin levels as relative to hepatotoxic control. Administration of MEAO (250 mg/kg) significantly (p<0.05) decreased creatinine levels as compared to hepatotoxic control. Silymarin (50 mg/kg) showed the only significant difference (p<0.05) in albumin levels, while MEAO (250 mg/kg) showed significant difference in hepatic biomarkers indicated above, as compared to normal control. MEAO (250 mg/kg) showed the comparable effects only with creatinine levels as compared to standard control. It demonstrates that, MEAO (250 mg/kg) showed mild hepatic protection in ethanol-treated rats as compared with silymarin.

3.3. Histopathological studies

The liver section of the Normal control group showed typical hepatic architecture with normal hepatic lobules, clear cytoplasm, eccentric nuclei, and no fatty changes. Figure 1 depicts the presence of black and white spots, which are considered as glycogen and vacuoles in the normal control [15]. In contrast to the Normal control group, hepatotoxic control (ethanol-alone treated group) showed structural damage with fat infiltration, hyperchromatic nuclei, and necrosis as shown in Figure 2. In comparison to the hepatotoxic control group, the silymarin-treated group exhibits mild inflammation and less degeneration of the hepatic parenchyma, and no indications of necrosis, in Figure 3. The MEAO-treated group shows mild fat deposition, necrosis and sinusoidal capillary dilation when compared to the hepatotoxic control group as observed in the Figure 4.

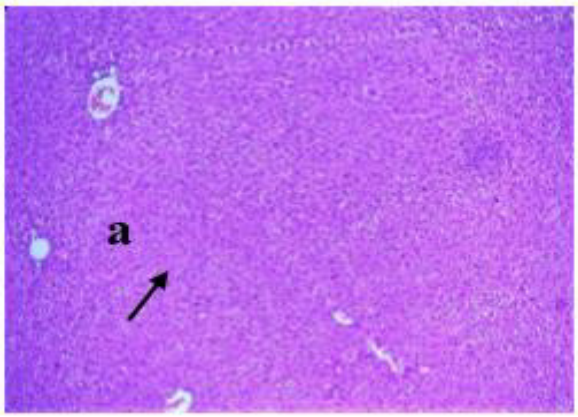

Fig 1: Normal Control shown a clear hepatic architecture with clear cytoplasm and presence of spots (a) are identified as glycogen and vacuoles. H& E staining at 40x magnification.

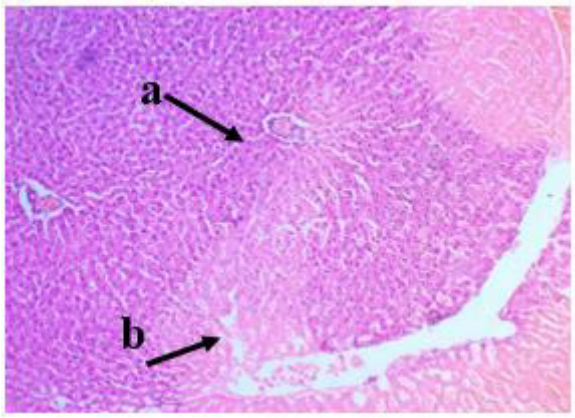

Fig 2: Hepatotoxic control (Ethanol 40%v/v2ml (40% v/v) shown a structural damage (a) with fat infiltration, hyperchromatic nuclei, and necrosis (b). H& E staining at 40x magnification

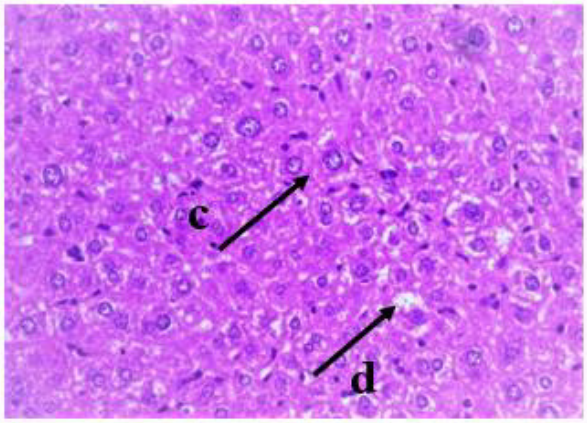

Fig 3: Silymarin (50 mg/kg) treated group shown less hepatic degeneration (c) with mild inflammation (d) H& E staining at 40x magnification.

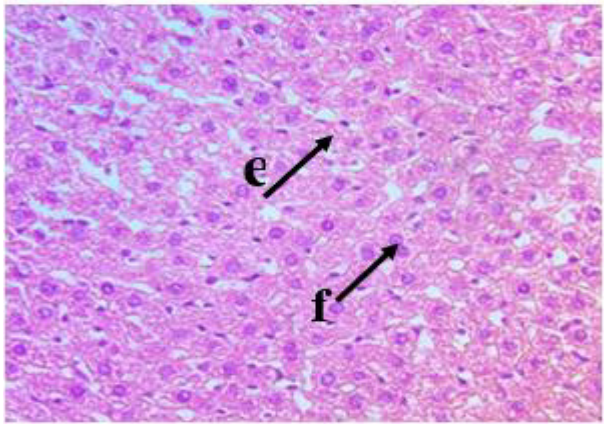

Fig 4: MEAO (250 mg/kg) treated group shown mild hepatic distortion (e) and fat deposition (f). H& E staining at 40x magnification.

