Research Article **CODEN: IJRPJK** **ISSN: 2319 – 9563**

International Journal of Research in Pharmaceutical and Nano Sciences

Journal homepage: **www.ijrpns.com**

EVALUATE HEPATOPROTECTIVE ACTIVITY OF THE EMU OIL

Gurram. Prasadachowdari[*1], Malempati Ramesh Babu[1], Purnema Devi[1], Dilsad[1], P. Srikanth[1], Suresh Yadav Katam[2]

[1*]Department of Pharmacology, Dr. Samuel George Institute of Pharmaceutical Sciences, Markapur, A P, India.
[2]Department of Pharmacology, Jaypore College of Pharmacy, A P, India.

ABSTRACT

Liver is the largest gland in the human body. It plays a major role in metabolism and has a number of functions in the body, including glycogen storage, plasma protein synthesis, and detoxification. Emu oil comes from a thick pad of fat on the back of the bird that was initially provided by nature to protect the animal from the extreme temperatures of its Australian homeland. The present study evaluated the Hepatoprotective activity of Emu oil (Dromaius novaehollandiae) Pods by using CCl4 and Paracetamol induced Hepaotoxicity in Wistar albino rats. In the present study Emu oil is used at arbitrary dose for 7 days in CCl4 and Paracetamol induced Hepaototoxicity. Parameters like SGPT, SGOT, ALT, ALP, Total Bilirubin and Direct Bilirubin in blood and Histopathology of liver were evaluated.

KEYWORDS

Emu oil, Hepatoprotective agent, Plasma protein, Bilurubin and CCl_4.

Author for Correspondence:

Gurram. Prasadachowdari,
Department of Pharmacology,
Dr. Samuel George Institute of Pharmaceutical Sciences, Markapur, Andhra Pradesh, India.

Email: prasadachowdrigurram@gmail.com

INTRODUCTION

Liver is the largest gland in the human body. It plays a major role in metabolism and has a number of functions in the body, including glycogen storage, plasma protein synthesis, and detoxification. It produces bile, an alkaline compound which aids in digestion, via the emulsification of lipids. It also performs and regulates a wide variety of high-volume biochemical reactions requiring specialized tissues[1].

Anatomy

The liver is the largest gland of the body enclosed within the right lower rib cage beneath the diaphragm. It is almost completely covered by visceral peritoneum as well as completely covered by a dense irregular connective tissue layer that lies deep to the peritoneum. Liver is divided in to two principle lobes, a large right lobe, and a smaller left lobe separated by falciform ligament. The right lobe is considered by many anatomists to include an inferior quadrate lobe and a posterior quadrate lobe[2]. The anatomy of liver shown in the Figure No.1.

Animal Models in Liver Diseases

By recent advances in science and increasing knowledge of liver pathology various animal models of liver diseases has come forth, various Hepatotoxicants are listed in the Table No.1.

CCL_4 Induced Hepatotoxicity

It is useful to divide the mechanism of CCl_4 into the following sequence.

Initial events

Secondary evoked mechanism

End stage pathological consequences

The initial event involves carbon-halogen bond cleavage, probably by a one-electron reduction of CCl_4, by a particular ferrous cytochrome P-450, to form chloride anion and trichloromethyl radical (CCl_3). Trichloromethyl peroxy radical ($OOCCl_3$) is probably generated and small quantity of CO may appear, mostly through dichlorocarbene intermediate.

In next stage, CCl_4-carbon is covalently bound to microsomal lipids and proteins. This placed CCl_4 into a general class of xenobiotics, the toxicity of which appears to depend on their metabolism and subsequent covalent bindings to cellular macromolecules. However, within the first hour, there is inhibition of movement of liver triglycerides to the plasma as very low-density lipoprotein (VLDL), polyribosomal desegregation and findings of protein synthesis set in well. Protein synthesis could not take place as the specific binding site is already occupied by cytochrome P-450 induced free radicals[3].

The peroxidative decomposition of lipids of the endoplasmic reticulum (ER) initiated by CCl_4 metabolism. Lipid peroxidation generates a wide variety of more or less toxic products, not organic radicals, which presumably could migrate from membrane sites near cytochrome P-450 to the other parts of the cell. It is reported that aminopyrin demethylase, cytochrome P-450 and glucose-6-phosphatase were virtually unaffected during anaerobic metabolism of CCl_4 despite covalent binding of large amounts of CCl_4. This states that CCl_4 Hepatotoxicity is primarily a matter of lipid peroxidation rather than covalent binding of CCl_4 cleavage products.

Paracetamol Induced Hepatotoxicity

Paracetamol (Acetaminophen), a widely used antipyretic and analgesic, which is safe at therapeutic dose, but it, is reported to induce liver injury in man and experimental animals when used in large doses[31]. The metabolic pathway for acetaminophen involves phase I and II reactions, glutathione detoxification and the formation of reactive intermediates, which disrupt cell macromolecules. If glucuronyl transferase and sulfotransferase are available, phase II reactions predominate, with only a small fraction of acetaminophen metabolized directly by cytochrome P-450, unless the quantity of acetaminophen exceeds the capacity of these phase II enzymes. At this point, an avidly arylating metabolite of acetaminophen, N-acetyl-p-benzoquinoneimine (NAPQI), is formed through cytochrome P-450 and may bind covalently to cell macromolecules, thereby disrupting mitochondrial and possibly nuclear function. The formation of covalent bonds is prevented if NAPQI can be detoxified by conjugation (through glutathione-S-transferase) to generate, through a series of steps, mercapturic acid, a harmless, water-soluble product excreted by the kidney. This arylation of hepatic macromolecules by NAPQI was protected *in-vitro* by addition of electrophilic sulfhydryl compounds such as glutathione and cysteine[3].

