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Evaluation of Hepatoprotective Activity of Leaves Extract of Caryeaarborea Against Paracetamol Induced Liver DamageIn Rats

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ABSTRACT:This research work is about, evaluation of hepatoprotective activity of leaves extract of Careyaarborea against paracetamol induced liver damage in Wistar rats. Paracetamol induced rats exhibited significant elevation of serum markerenzymes SGPT, SGOT, ALP, ACP and elevated concentration of bilirubin (Total and Direct)indicates hepatic damage when compared with normal control group animals. The dose dependent significant fall in elevated SGPT, SGOT, ALP, ACP, TB and DB levels demonstrated in animals treated with methanolic extract of CaryeaArborea leaves.

Liver sections of normal control group subjected to histopathologicalexamination showed normal architecture of heaptocytes. In paracetamol (hepatic control group) intoxicated rats the disruption of hepatic globular architecture and hepatic cells and fatty degeneration like ballooning of hepatocytes, fatty cyst,infiltration of lymphocytes, proliferation of kuffer cells and congestion of liversinusoids were observed. There was marked reduction in the changes of liverhistopathology monitored with the test extract treated groups.

KEYWORDS: Hepatoprotective, Careyaarborea, Paracetamol.

I. INTRODUCTION

The Greek word for liver is hepar, so medical term related to liver often startwith hepato or hepatic. Liver has a pivot role in regulation of physiological processes.It is involved in several vital functions such as metabolism, secretion and storage. Furthermore, detoxification of a variety of drugs and xenobiotics occurs in liver. Thebile secreted by liver has, among other things, an important role in digestion. Liver diseases are among the most serious ailment. They may be classified as acute orchronic hepatitis and cirrhosis.Liver diseases are mainly caused by toxicchemicals (certain antibiotics, chemotherapeutics, peroxidised oil, aflatoxin, carbontetrachloride, chlorinated hydrocarbons, etc.), excess consumption of alcohol,infection and autoimmune disorder. Most of hepatotoxic chemicals damage liver cellsmainly by including peroxidation and other oxidative damages in liver. Enhancedlipid peroxidation produced during the liver microsomal metabolism of ethanol mayresult in hepatitis and cirrhosis [1].

Hepatic injury is always associated with cellular necrosis, increase in tissuelipid peroxidation depletion in the tissue glutathione (GSH) levels. In addition, serumlevels of many biochemical markers like serum glutamate oxaloacetate transaminase (SGOT/AST) and serum glutamate pyruvate transaminase (SGPT/ALT) triglycerides,cholesterol, bilirubin and alkaline phosphatase are elevated.

Careyaarborea is a deciduous tree about 20 m high and is known as'Padmaka' in Ayurveda.[2] The accepted botanical name is kumbi[3] and "Wild guava" inEnglish.[4] The therapeutical importance of Careyaarboreais mentioned in MateriaMedica, Ayurveda, Siddha and Unani system of medicines. The stem bark of Careyaarboreais traditionally used in the treatment of tumours, bronchitis, skin disease,epileptic fits, astringent antidote to snake venom, abscesses, boil and ulcer [5]. Infusion of the flower is used after childbirth to heal rupture caused by childbirth.

The plant has been extensively investigated and chemical constituents fromthe barks, leaves and seeds of the plant have previously been reported to includetriterpenoids[6], flavonoid[7], coumarin[8], saponins and tannins[9].The different parts of plant has been reported to possesses variouspharmacological activities viz-Anticancer[10], Antimicrobial[11], CNS depressant[12], Antiulcer[13], Antidiarrhoeal[14]. Hepatoprotective activity of stem bark extract of Careyaarborea documentedin literature [15].However, no scientific

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data is available on hepatoprotective activity ofCareyaarborealeaves extract in literature till date. Hence, the present research workwas undertaken to evaluate the hepatoprotective activity of Careyaarborealeavesextract againstparacetamol induced liver damage in rats.

II. MATERIALS AND METHODS

Plant material collection:

For this study, Careyaarborealeaves were collected from Khanapur Forest,Belagavi Dist, after it was authenticated by Dr. P.D. Needagi, HOD and Professor,SB Arts and KCP Science College, Vijayapur, Karnataka.