4. DISCUSSION

The study reveals that the MEAO has a variety of phytochemical constituents that may be responsible for hepatoprotective actions. The phytochemical screening could help in the identification and separation of bioactive ingredients, which could lead to the development of drugs from medicinal plants. Further, toxicity study unveils that *A. odoratissima* does not cause any behavioral abnormalities, toxicity, or mortality in rats. The results clearly evidence that ethanol feeding effectively induces hepatic toxicity in normal rats. Early clinical signs of ethanol-induced hepatic injury are manifested by increased SGPT, SGOT and ALP levels. These hepatic enzymes are released into the circulation as a result of loss of the hepatocyte membrane integrity [16-18]. In addition, ethanol elevated bilirubin levels due to inadequate absorption of unconjugated bilirubin by the liver, is a sign of hepatic toxicity [19-21]. Further, abnormal bilirubin levels indicate that biliary constituents escape through tight junctions between the biliary canaliculi and sinusoids and enter the plasma [22]. The detrimental effects of ethanol on hepatic enzymes are similar to the findings of the Dinesh K et al and Sharma Set al [23, 24]. Treatment with MEAO (250 mg/kg) showed a significant decrease only in SGPT and ALP levels, as compared to hepatotoxic control. Total protein measures the total number of various proteins in the circulation. In chronic alcoholics, the

digestion and absorption of proteins are impaired. As a result, decreased TP levels indicate protein deficiency, which could be caused by liver dysfunction, hepatic diseases, and or increased amino acids uptake by extra-hepatic tissues. Hepatic tissues synthesize albumin, which is decreased in liver disorders[25]. Albumin helps in the maintenance of blood volume and flow. It also helps in the distribution of various hormones, vitamins, and drugs in the body. Thus, ethanol-induced hypoalbuminemia could be an outcome of hepatocellular dysfunction, and these findings are consistent with the Teschke R et al [26]. Administration of MEAO, trivially elevated TP and albumin levels. Ureagenesis plays an important role in nitrogen-homeostasis in humans. In general, urea synthesis is linked to liver function and has been shown to decrease in liver cirrhosis [27]. Conversely, stressful and inflammatory conditions, hyperglycemia, and chronic alcohol and its metabolite acetaldehyde may up-regulate the urea synthesis, resulting in hyperammonemia. Thus, uremia in this study could be possibly due to alcohol-induced hepatic inflammation, renal dysfunction or acetaldehyde and these results are comparable to the Azam LF et al and Palizgir MT et al [28, 29]. However, treatment with MEAO (250 mg/kg) minimally diminished the urea synthesis in hepatotoxic animals. Ethanol ingestion elevated plasma creatinine levels while decreasing albumin levels. The study findings are similar to the observations documented by Azam LF et al and Palizgir MT et al [28, 29]. Interestingly, MEAO substantially reduced the creatinine levels in hepatotoxic animals. In this study, ethanol feeding caused histopathological changes in the liver by increasing cell inflammation. However, Silymarin-treated rats demonstrated protective effects and improved liver tissue in hepatotoxic rats. The protective and therapeutic effects of MEAO (250 mg/kg) appear to be mediated by alleviating hepatic tissue injury and inflammation.

5. CONCLUSION

Present study findings reveal that MEAO has shown the presence of various phytochemicals and was found to be safe up to a test dose of 2000 mg/kg. A low dose of MEAO (250 mg/kg) has been shown to partially reverse the leakage of various hepatic cellular enzymes or hepatocellular injury caused by ethanol feeding. Furthermore, histomorphological studies reveal that MEAO (250 mg/kg) can reverse fat deposition and necrosis. Therefore, the study concludes that the dose of MEAO has exhibited mild beneficial effects as compared with ethanol-induced liver toxicity, as evidenced by the partial alleviation of biochemical and histopathological changes. Further studies are needed to understand the actions of MEAO alone and or in combination with silymarin in order to evaluate its therapeutic potential in hepatotoxicity models.

6. ACKNOWLEDGMENT

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7. AUTHORS CONTRIBUTION STATEMENT

The authors confirm contribution to the paper as follows. Mr. Byna Jayachandrababu and Dr S Rajaram were involved in conceptualization of research topic and study design. Byna Jayachandrababu performed the entire study and collected the data. Dr. M H R K Gupatha Bayya and Dr. Jitendra R Zaveri were involved in statistically analysis, results interpretation and drafted the manuscript. Mr. Byna Jayachandrababu also prepared the tissue slides for the histopathological analysis and Dr. Goverdhan Singh interpreted and compared the pathological findings among the groups of the study. All authors reviewed the results and approved the final version of the manuscript.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

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