The present study evaluated the Hepatoprotective activity of Emu oil (Dromaius novaehollandiae)

Pods by using CCl4 and Paracetamol induced Hepaotoxicity in Wistar albino rats. In the present study Emu oil is used at arbitrary dose for 7 days in CCl_4 and Paracetamol induced Hepaototoxicity. Parameters like SGPT, SGOT, ALT, ALP, Total Bilirubin and Direct Bilirubin in blood and Histopathology of liver were evaluated.

MATERIAL AND METHODOLOGY

Chemicals and Reagents

Cetyl tri methyl ammonium Bromide, (Hexadecyl trimethyl ammonium Bromide), Riboflavine ($C_{17}H_{20}N_4O_6$) and Dextran sulphate sodium salt from SRL Pvt. Ltd., Mumbai.

Thiobarbituric acid ($C_4H_4N_2O_2S$), Trichloroacetic acid (CCl_3COOH), EDTA disodium salt [$CH_2N(CH_2COOH)CH2COONa]_2$ $2H_2O$, Sodium azide (NaN_3), Sulphanilamide ($C_6H_6N_2O_3S$), Sodium Nitroprusside (Na_2 [Fe (CN) $_5$ NO] $_2$ H_2O), Glutathione (Reduced) ($C_{10}H_{17}N_3O_6S$) and N-1 Naphthyl ethylene diamine dihydrochloride ($C_{12}H_{16}Cl_2N_2$) provided by Qualikems Fine Chemicals Pvt. Ltd., Vadodara.

Sodium hydroxide, Sodium dihydrogen phosphate and Disodium hydrogen phosphate provided by SD Fine Chemicals, Mumbai.

Hydrogen Peroxide from SDFCL, Mumbai, 1, 1, 3, 3,-Tetraethoxy propane from Sigma Aldrich, St. Louis, USA and o- Dianisidine from LOBA Chemicals Pvt. Ltd., Mumbai.

Experimental design

Carbon tetrachloride (CCl_4) induced hepatotoxicity[4]

Procedure

Wistar albino Rats (180-220g) were used. All the animals were divided into the five. groups each group consisting of 6 rats and they received the treatment as follows.

Group I : Vehicle Control received distilled water (10ml/kg p.o.)

Group II : Animals Received CCl_4 (0.5ml/kg i.p. with Arachis oil)

Group III : Animals received Liv -52 (2ml/kg p.o.).

Group IV : Animals received of *Emu oil.* (0.5ml/lit p.o).

Group V : Animals received of Emu oil (1.0 ml/lit p.o).

Emu oil and vehicles (in distilled water) were administered orally for 7 days. Hepatotoxicity was induced in group IInd, IIIrd, IVth, and Vth, by an injection of CCl_4 (0.5 ml/kg, 1:1 with Arachis oil i.p.) on one day before the dosing of test and standard drugs. On the 8th day blood sample from all Groups of rats were obtained by puncturing retro-orbital plexus. Serum was separated by centrifugation at 3000 rpm at room temperature for 20 min and subjected to biochemical estimations viz. SGPT, SGOT, ALP, Total Bilirubin and Direct Bilirubin.

The livers of all animals were removed and processed for Histopathological investigations.

Paracetamol induced hepatotoxicity[5-20]

Procedure

Wistar albino rats (180-220g) were used. All the animals were randomly divided into the five groups each group consists of 6 animals and they received the treatment as follows

Group I : Vehicle Control received (Distilled water p.o.)

Group II : Animals received Paracetamol (750mg/kg p.o.)

Group III : Animals received (Liv-52 2ml/kg p.o.)

Group IV : Animals received of *Emu oil*. (0.5ml/lit p.o.)

Group V : Animals received of *Emu oil*. (1.0ml/lit p.o).

The vehicle (Distilled water) and Emu *oil* were administered orally for 7 days. Paracetamol suspension (1% CMC) was administered in a dose of 2ml/lit p.o on one day before the dosing of test and standard drugs. On the 8th day blood sample from all Groups of rats were obtained by puncturing retro-orbital plexus Serum was separated by centrifugation at 3000 rpm at room temperature for 20 min and subjected to biochemical estimations viz. SGPT, SGOT, ALP, Total Bilirubin and Direct Bilirubin.

The livers of all animals were removed and processed for Histopathological investigations. Hepatoprotective activity of Emu oil against Carbon Tetrachloride induced Hepatotoxicity showed in the Table No.2.

Effect of *Emu oil* on SGPT, SGOT, ALP, Total Bilirubin and Direct Bilirubin level in CCl_4 induced Hepatotoxicity in rats are showed in the Figure No. 2, 3 and 4.

Histopathological changes of Liver in CCL_4 induced Hepatotoxicity in rats showed in the Figure No.5.

Hepatoprotective activity of Emu oil against Paracetamol induced Hepatotoxicity showed in the Table No.3.

Effect of *Emu oil* on SGPT, SGOT, ALP, Total Bilirubin and Direct Bilirubin level in Paracetamol induced Hepatotoxicity in rats are showed in the Figure No. 6, 7 and 8.

Histopathological changes of Liver in Paracetamol induced Hepatotoxicity in rats showed in the Figure No.9.

