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Research Article

Pharmacology for Drug Discovery

Effect of aqueous extract of *Colocasia esculenta* against high-fat diet-induced non-alcoholic fatty liver disease in rats

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Abstract: Non-alcoholic fatty liver disease (NAFLD) is becoming a major public health problem due to the increasing incidence of obesity and diabetes in India for the last two decades. The present work is aimed to investigate the effect of aqueous extract of *Colocasia esculenta* against (AECE) high-fat diet-induced non-alcoholic fatty liver disease in Wistar albino rats. Wistar albino rats were assigned in to four groups as follows; Group I: Normal conventional diet, Group II: High fat diet, Group III & Group IV: Combined high fat diet and different doses of *Colocasia esculenta* (200 and 400 mg/kg, p.o) for 4 weeks.Treatment with aqueous extract of *Colocasia esculenta*(200mg/kg, 400mg/kg bw) significantly reduced the body weight and weight gain. In addition, it also significantly reduced the serum triglycerides, total cholesterol, LDL cholesterol, VLDL cholesterol and showed a significant increase in HDL cholesterol as well as significant improvement in liver cholesterol and triglyceride content. AECE treated rats exhibited a significant beneficial effects on levels of liver function markers and oxidative stress and also reversed the histopathological changes.The study suggested the therapeutic potential of AECE against NAFLD, acting in part through anti-obesity, antioxidant mechanisms, and liver marker enzymes.

Keywords: Colocasiaesculenta; High-fat diet; Non-alcoholic fatty liver disease, Anti-oxidant effect.

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1. INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a state defined by lipid content increase (5-10%) in liver tissue without significant chronic alcoholism [1]. It is the largest of frequent liver disease in the globe, touching up to 30% of the adult people and 70-80% of individuals who are obese and diabetic[2]. It comprises a disease continuum ranging from benign hepatic steatosis to non-alcoholic steatohepatitis with inflammation (NASH) and liver cirrhosis. NAFLD is a multi factorial disease with compound pathophysiology. The scientific markers of NAFLD include obesity, insulin resistance (IR), and dyslipidemia[3]. Study screening shows that the severity of fat accumulation in the liver is an excellent interpreter of the probability of development of NAFLD to NASH led to the scheme of the two knock hypothesis[4]. The initial drive involves the succession of hepatic steatosis and the destruction in the key enzymes in modifiable Triglyceride (TG) and free fatty acids (FFA) metabolism, expose the liver to a second undefined drive, resulting in more severe liver injury[5]. These days, the methods for treating NAFLD taking place are balanced diet, exercise, and medicines including metformin, statins, and fibrates. However, these drugs have some undesirable effects or contraindications and no harmony exists on the most effective drug therapies[6].In current years, the profit of plant extracts on NAFLD development have received increasing concentration due to their reward, whereas plants are broadly available around the world, their extracts have low or negligible side effects and they showed superior antioxidant properties.[7]*Colocasia esculenta* is an herbaceous perennial plant belonging to the "Araceae" family. The *Colocasia esculenta* has been reported antihyperlipidaemic, anti-inflammatory, antihypertensive, antidiabetic, antioxidant, hepatoprotective, anti-inflammatory, antimicrobial, anthelminthic activities.[8] However, the outcome of leaves of Colocasia esculenta against non-alcoholic fatty liver disease have not been reported. For this reason, the present work is aimed to explore the effect of aqueous extract of *Colocasia esculenta* against (AECE) in high fat diet induced non-alcoholic fatty liver disease in wistar albino rats.

2. MATERIALS AND METHODS

2.1 Collection and authentication of Plant material

The leaves of *Colocasiaesculenta*were collected from Nellore region (Dist. Nellore, Andhrapradesh, India). The plant variety was valid (RIPER/PHCOG/003/2017) confirmed and authenticated by Botanist Dr.J. Raveendra reddy, Raghavendra Institute of Pharmaceutical Education and Research (RIPER), Anantapuramu, Andhra Pradesh, India.