Preparation of extract:

Fresh leaves were cleaned, cut in to small pieces, shade dried at roomtemperature and powdered using grinder. Then the powdered material was extractedwith methanol by Soxhlet extraction procedure at temperature between60 - 70^0 C.Thereafter, the extract was concentrated using rotary flash evaporator. The yield wasfound to be 10 %. The dried extract was stored in refrigerator below 10^0C for furtherstudies.

Preliminary phytochemical screening[16,17]

The preliminary phytochemical investigation ofmethanolic extract of Careyaarborealeaves (MECAL)was carried out for detection of various phytoconstituents. Tests for presence of phytochemicals were performed following standard methods described by Dr. Kokate C.K and Dr. Khandelwal K.R.

Animals used:

Albino rats (Wistar strain) weighing 150-200 g of both sex and Swiss miceweighing 20-25g of either sex were used in the present study. They were procuredfrom Venkateshwar enterprises, Rajajinagar, Bangalore. The animals wereacclimatized for ten days under standard laboratory condition. They were housed inpolypropylene cage and maintained at $27^oC \pm 2^0C$, relative humidity 65 ± 10% under12 hr light/dark cycle. The animals were fed with rodent pellet diet and water assufficient. The study protocol was approved from the Institutional Animal Ethics Committee (IAEC) before initiation of the experiments. [Ref No. BLDE/BPC/2019-20/645 dated 21/09/2019]

Determination of acute toxicity [18]

The acute toxicity (LD50) of MECAL was determined by fixed dose methodOECD guide line no. 423. The female albino mice weighing between 20-25g werefasted for overnight prior to experiment. $1/10^{th}$ and $1/5^{th}$ LD50 cutoff value of theextract were selected as screening doses.

Evaluation of Hepatoprotective Activity

Paracetamol induced hepatotoxicity [19,20]

Albino rats (Wistar strain) weighing 150 – 200g were put into five different groups of six animals each

Group I: Normal control(received vehicle 1 ml/kg; p.o.)
Group II: Hepatic control(Paracetamol 500 mg/kg; p.o.)
Group III: Standard drug (Silymarin 25 mg/kg; p.o.)
Group IV:250mg/kg of MECAL ($1/10^{th}$)
Group V: 500mg/kg of MECAL ($1/5^{th}$)

Animals were treated as shown above for a period of 07 days. Paracetamolwas administered to all groups except group I (Normal Control) daily at the dose of 500 mg/kg p.o. Group III received standard drug Silymarin (25 mg/kg p.o.) where as groups IV & V treated with methanolic extract of careyaarborea leaves (MECAL) at graded doses 250 and 500mg/kg simultaneously for a period of 7 days.

During this period of treatment, the rats were maintained under normal diet and water. 18 hours after the last dose of paracetamol i.e. on 8^{th} day blood was collected by cardiac puncture under mild ether anesthesia using disposable syringe and needle. Blood was allowed to clot at room temperature for 30 minutes and subjected to centrifugation at 3000 rpm for 15 minutes for estimation of biochemical parameters.

Estimation of biochemical parameters include:

01. Serum Glutamate Pyruvate Transaminase (SGPT)[21]
02. Serum Glutamate Oxaloacetate Transaminase (SGOT)[22]
03. Serum Alkaline Phosphatase (ALP)[23]
04. Serum Direct Bilirubin[24]
05. Serum Total bilirubin[25]

Later all the animal were sacrificed and liver tissues were dissected out for morphological &histopathological studies.

Study of morphological parameters[15].

- ✓ Wet liver weight
- ✓ Wet liver volume

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Histopathological studies [26]

Processing of isolated liver (Modified Luna's method 1960)

The animals were sacrificed and the liver of each animal was isolated. The isolated liver was cut in to small pieces and preserved and fixed in 10% formalin for two days. Following this was the washing step where by the liver pieces were washed in running water for about 12 hrs. This was followed by dehydration with isopropyl alcohol of increasing strength (70%, 80% and 90%) for 12 hrs. each. Then the final dehydration is done using absolute alcohol with about three changes for 12 hrs. each.The cleaning was done by using chloroform with two changes for 15 to 20 minutes each. After clearing the liver pieces were subjected to paraffin infiltration in automatic tissue processing unit.