RESULTS AND DISCUSSION

Drug induced liver disorders are the major problem now a days. Therefore, in the present study Paracetamol and CCl_4 were employed as toxicants to induce liver fibrosis. The protective role of *Emu oil* against the above stated Hepatotoxicants was studied. The extent of toxicity was estimated by serum marker enzymes level and Histopathological studies. Above models are widely used to evaluate Hepatoprotective potential of an agent.

Table No.1: Shows Various Hepatotoxicants that induce various diseases

S.No	Agent/ model	Type of liver disease
1	4-nitroquinolone -1-oxide	Cancer by oxidative stress
2	Alfatoxin-B	Liver cancer
3	Allylalcohol	Focal liver necrosis
4 5	Anti-tubercular drugs in combination such as isoniazide, rifampin and pyriznamide	Drug induced hepatotoxicity to produce degeneration, necrosis and fibrosis
6	Carbontetrachloride (CCl_4)	Centrilobular necrosis, fatty liver and fibrosis
7	Paracetamol	Drug induced hepatitis and necrosis
8	D-L-Galactosamine(GalN)	Hepatitis
9	Dimethylhydrazine	Cancer by oxidative stress
10	Doxorubicin	Oxidative hepatotoxicity
11	Ethylalcohol	Fibrosis, hepatitis, cirrhosis and fatty liver
12	Inhibiting protein hydroxylation	Fibrosis
13	Lectin	Fibrosis
14	Thioacetamide (TAA)	Cirrhosis and fibrosis

Table No.2: Hepatoprotective activity of *Emu oil* against Carbon Tetrachloride induced Hepatotoxicity

S.No	GROUP	TREATMENT	DOSE	SGPT	SGOT	ALP	Total Bilirubin	Direct Bilirubin
1	I	Control(distilled water)	10ml/kg	22iu/l	41.50±5.20	152.83±6.48	0.493±.0367	0.074±.007
2	II	CCL4	0.5ml/kg	*112IU/L	*262IU/L	325.16±8.89$^{###}$	0.7mg/dl	0.2mg/dl
3	III	Liv-52	2ml/kg	81.16±4.45***	57.50±5.36**	188.33±8.07***	0.667±.0312*	0.093±.009^{NS}
4	IV	Emuoil	0.5ml/lit	*75IU/L	*90IU/L	210.67±7.59***	0.8mg/dl	0.3mg/dl
5	V	Emuoil	1.0ml/lit	*50IU/L	*45IU/L	233.33±7.74***	0.7mg/dl	0.2mg/dl

Table No.3: Hepatoprotective activity of *Emu oil* against Paracetamol induced Hepatotoxicity

S.No	Group	Treatment	Dose	SGPT	SGOT	ALP	Total bilirubin	Direct bilirubin
1	I	Control (distilled water)	10ml/kg	74±5.62	44.66±7.99	153.5±4.51	0.480±.0511	0.074±.010
2	II	Paracetamol	750mg/kg	*88IU/L	*256IU/L	320.16±7.63###	*1.2mg/dl	*o.3mg/dl
3	III	Liv-52	2ml/kg	88.16±6.62*	59.16±4.35**	179.16±7.98***	0.639±.0499**	0.105±.011NS
4	IV	Emuoil	0.2ml/kg	*76iu/l	*178iu/l	193.16±4.99***	0.8mg/dl	0.2mg/dl
5	V	Emuoil	0.5ml/kg	*50iu/l	*55iu/l	206.16±6.31***	0.6mg/dl	0.2mg/dl