2.2 Extraction procedure

The leaves were dehydrated under the shade and powdered using a grinder mixer. The powdered material (1000 g) was waterlogged in cold distilled water for 72 hrs and then filtered. The obtained filtrate was evaporated on water bath to obtain the solid reddish coloured dry mass of 30g. The extract was then preserved in the desiccators.[9]

2.3 Animals

Male wistar rats, weighing between 180 and 200g were obtained from the Raghavendra enterprises, Bangalore, India. All the animal experiments were conducted according to the protocols agreed by the Institutional Animal Ethical Committee (Protocol No: 878/ac/05/CPCSEA/001/2017). All animals were maintained under ample conditions at an ambient temperature of 21±2° c, and were subjected to 12 h light and dark cycle. They were fed with normal pellet diet and water in quantity sufficient. Animals were kept for 7 days in laboratory for acclimatization. Experimental animals were maintained at CPCSEA recommended conditions during acclimatization and experimentation period.

2.4 Treatment schedule

2.4.1 Experimental protocol

Male albino wistar rats were allocated in to four groups of six animals each and received the following interventions for the period of 4 weeks. High fat diet prepared daily according to the composition given in the earlier reports. [10]

Group I: Rats assigned with normal diet
Group II: Rats assigned with high fat diet only
Group III: Rats assigned with high fat diet and aqueous extract of Colocasia esculenta, 200mg/kg, p. o
Group IV: Rats assigned with high fat diet and aqueous extract of Colocasia esculenta, 400mg/kg, p. o.[11]

2.5 Measurement of Physiological parameters

Body weight was recorded before and after the study protocol and as well as cumulative food intake was noted.

2.6 Blood collection and separation of serum

At the end of the study, blood samples were collected from all the overnight fasted animals by retro orbital sinus puncture into Eppendorf tubes and permitted to clot at room temperature. The clotted blood samples were subjected for centrifugation at 5000rpm for 15 min by using Remi cooling centrifuge. The separated clear serum was collected and stored at 2-8 ℃ in the refrigerator.

2.7 Measurement of serum parameters

The following serum parameters were estimated by using ERBA diagnostic kits with a semi auto analyser (ERBA diagnostics, Germany). The list of parameters includes Triglycerides (TG), Total cholesterol (TC), Low density lipoprotein cholesterol (LDL-C), High density lipoprotein cholesterol (HDL-C), Very low density lipoprotein cholesterol(VLDL-C), Serum glutamate pyruvate transaminase (SGPT), Serum glutamate oxaloacetate transaminase (SGOT), Alkaline phosphatase (ALP).[12]

2.8 Estimation of Liver Anti-oxidants, hepatic lipids

The animals were euthanized by general anaesthesia (sodium pentobarbital 120 mg/kg, I.P) followed by cervical dislocation and the liver was immediately taken out by abdominal opening. The part of liver tissue was immediately homogenized in the cold phosphate buffer saline (50 mM, pH 7.4) with Remi tissue homogenizer at cold condition. The 10% w/v crude tissue homogenate subjected for centrifugation by cooling Remi centrifuge at 10000 rpm for 20 minutes. Supernatant of the tissue homogenate was collected in to test tubes and is utilised for estimation of

superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and malondialdehyde (MDA) and as well as estimation of liver tissue cholesterol (TC) and Triglycerides (TG) content were determined by ERBA diagnostic kits.[13,14]

2.9 Histopathology

Small pieces of liver were fixed directly in 10% buffered formalin for histopathological study. Liver tissue was serial dehydrated with alcohol and paraffin wax embedded tissue was sectioned in to 4-5 µ size slices. The slices were stained with hemtoxylin and eosin stain.[15]

3. STATISTICAL ANALYSIS

All the data are expressed as mean ± SEM. Statistical significance among the groups was tested using one-way ANOVA followed by the bonferroni multiple comparisions test as appropriate using computer based fitting program (Prism, Graph pad). Differences were considered to be statistically significant when $P < 0.05$.

Table 1: Effect of Aqueous Extract of ColocasiaEsculenta (AECE) on Physiological Parameters

Group	Initial body weight(g)	Final body weight (g)	Weight gain (g)	Food intake (g)
Group I	238.3 ± 7.92	356.7 ± 14.53	118.8 ± 3.06	23635 ± 1560
Group II	245.0 ± 6.70^{ns}	493.3 ± 18.38**	244.7 ± 4.21***	29632 ± 337.9^{ns}
Group III	225 ± 7.63^{ns}	381.7 ± 23.30$^{\#}$	148.3 ± 2.78$^{\#\#\#}$	24487 ± 1486^{ns}
Group IV	240 ± 7.30^{ns}	396.7 ± 20.60^{ns}	151 ± 3.07$^{\#\#\#}$	25337 ± 712.5^{ns}

Group 1= Normal control, Group 2= High fat diet control group, Group 3= Aqueous extract of Colocasiaesculenta (200mg/kg), Group 4= Aqueous extract of Colocasiaesculenta (400mg/kg), values are expressed mean±SEM (n=6). *** P<0.001 compared with normal control; **P<0.01 compared with normal control; ns- non significant compared with normal control; $^{\#\#\#}$P<0.001 compared with HFD control; $^{\#}$ P<0.05 compared with HFD control; ns- non significant compared with HFD control.