The liver pieces were washed with running water to remove formalin completely. To remove the water, alcohol of increasing strengths was used since it is a dehydrating agent. Further alcohol was removed by using chloroform and chloroform removed by paraffin infiltration.

Embedding in paraffin vacuum mould

Hard paraffin was melted and the hot paraffin was poured into L-shaped blocks. The liver pieces were then dropped into the molten paraffin quickly and allow to cool.

Sectioning & Staining

The blocks were cut using microtone to get sections of thickness of (5µ). The sections were taken on a microslide on which a egg albumin (sticking substance) was applied. The sections were allowed to remain in a oven at 60^0C for 1 hr. Paraffin melts and egg albumin denatures, thereby fixes tissues to slide. Eosin is an acid stain. Hence it stains all the cell constituents pink which are basic in nature. Hemotoxylin basic stain which stains all the acidic cell components blue.

III.RESULTS

Phytochemical Screening

The results of preliminary phytochemical screening on MECAL are summarized in Table-1

Determination of acute toxicity studies

In acute toxicity studies, MECAL did not produce any mortality of the animals at dose of 2000 mg/kg. Hence, 2500 mg/kg was fixed as LD_{50} cut off value as per fixed dose method, OECD (Organization for Economic Corporation Development) guideline No 423 (Annexure 2d). The screening doses selected for hepatoprotective activity of test extract of title plant were:

250 mg/kg – $1/10^{th}$ dose of 2500 mg/kg b.w.

500 mg/kg – $1/5^{th}$ dose of 2500 mg/kg b.w.

Effect of MECAL on biochemical markers in Paracetamol induced rat liver injury

Paracetamol challenged rats exhibited significant elevation of serum marker enzymes SGPT, SGOT, ALP, ACP and elevated concentration of bilirubin (Total and Direct) indicates hepatic damage when compared with normal control group animals. The dose dependent significant fall in elevated SGPT, SGOT, ALP, ACP, TB and DB levels demonstrated in animals treated with MECAL The standard drug Silymarin also exhibited the significant hepatoprotective activity . The results are given in Table-2.

Effect of MECAL on wet liver weight & volume in Paracetamol toxicated rat liver injury

The significant increase in wet liver weight and volume were observed in rats exposed to paracetamol over normal control animals. However, the wet liver weight and volume of the animals treated with test extracts of both doses and standard silymarin demonstrated significant reduction in weight and volume of liver as compared to hepatic control group. The results are given in Table-3.

Effect of MECAL on histopathological profile in Paracetamol induced rat liver injury

Liver sections of normal control group subjected to histopathological examination showed normal architecture of heaptocytes. In Paracetamol (hepatic control group) intoxicated rats the disruption of hepatic globular architecture and hepatic cells and fatty degeneration like ballooning of hepatocytes, fatty cyst, infiltration of lymphocytes, proliferation of kuffer cells and congestion of liver sinusoids were observed. There was marked reduction in the changes of liver histopathology monitored with the test extract treated groups (Fig: 1 - 5).

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Table - 1
The results of preliminary phytochemical screening on MECAL

Sl. No.	Phytochemical Constituents	Inference
01	Carbohydrate	+
02	Proteins	+ +
03	Alkaloids	+
04	Tannins	+ + +
05	Flavonoids	+ + +
06	Lipids	+

+ :Indicates presence
+ + :More clarity
+ + + :Better response

Table - 2
Effect of MECAL on biochemical parameters in Paracetamol induced rat liver injury

Groups	Units in IU/L				Units in mg/dL	
	ALT (SGPT)	AST (SGOT)	ALP	ACP	TB	DB
Normal control	103.84±3.29	112.32 ±6.58	242.36 ±4.11	25.16±1.21	0.298±0.02	0.269 ±0.012
Hepatic control (Paracetamol)	239.25 ±8.1[@]	280.29 ±8.13[@]	460.56 ±9.22[@]	54.13 ±1.25[@]	0.526 ±0.01[@]	0.598 ±0.013[@]
Standard (Silymarin)	125.25±7.10***	130.84±7.69***	285.69±7.26***	31.36±1.98***	0.310±0.01*	0.354±0.031***
MECAL 250 mg/kg	201.95 ±8.12**	240.12 ±6.51**	410.36 ±8.63***	40.32 ±1.91***	0.325 ±0.03*	0.452 ±0.013***
MECAL 500 mg/kg	180.78 ±6.23***	198.14 ±7.6***	354.14 ±6.31***	39.32 ±1.23***	0.311 ±0.02*	0.391 ±0.014***

Results are Mean ± SEM, n = 6, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ compared to Paracetamol control.