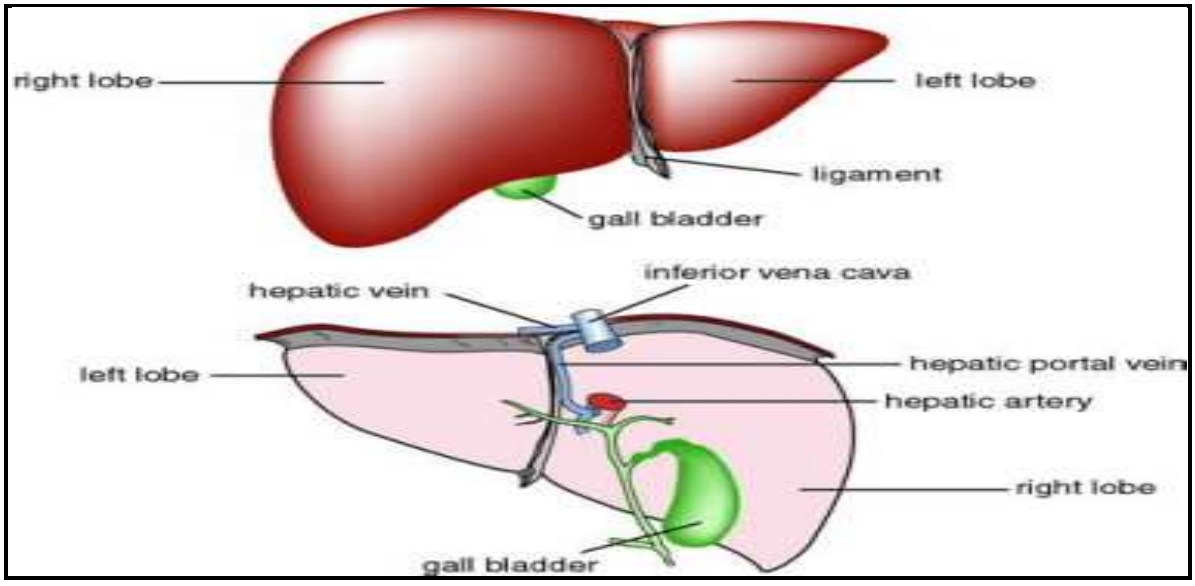


Figure No.1: External Anatomy of liver

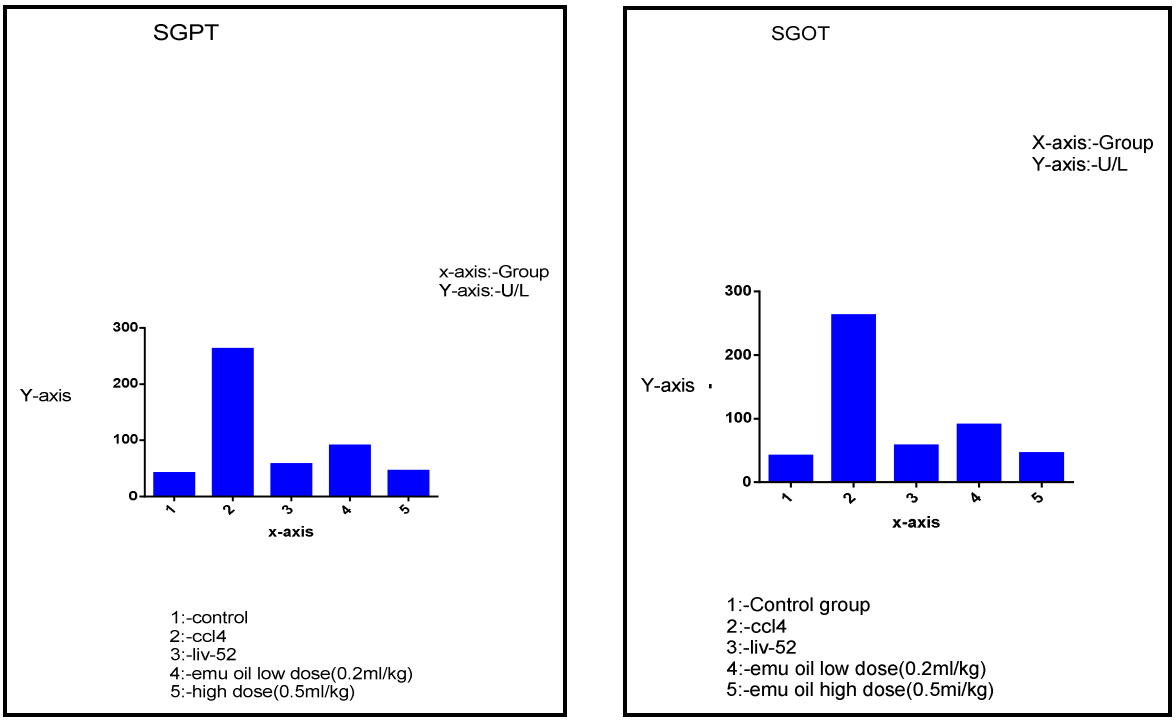


Figure No.2: Effect of *Emu oil* on SGPT and SGOT level in CCl_4 induced Hepatotoxicity in rats

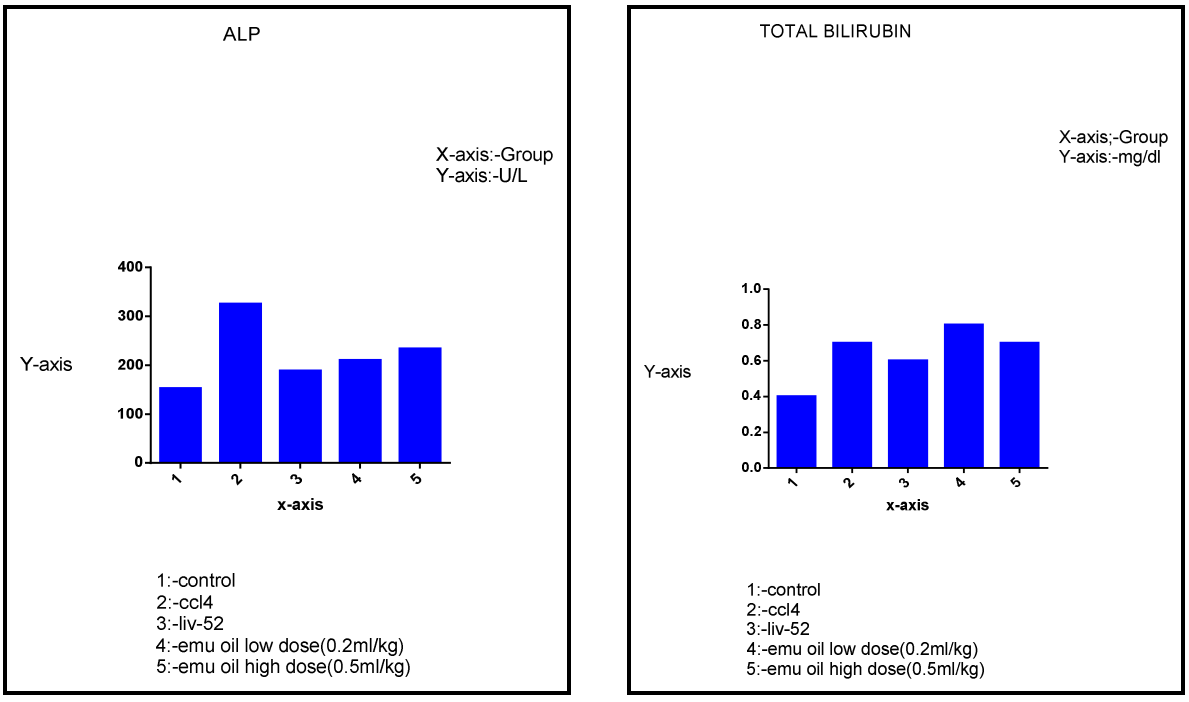


Figure No.3: Effect of Emuoil on ALP level and Total Bilirubin in CCl_4 induced Hepatotoxicity in rats