Table 2: Effect of Aqueous Extract of ColocasiaEsculenta (AECE) on serum Lipid Profile

Group	Triglycerides (mg/dl)	Total cholesterol (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)	VLDL-C (mg/dl)
Group I	57.67 ± 2.04	77.67 ± 7.75	39.83 ± 1.40	67.50 ± 2.14	16.0 ± 2.59
Group II	174.5 ± 3.75***	173.8 ± 3.50***	69.67± 2.90***	24.83 ± 1.97***	53.6 ± 4.13***
Group III	55.67 ± 3.32$^{\#\#\#}$	65.50 ± 5.42$^{\#\#\#}$	43.0± 2.36$^{\#\#\#}$	70.0 ± 1.39$^{\#\#\#}$	22.1 ± 1.72$^{\#\#\#}$
Group IV	69.50 ± 4.52$^{\#\#\#}$	104.50 ± 6.84$^{\#\#\#}$	51.0±4.44$^{\#\#}$	39.17 ± 3.74$^{\#\#}$	36.67 ± 1.30$^{\#\#}$

Group 1= Normal control, Group 2= High fat diet control group, Group 3= Aqueous extract of Colocasiaesculenta (200mg/kg), Group 4= Aqueous extract of Colocasiaesculenta (400mg/kg), values are expressed mean±SEM (n=6). ***P<0.001 compared with normal control; ###P<0.001 compared with HFD control; $^{\#\#}$ P<0.01 compared with HFD control

Table 3: Effect of Aqueous Extract of ColocasiaEsculenta (AECE) on Liver cholesterol and triglycerides content

Group	Liver Cholesterol Content (mg/dl)	Liver Triglyceride content (mg/dl)
Group I	91.67 ± 4.59	83.3± 2.47
Group II	263.3± 24.04***	208.7 ± 35.75*
Group III	97.17± 3.56$^{\#\#\#}$	89.17± 3. 00$^{\#}$
Group IV	158.3± 3.33$^{\#\#\#}$	132.8± 6.88^{ns}

Group 1= Normal control, Group 2= High fat diet control group, Group 3= Aqueous extract of Colocasiaesculenta (200mg/kg), Group 4= Aqueous extract of Colocasiaesculenta (400mg/kg), values are expressed mean±SEM (n=6). *** P<0.001 compared with normal control; *P<0.05 compared with normal control; $^{\#\#\#}$P<0.001 compared with HFD control; $^{\#}$ P<0.05 compared with HFD control; ns- non significant compared with HFD control.

Table 4: Effect of Aqueous Extract of ColocasiaEsculenta (AECE) on Serum Liver marker Enzymes

Group	SGPT	SGOT	ALP
Group I	93.33 ± 4.41	102.3± 7.55	101.3 ± 3.18
Group II	317.80± 7.95***	310.5 ± 6.29***	149.2 ± 6.37**
Group III	57.17± 8.16$^{\#\#\#}$	125.7± 3.63$^{\#\#\#}$	90.0 ± 9.66$^{\#\#\#}$
Group IV	132.8± 2.104$^{\#\#\#}$	145.0± 7.63$^{\#\#\#}$	118.2 ± 4.95^{ns}

Group 1= Normal control, Group 2= High fat diet control group, Group 3= Aqueous extract of Colocasiaesculenta (200mg/kg), Group 4= Aqueous extract of Colocasiaesculenta (400mg/kg), values are expressed mean±SEM (n=6). ***P<0.001 compared with normal control; ** P<0.01 compared with normal control; $^{\#\#\#}$P<0.001 compared with HFD control; ns- non significant compared with HFD control.