Table - 3
Effect of MECAL on wet liver weight and volume in Paracetamol induced rat liver injury

Groups	Wet Liver/ 100 gm	
	Weight	Volume

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Normal control	3.54 ±0.05[@]	3.91 ±0.08[@]
Hepatic control	5.68 ±0.09***	5.93 ±0.03***
Standard Silymarin	3.86 ±0.03***	4.10±0.02***
MECAL 250 mg/kg	4.80 ±0.09***	4.95±0.03***
MECAL 500 mg/kg	4.10±0.05***	4.43 ±0.07***

Results are Mean ± SEM, n = 6, ***p < 0.001 compared to Paracetamol control.

Effect of MECAL on Histopathological profile against Paracetamol induced rat liver injury

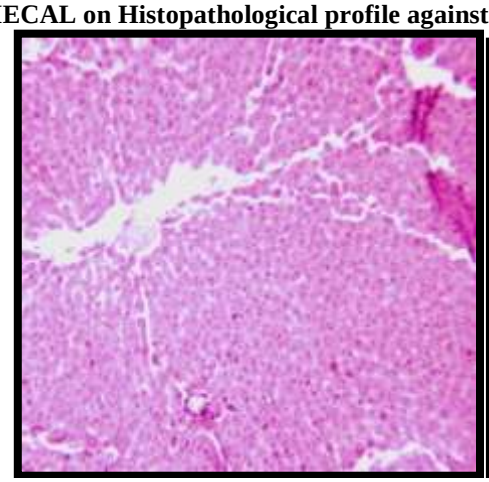

Fig – 1 -Normal control

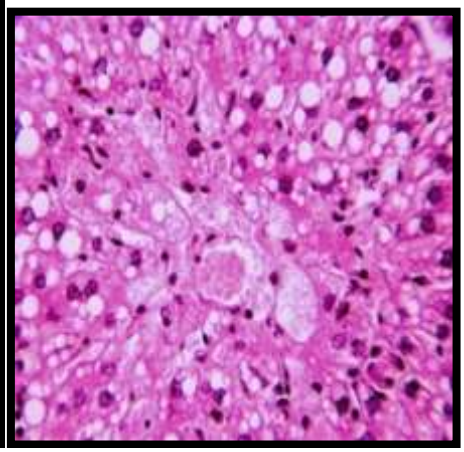

Fig – 2 - Paracetamol control

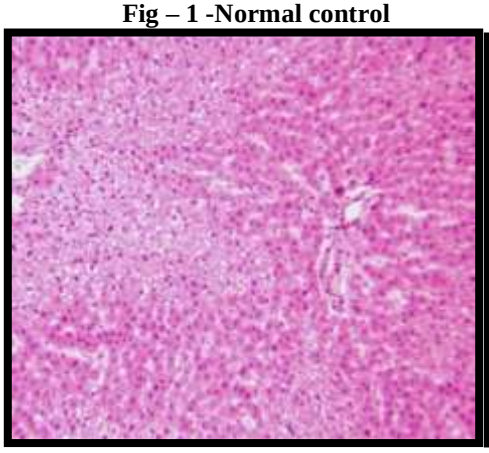

Fig – 3 –Standard

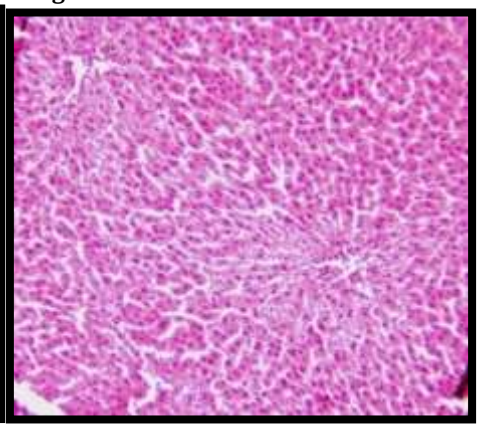

Fig – 4 –MECAL 250 mg/kg

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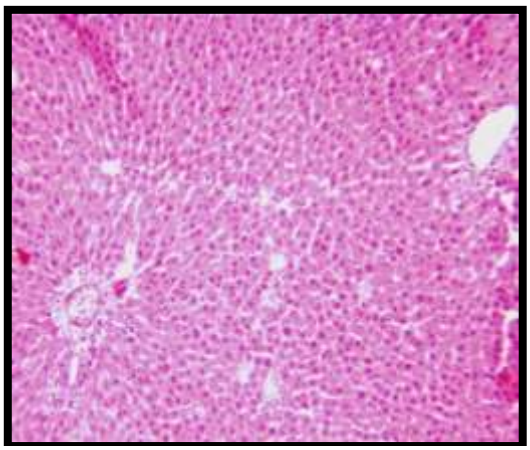

Fig – 5 – MECAL 500 mg/kg

IV. DISCUSSION

In the current study, MECAL has been screened hepatoprotective property using paracetamol intoxicated rat liver injury. To investigate hepatoprotective activity of the test extract, liver function tests (LFT), weight and volume of wet liver and histopathological examination of liver tissues were examined.

Hepatic function in the laboratory can be assessed by measuring the serum level of ALT (SGPT), AST (SGOT), ALP, ACP, TB and DB which are known to present in the cytoplasm[27]. When there is hepatotoxicity, these enzymes along with bilirubin enters into the blood stream and serve as an indicator for the liver injury [28]. Increased folds of serum concentration of ALT (SGPT), AST (SGOT), ALP, ACP, TB and DB which was monitored in paracetamol exposed rats in our laboratory study are indications of liver dysfunction and also denotes the damage of hepatic cells.

The significant reversal of these serum enzymes and bilirubin levels against paracetamol challenge demonstrated by the test extract may be due to inhibiting the leakage of intracellular enzymes by its membrane stabilizing activity.

Hepatoprotective property of MECAL was further evident by histopatholocgical examination of liver tissue which supports the results of biochemical parameters. The studies on histopathological examination demonstratedthe disruption of hepatic globular architecture and hepatic cells and fatty degeneration like ballooning of hepatocytes, fatty cyst, infiltration of lymphocytes, proliferation of kuffer cells and congestion of liver sinusoids in paracetamol toxicated animals. There was marked reduction in the changes of liver histopathology monitored with the test extract treated groups compared to hepatic control group.