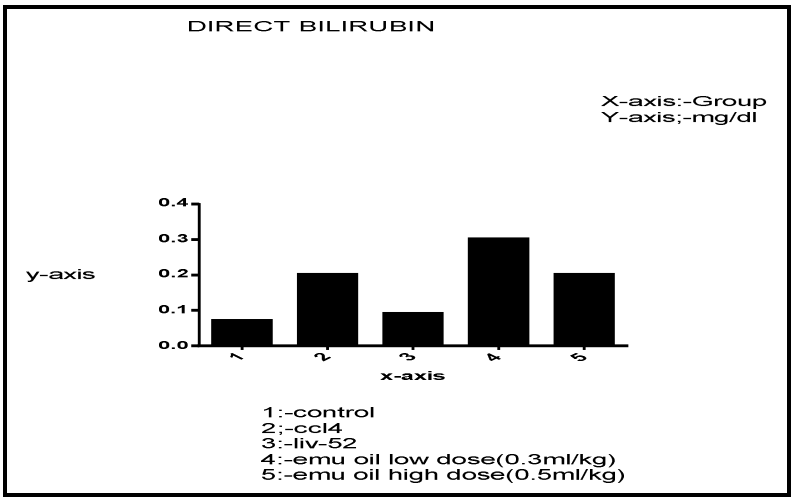
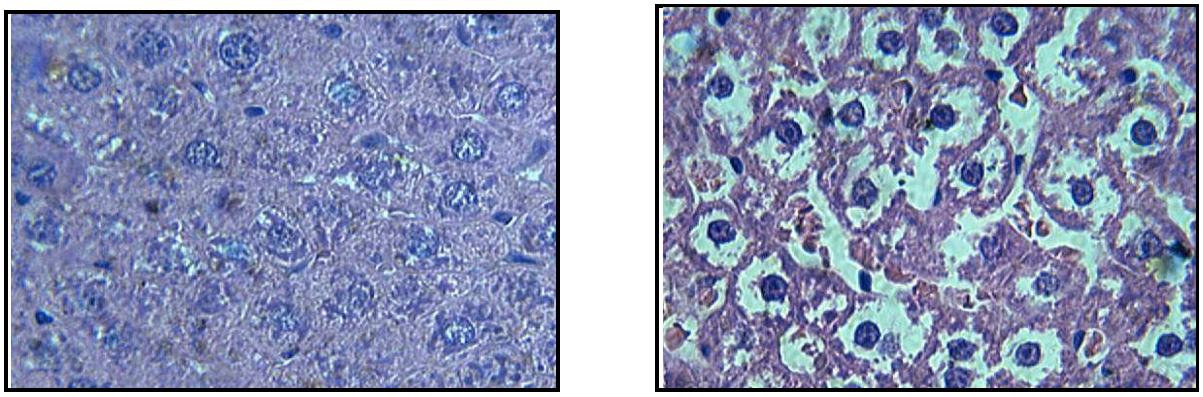
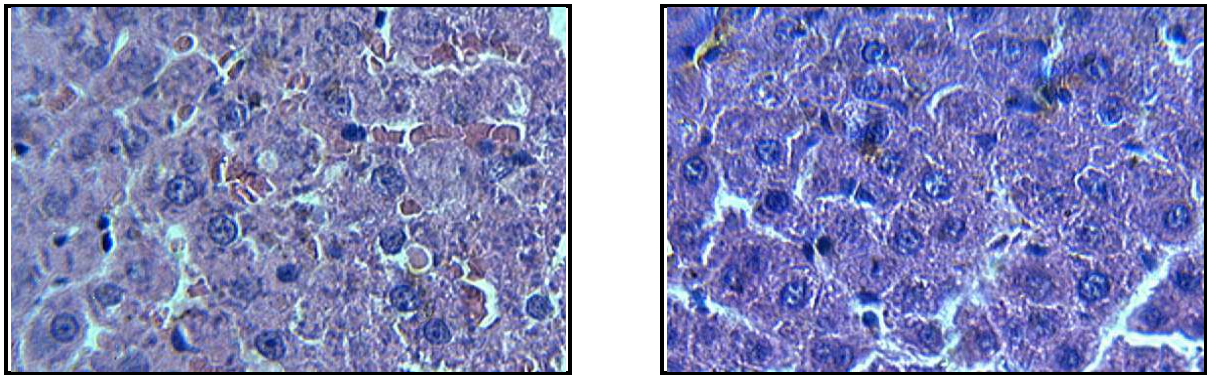


Figure No.4: Effect of *Emu oil* on Direct Bilirubin level in CCl_4 induced Hepatotoxicity rats



Normal control liver showed normal CCl4 treated rat showed a enlarged hepatocyte with vacuoles. Kupffer cells are compressed wit centrilobular necrosis in parenchyma and collection of lymphocytes



Liv-52 treated rat showed intact Architecture of liver with few vacuoles in cytoplasm .central veins exhibited Emuoil 1ml/lit treated rat showed normal central vein with few vacuoles in cytoplasm. Kupffer cells compressed with areas of necrosis