Table 5: Effect of Aqueous Extract of ColocasiaEsculenta (AECE) on Liver Anti-oxidants

Group	Superoxide Dismutase (U/mg protein)	Catalase (µmol of H_20_2 decomposed/min/mg protein)	Reduced Glutathione (µg/g tissue)	Lipid peroxidation
Group I	26. 33 ± 3.52	59.00 ± 3.18	68.00 ± 3.55	13.33 ± 1.74
Group II	12.00 ± 1.88*	31.50 ± 4.06**	29. 00 ± 2.73**	68.83 ± 2.57***
Group III	28.83 ± 3.27$^{\#}$	57.33 ± 3.69$^{\#\#}$	61.83 ± 3.01$^{\#\#\#}$	18.50 ± 3.51$^{\#\#\#}$
Group IV	14. 50 ± 1.23^{ns}	36.17 ± 2.82^{ns}	49.67 ± 2.90$^{\#\#}$	41.17 ± 3.08$^{\#\#\#}$

Group 1= Normal control, Group 2= High fat diet control group, Group 3= Aqueous extract of Colocasiaesculenta (200mg/kg), Group 4= Aqueous extract of Colocasiaesculenta (400mg/kg), values are expressed mean±SEM (n=6). ***P<0.001 compared with normal control; ** P<0.01 compared with normal control; *P<0.05 compared with normal control; $^{\#\#\#}$P<0.001 compared with HFD control; $^{\#\#}$P<0.01 compared with HFD control; $^{\#}$ P<0.05 compared with HFD control; ns- non significant compared with HFD control.

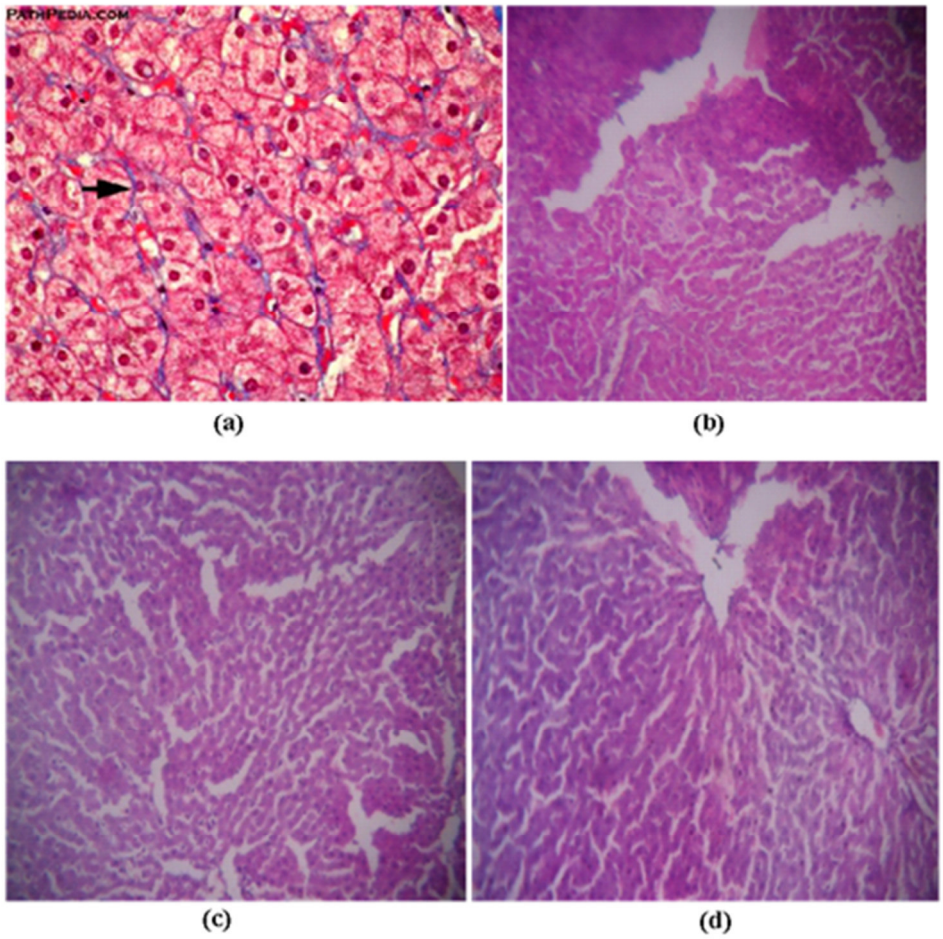

Fig 1: Histology of hepatic tissue sections dyed with haematoxyline-eosin (HE). (a) Norma diet control liver (100x) (b)HFD fed control rats liver (c) HFD fed AECE treated rats (200mg/kg body weight) liver (100x) (d) HFD fed AECE treated rats (400mg/kg body weight) liver (100x)