Liver is the most adversely affected organ because of consumption of alcohol in human beings. Different mechanisms are associated with the pathological changes of alcohol intoxicated liver injury, and oxidative stress was one of them [29]. ROS is one kind of peroxidants including hydroxyl radical, superoxide radical, and hydrogen peroxide, which are frequently generated spontaneously during metabolism. Normally produced ROS is rapidly eliminated by the antioxidant defense system. The antioxidant defense system is able to scavenge ROS and terminate chain reaction of free radicals in vivo. Exposure to alcohol can result in excessive accumulation of ROS and leads to heaptocellular damage. Increased storage of ROS could cause lipid peroxidation of hepatocytes, which considered as the primary mechanism concerned with alcohol exposed liver injury [30]. In the present investigation the protective effect of MECAL on alcohol mediated liver toxicity in rats was evaluated. Elevated levels of serum marker enzymes SGPT, SGOT, ALP, ACP and bilirubin (Total and Direct) evidenced the hepatic damage in alcohol challenged animals. Treatment with MECAL significantly lowered the elevated concentrations of these serum markers and bilirubin levels in animals against alcohol challenged hepatic injury, thereby exhibits significant hepatoprotective efficacy. The antioxidant property of Careyaarboreahas been documented in the literature[31] which could reasonably responsible for heaptoprotective property of the title plant.

Medicinal plant extracts containing flavonoids and tannins claimed to be having heaptoprotective activity [32]. The presence of these chemical substances in the test extract could be the

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reason for exhibiting the significant hepatoprotective activity against paracetamol and alcohol mediated liver damage in our study.

V. CONCLUSION

The findings of the present study suggest that MECAL demonstrated significant hepatoprotective activity in dose dependent manner. Further studies are required to isolate the active principle responsible for observed hepatoprotective activity of the test extract.

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