Figure No.5: Histopathological Changes of Liver in Ccl_4 Model

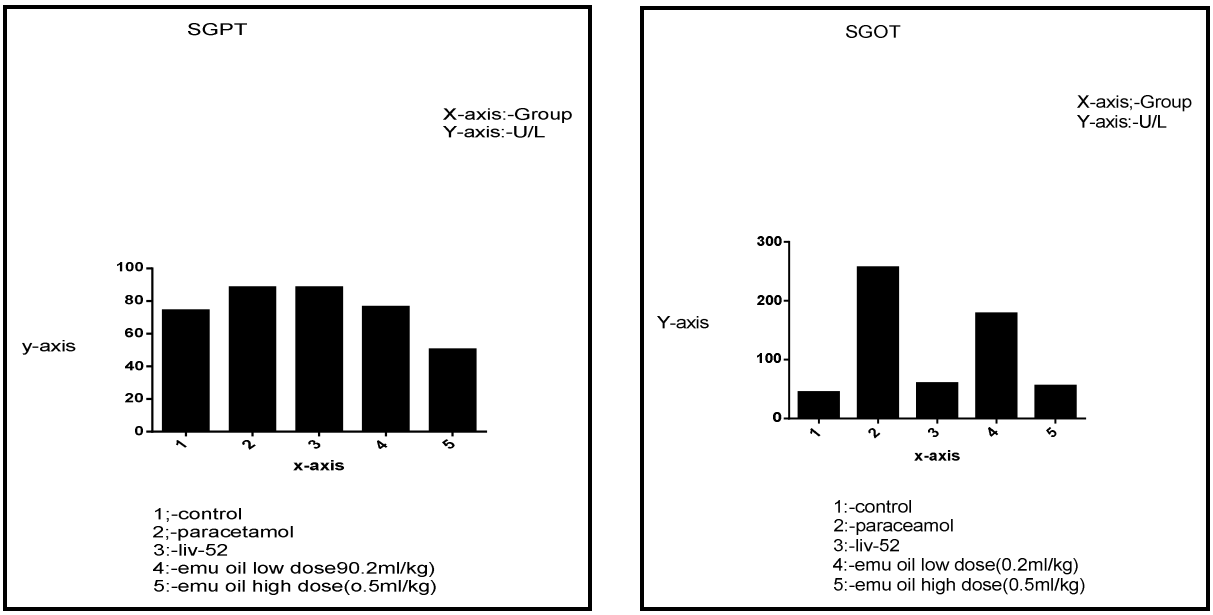


Figure No.6: Effect of *Emu oil* on SGPT and SGOT levels in Paracetamol induced Hepatotoxicity in rats

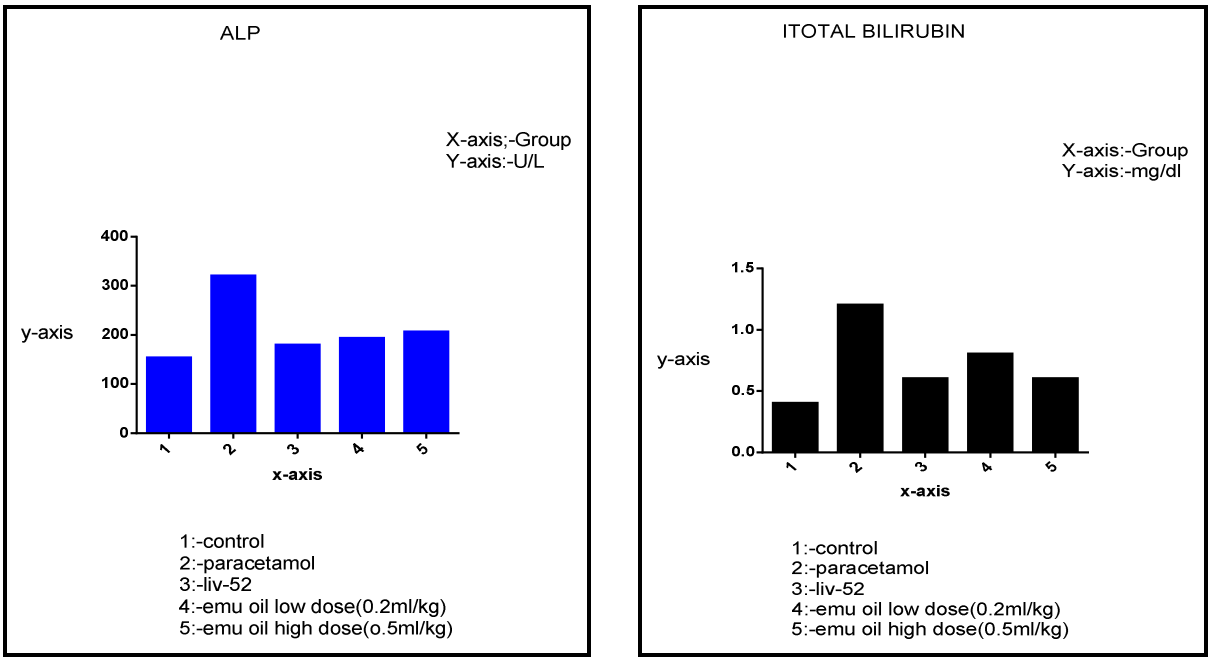


Figure No.7: Effect of *Emu oil* on ALP and Total Bilirubin level in Paracetamol induced Hepatotoxicity in rats