4. RESULTS

4.1 Effect of the aqueous extract of the Colocasia esculenta (AECE) on physiological parameters

Body weight was measured at initial and final day of the treatment procedure. In initial body weight, there is no significant difference between HFD control group and normal control group as well as between HFD control group and test groups. In final body weight the HFD control rats showed significant increase in body weight compared with normal control rats (**P<0.01) where as HFD+AECE (200mg/kg) treated rats showed significant decrease in body weight compared with HFD rats ($^{\#}$ P<0.05). HFD control rats showed significant body weight gain compared with normal control rats (*** P<0.001). HFD+AECE (200mg/kg) treated rats showed significant decrease in body weight gain compared with HFD rats ($^{\#\#\#}$P<0.001) HFD+AECE (400mg/kg) treated rats showed significant decrease in body weight gain compared with HFD rats ($^{\#\#\#}$P<0.001). (Table 1)

Food Intake

There is no significant difference in food intake between HFD control and normal control and between HFD +AECE (200mg/kg), HFD +AECE (400mg/kg) between HFD control. (Table 1)

4.2 Effect of the aqueous extract of the Colocasia esculenta (AECE) on serum lipid parameters

HFD control rats showed significant increase in serum triglycerides, total cholesterol, LDL cholesterol, VLDL

cholesterol (***P<0.001) and showed significant decrease in HDL cholesterol as compared to normal fed control rats (***P<0.001). Treatment of high fat diet fed rats with aqueous extract of Colocasia esculenta at doses 200, 400mg/kg body weight produced significant decrease in serum triglycerides (###P<0.001, ###P<0.001 respectively), total cholesterol (###P<0.001, ###P<0.001respectively), LDL cholesterol (###P<0.001, ## P<0.01 respectively), VLDL cholesterol (###P<0.001, ## P<0.01 respectively) and showed significant increase in HDL cholesterol (###P<0.001, ## P<0.01 respectively) as compared to high fat diet fed control rats.(Table 2)

4.3 Effect of the aqueous extract of the Colocasia esculenta (AECE) on liver cholesterol content and liver triglyceride content

High fat diet fed control rats produced significant increase in liver cholesterol content, triglyceride content as compared to normal diet fed control rats (*** P<0.001, *P<0.05 respectively). High fat diet fed control rats treated with AECE at doses 200, 400 mg/kg body weight produced significant decrease in liver cholesterol content as compared to high fat diet fed control rats (###P<0.001, ###P<0.001 respectively). High fat diet fed control rats treated with AECE at doses 200 mg/kg body weight produced significant decrease in liver triglyceride content as compared to high fat diet fed control rats (# P<0.05). No significant difference was found between HFD control rats treated with AECE (400mg/kg) and high fat diet fed control rats in case of liver triglyceride content. (Table 3)

4.4 Effect of the aqueous extract of the Colocasia esculenta (AECE) on serum enzyme markers of liver

High fat diet fed control rats produced significant increase in serum SGPT, SGOT, ALP as compared to normal diet fed control rats (***P<0.001, ***P<0.001, **P<0.001 respectively). High fat diet fed control rats treated with AECE at doses 200, 400 mg/kg body weight produced significant decrease in SGPT(###P<0.001) and SGOT (###P<0.001). High fat diet fed control rats treated with AECE at dose 200 mg/kg body weight produced significant decrease in ALP (###P<0.001). No significant difference was found between HFD control rats treated with AECE (400mg/kg) and high diet fed control rats in case of ALP. (Table 4)

4.5 Effect of the aqueous extract of the Colocasia esculenta (AECE) on liver oxidant parameters

High fat diet fed control rats produced significant decrease in liver superoxide dismutase (*P<0.05), catalase (** P<0.01), reduced glutathione (** P<0.01) and increase in lipid peroxidation (***P<0.001) as compared to normal diet fed control rats. High fat diet fed control rats treated with AECE at dose 200 mg/kg body weight produced significant increase in liver superoxide dismutase (# P<0.05), catalase (##P<0.01), reduced glutathione (##P<0.001) and decrease in lipid peroxidation (###P<0.001) as compared to high fat diet fed control rats. High fat diet fed control rats treated with AECE at doses 400 mg/kg body weight produced significant increase in liver reduced glutathione (##P<0.001) and decrease in lipid peroxidation (###P<0.001). No significant difference was found between HFD control rats treated with AECE (400mg/kg) and high fat diet fed control rats in case of liver superoxide mutase, reduced glutathione. (Table 5)