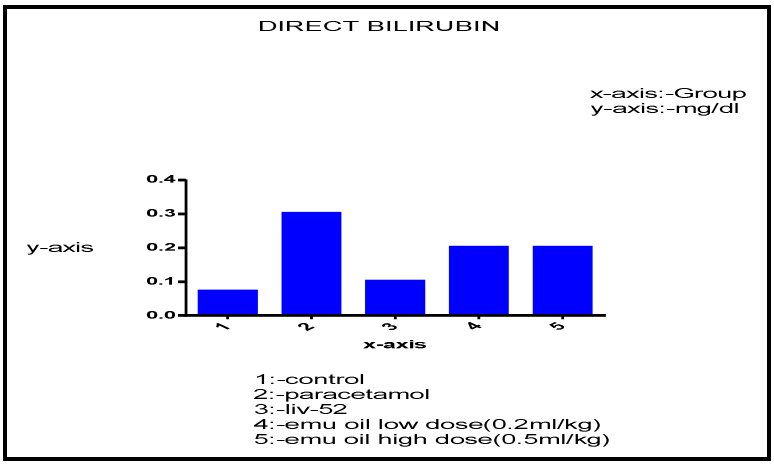
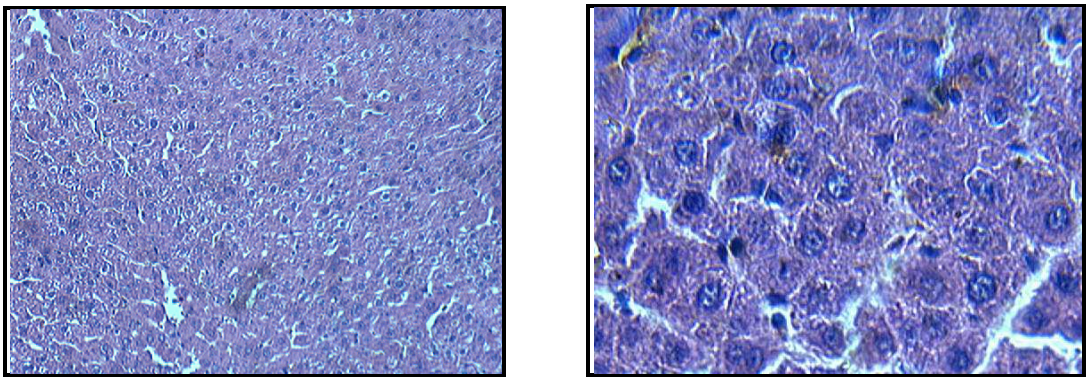
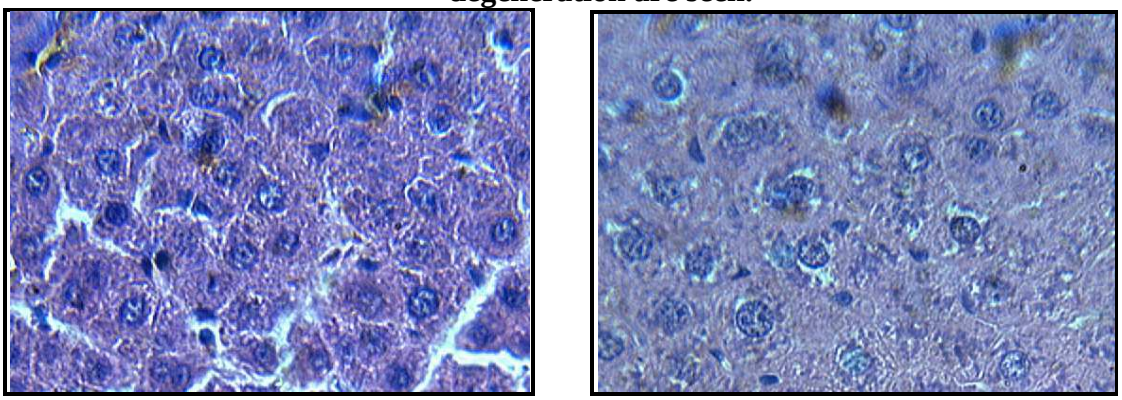


Figure No.8: Effect of *Emu oil* on Direct Bilirubin level in Paracetamol induced Hepatotoxicity in rats



Normal control liver showed normal histology showing prominent central vein, portal triads, Paracetamol treated rat showed focal areas of liver cell degeneration, lymphocytic infiltration, fatty degeneration are seen.



Liv-52 treated rat showed few areas of fatty change and restoration of cells Emuoil 0.5ml/lit treated rat showed Small areas of cell degeneration and almost normal histology. Emuoil 1ml/lit treated rat showed Small areas of cell degeneration lymphocyte infiltration are seen.

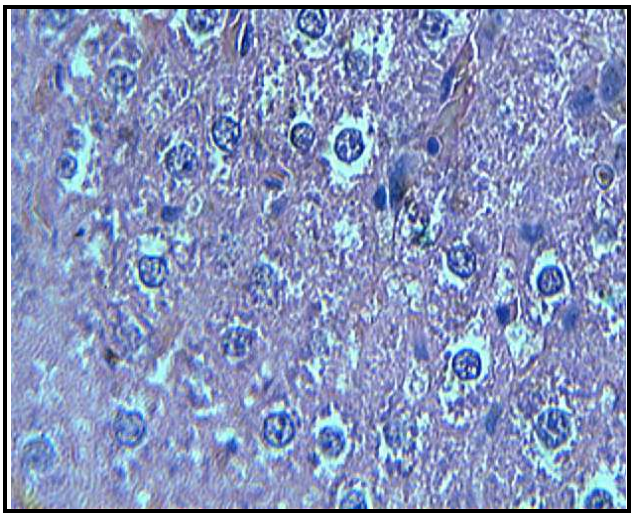


Figure No.9: Histopathological Changes of Liver in Paracetamol Model

CONCLUSION

The selected Bird *Emu oil* was widely used as Hepatoprotective in traditional and ayurvadic system of medicine. The present study was designed with the objective to evaluate the Hepatoprotective potential of Emuoil in CCl_4 and Paracetamol induced Hepatotoxicity Emuoil 1.0ml/lit showed a significant hepatoprotection on the various Hepatotoxicant induced liver toxicity. These findings were supported by the significant reduction in the elevated biochemical parameters. This was further supported by the Histopathological studies of liver tissue that showed reversal of the altered architecture as well as maintenance of normal architecture. This Hepatoprotective effect was observed in a dose dependent manner and maximum therapeutic activity i.e. the Hepatoprotective activity was evident in Emuoil 0.5ml/lit dose level.

ACKNOWLEDGEMENT

Our college Dr. Samuel George Institute of Pharmaceutical Sciences, Markapur and all our lecturers help and provide all the facilities and supports for doing this research work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

REFERENCES

1. Guyton C, John E. Hall, "Text book of Medicinal Physiology, John E Hall 2004, *Text Book of Medicinal Physiology,* 13th edition, 1995, 20.
2. Gray's Anatomy. The anatomical basis of medicines and surgery, *Anatomy Research International*, 34(4), 1995, 15-32
3. Ferret P J. Detoxification of Reactive Oxygen Species by a Nonpeptidyl Mimic of Superoxide Dismutase Cures Acetaminophen - Induced Acute Liver Failure in the Mouse, *J. Hepatoloty*, 33(5), 2001, 1173-1180. 13.
4. Chaudhari B P, Chaware V J, Joshi Y R, Biyani K R. Hepatoprotective activity of Hydroalcoholic extract of *Momordica charantia* Linn. Leaves against Carbon tetra chloride induced Hepatopathy in Rats, *International Journal of ChemTech Research,* 1(3), 2009, 355-358.
5. Tai-Nin Chau. Drug-induced Liver Injury: An Update, *Medical Bulletin*, 13(3), 2008, 23-26. 12.
6. Agarwal M, Srivastava V, Saxena K K and Kumar A. Hepatoprotective activity of *Beta vulgaris* against CCl_4 induced hepatic injury in rats, *Fitoterapia*, 77(2), 2006, 91-93.
7. Ahemed B, Alam T, Khan S A. Hepatoprotective activity of *Luffa echinata*

fruits, *Journal of Ethnopharmacology*, 76(2), 2001, 187-189.

8. Ajih T A, Janardhan K K. Antioxidant and antihepatotoxic activities of *Phellinus rimosus* (Berk) Pilat, *Journal of Ethnopharmacology*, 81(3), 2002, 387-391.
9. Anwar U, Hassan, Gilani and Khalid, Hussain, Janbaz. Preventive and Curative Effect of *Artemisia absinthium* on Acetaminophen and CCl_4 induced Hepatotoxicity, *J Pharmacol*, 26(2), 1995, 309-315.
10. Manjunatha B K. Hepatoprotective activity of *pterocarpus sntalinus l.,f* an endangered medicinal plant, *International Journal of Pharma Sciences and Research*, 1(7), 2010, 265-270
11. Harishkumara B K. Traditional knowledge system (medicine): tool conservation, *Traditional Knowledge System*, 7(3), 2009, 45-46.
12. Bhattacharya D, Pandit S, Mukherjee R, Das N and Sur T K. Hepatoprotective effect of Himolive, a polyherbal formulation in rats, *Indian J Physiol Pharmacol*, 47(4), 2003, 435-440.
13. Chatterjee T K. Medicinal plants with Hepatoprotective properties in Herbal opinions, *Books and Allied (p) Ltd.Culcutta*, 3rd edition, 2000, 135.
14. Chattopathyay R R. Possible mechanism of hepatoprotective activity of *Azadirachta indica* leaf extract: Part II, *J. Ethnopharmacology*, 89(2-3), 2003, 217-219.
15. Mandeep Singh Dhingra. Drug Induced Liver Injury, *Evidence based practice*, 7(1), 2006, 1-30.
16. Grahame - Smith D G, Azonson J K. The drug therapy of gastro-intestinal, hepatic and biliary disorders Chaper 23 In: Oxford Textbook of Clinical Pharmacology and Drug Therapy, *Oxford University Press, Oxford*, 1st edition, 1984, 383 - 385.
17. Gujrati V, Patel N, Rao N, Kumar K N, Gouda T S, Shalam M D. Hepatoprotective activity of alcoholic and aqueous extract of leaves of *Tylophora indica* in rats, 4(2), 2007, 20.
18. Guntupalli, Saketh R M D, Steingrub, Jay M D. Hepatic disease and Pregnancy: An overview of diagnosis and management Critical Care Medicine, 33(10), 2005, 112-116.
19. Guntupalli M M, Chandana V R, Palpu P and Annie S. Hepatoprotectiv effect of rubiadin, a major constituent of *Rubia cardifolia* Linn, *J.Ethnopharmacology*, 103(3), 2005, 484-490.
20. Gupta Ajay Kumar, Misra Neelam. Hepatoprotective Activity of aqueous ethanolic Extract of *Chamomile capitula* in Paracetamol Intoxicated Albino Rats, *American Journal of Pharmacology and Toxicology*, 1(1), 2006, 17-20.

Please cite this article in press as: Gurram Prasadchowdari. *et al.* Evaluate hepatoprotective activity of the emu oil, *International Journal of Research in Pharmaceutical and Nano Sciences*, 3(5), 2014, 397-407.