4.6 Effect of the aqueous extract of the Colocasia esculenta (AECE) on liver histopathology

High fat diet fed control rats liver showed hepatocytes showing fatty change and severe hepatocellular ballooning. High fat diet fed control rats treated with AECE at dose 200 mg/kg body weight liver showed near normal structure. High fat diet fed control rats treated with AECE at dose 200 mg/kg body weight liver showed moderate improvement (Figure1)

5. DISCUSSION

Non-alcoholic fatty liver disease (NAFLD) has becoming a major health issue faced by the society and is characterized by excessive deposition of saturated fat in liver due to consumption of westernized diet contain high calories fat. NAFLD is most prevalent especially in developed countries with cost of urbanization and life style modifications.[16].NAFLD has multistage progressive disease ranging from steatosis, steatohepatitis to advanced stage including fibrosis, cirrhosis and hepatocellular carcinoma.[17] . Apart from lifestyle changes, effective treatment of NAFLD is still vague, as result researchers look forwarding to effective drugs for NAFLD. Intake of fruits and vegetable rich in polyphenolic compounds reverse the progression of NAFLD, is observed from various epidemiological and clinical data. [18] With proof of health benefits of *Colocasiaesculenta*due to its high phenolic content we tried to explore the beneficial effect in NAFLD.[19] Prolonged intake of high fat diet allows the rats to develop obesity, is associated with accumulation fat in liver and high visceral fat, similar characteristic generally observed in patients of NAFLD.[20] Significant increase in weight gain were observed in high fat diet consumed rats and we observed weight loss property of *colocasia esculenta* that is reflected by significant decrease in body weight in AECE treated animals, particularly at the dose of 200 mg/kg. Polyphenolic compounds are known to be as weight controllers, and are rich in the aqueous extract of *Colocasia esculenta* and it is observed with qualitative phytochemical analysis.[21]Feeding of high fat diet in rats successfully established dyslipidaemia,which is the common problem in NAFLD condition. Dyslipidaemia induced by high fat diet is efficiently balanced by aqueous extract of *Colocasia esculenta* at the dose of 200mg/kg, and was demonstrated as significant decrease in TC, TG, VLDL, LDL and increase in HDL compared to high fat feeding rats and this is known to be resulting from anti-dyslipidaemic effects of flavonoids, which are noticed in our plant extract. [22]It is a known fact, from literature that accumulation of fat in liver due to high fat diet initially causes hepatic steatosis and it progress to liver failure. Molecular basis of fat diet induced liver damage result from combination of hepatocyte lipotoxicity, oxidative stress and pro-inflammatory cytokines, which is well described by multiple hit hypotheses[23]. Deleterious effect of hepatocyte lipotoxicity is observed by significantly increased in hepatic tissue damage marker that includes SGPT, SGOT and ALP in high fat feeding rats and it is normalized by administration of aqueous extract of *Colocasiaesculenta, where* more significant changes were observed at 200 mg/kg. This indicate the hepatoprotective effective of extract, may be result of anti-dyslipidemic effect. Further hepatoprotective effect of extract is also demonstrated by balancing of oxidative defensive

factors and hepatocyte lipid peroxidation induced by high fat diet.[24]Thismay bedue to increase in catalase, reduced glutathione, superoxide dismutase and decrease in lipid peroxidation compared with high fat diet model.[25]

6. CONCLUSION

The present study supported the traditional claim of Colocasia esculenta for liver disorders with scientific justification, particularly documented for beneficial effect in high fat diet induced non-alcoholic fatty liver in albino wistar rats. Protective abilities of aqueous extract of Colocasia esculenta in fatty diet induced hepatic damage in rats through its anti-obesity, anti-hyperlipidemic, antioxidant and hepatoprotective effects. The above multiple effects may be result of abundant presence of flavonoids in aqueous extract of Colocasia esculenta. So it is considered as good therapeutic agent in management of non-alcoholic fatty liver disease. Apart from these preliminary studies, it needs more preclinical and clinical studies warranted for its therapeutic benefits.

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8. AUTHORS CONTRIBUTION STATEMENT

Dr. K. Somasekhar Reddy conceptualized, designed and gathered the data related to this work. Dr. B. Pradeepkumar and Mr. A. Sudheer reviewed, analyzed these data and gave the necessary inputs towards the scheming of the manuscript. All authors discussed the sections of methodology, results and contributed to the final manuscript.

9. CONFLICT OF INTEREST

Conflict of interest declared none.

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