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Potential Efficacy Of *Citrus limon* On Protein And Creatinine Profile In Ketamine treated Zebra fish, *Danio rerio*

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ABSTRACT

This paper determines the toxicity of ketamine and its recuperation by phytochemicals of *Citrus limon* leaves extract. Fishes were collected, acclimatized & divided into 6 groups of 5 fishes each. Ist group served as control whereas, IInd group was treated with ketamine alone. IIIrd & IVth groups were treated with different concentrations of phytochemical and Vth & VIth groups were given ketamine with Phytochemical. After 96 hours total protein (TPRO) & creatinine levels were tested using autoanalyser from supernatant of liver and muscle tissues. Ketamine showed adverse effect on the protein concentration as well as in creatinine concentration, whereas at different concentrations, phytochemicals did not have any adverse impact and also helped in the suppression of toxic effect of ketamine. Some behavioural changes were observed after the treatment with ketamine. However such aberrant behaviour was absent in control fishes.

Figure : 00 References : 30 Tables : 02
KEY WORDS : *Citrus limon*, Creatinine, Ketamine, Total Protein (TPRO), Zebrafish.

Introduction

The liver is one of the most important organs in the body, performing a fundamental role in the regulation of diverse processes, like metabolism, storage, detoxification *etc* because of which the hepatic diseases are at the urge of having the main threats to public health worldwide[1]. Liver also plays major role in the biochemical processes of growing, supply of energy to different body parts and providing nutrients. Along with this, it aids in metabolism of carbohydrates and fats, in the secretion of bile, and in the storage of vitamins[2]. Despite enormous advances in hepatoprotective drugs, there are no such effective drugs which completely stimulate hepatic function, offer complete protection to the body organs, or aid in regenerating hepatic cells[5]. Additionally, some drugs can induce adverse or side effects. Ketamine which is a short acting general anaesthetic that is injected into patients to produce loss of consciousness before and during surgery or used in other medical procedures. The off-label use of ketamine has also been utilised for the successful treatment of chronic and acute pain, procedural sedation and alcohol withdrawal management[13]. The recreational misuse of ketamine can be physically hazardous and possesses many dangerous side-effects on the users especially at the higher dosage[22] . Ketamine is noted to induce stress, and can affect the physiological process of liver[26] .

Thus, it is necessary to identify alternative pharmaceuticals, with the aim of these agents being more effective and less toxic. The use of some plants have played fundamental roles in human health care. Approximately 80% of the world's population has employed traditional medicine for health care, which is based predominantly on plant materials[8]. The properties which are responsible for the beneficial effects of plants could be attributed to the presence of chemical compounds or substances that are biologically active secondary metabolites and non-essential nutrients for

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TABLE-1 : Estimation of Total Protein (TPRO) in Liver & Muscle tissues of zebrafish following 96 hours of exposure.

Biological Parameter (TPRO)	Control	Ketamine 12.5mg/L	Phytochemicals		Ketamine + Phytochemicals	
			1ml/L	2ml/L	1ml/L	2ml/L
	Group-I	Group-II	Group-III	Group-IV	Group-V	Group-VI
Liver (mg/dL) S.D±	2.515 ±0.08	1.86 ±0.620	2.54 ±0.325	3.14 ±0.085	3.68 ±0.257	3.62 ±0.235
Muscle (mg/dL) S.D±	2.74 ±0.0351	2.485 ±0.240	2.75 ±0.3711	2.78 ±0.383	3.18 ±0.175	3.32 ±0.217

life, called phytochemicals[10]. They have been shown to exert their positive antioxidant benefits in terms of favoured performance, production quality and enhanced endogenous antioxidant system. Some workers showed the antioxidant properties of *Citrus limon* against hepatic diseases in rats [3,6,21]. The work of *Citrus limon* leaves along with ketamine on zebrafish are scarce, therefore in the present investigation we have taken ketamine as sedative & *Citrus limon* leaves extract as phytochemical in zebrafish.

Materials and Methods

1. Collection and maintenance of fishes

The fishes *Danio rerio* were collected from local fish market of Jhansi and transferred to laboratory conditions. Before acclimatization fishes were treated with 0.2% KMnO4 solution to check the dermal infection for 2-4 minutes. After treatment fishes were acclimatized in laboratory conditions for 10 days at 28°C to 32°C temperature and pH ±7.2. They were fed with standard commercial diet.

2. Preparation of ketamine solution and *Citrus limon* leaves extract

Ketamine injection IP 50mg/ml was purchased from a nearby medical store with trade name Ketarays. Solution was prepared by dissolving 0.25 ml of ketamine in 1 litre normal water (12.5mg ketamine/ Litre of water), after every 24 hours fresh solution of 0.25ml of ketamine in 1L was prepared. For *C.limon* Leaves extract, leaves were collected from a tree and dried in shade. After the leaves were totally dried they were ground into a fine powder . Now 20g of powder was mixed with 50 ml water and boiled for 10 to 15 minutes. Solution was filtered and was made up to 50ml. From this stock solution different concentrations of extract were used in experiment.

3. Experimental design

The exposure protocol employed in the present study was to analyse the effect of ketamine toxicity and its recuperation by *Citrus limon* leaves extract on zebrafishes after 96 hours.

Fishes were divided into 6 groups of five fishes each as-

Group1:- Untreated *i.e.* Control

Group2:-Received sedative (ketamine) in concentration 12.5mg/L

Group3:-Subjected to phytochemical with concentration 1 ml/L

Group4:-Also received phytochemical with another concentration *i.e.,* 2ml/L

Group5:-Received both ketamine (12.5mg/L) and phytochemical with concentration (1ml/L)

Group 6:-Received sedative (12.5mg/L) and phytochemical with concentration (2ml/L).

After 96 hours of exposure period fishes were sacrificed 0.01g of liver and 0.10g of muscle tissues were collected from each group and homogenized in 1ml of distilled water separately. After centrifugation at 3000 rpm for 10 minutes supernatants were collected to be used for protein and creatinine estimation.

Results and Discussion

The results of Protein levels in liver and muscle tissues of zebrafish when treated with ketamine for 96 hours are presented in Table-1. Total protein concentration in Liver & Muscle tissues of control fish was estimated to be 2.515 mg/dL ±0.08 & 2.74 mg/dL ±0.0351, which decreased when treated with ketamine

i.e.,1.86mg/dL ±0.620 in Liver and 2.485 mg/dL ±0.240 in Muscle .

Studies show that protein metabolism significantly occurs in liver and diseased liver will show decreased level of protein synthesis and hence decrease in whole body metabolism[16]. Sedative induced stress decreases metabolic rate requiring higher amount of energy and due to which concentration of protein declined in tissues[15,19]. When treated with 1ml/L of *Citrus limon* leaves extracts the TPRO concentration was 2.54 mg/dL in liver & 2.75 mg/dL in muscle tissues and in 2ml/L concentration of *Citrus limon* it was 3.14 mg/dL in liver & 2.78 mg/dL in muscle tissues. Phytochemicals are capable of causing more increase in concentration of protein in tissues, hence showing positive effect on increasing the protein content as antioxidants are present in phytochemicals. In combination therapy of ketamine with 1ml/L and 2ml/L of *Citrus limon* leaves extract, TPRO concentration level increased in comparison to control in liver and muscle tissues both (Tables- 1 & 2) because antioxidant present in phytochemicals suppresses the oxidative stress increases the metabolic activity therefore lesser energy is used for proper functioning of liver[12,24].

The results of Creatinine levels in liver and muscle tissues of zebrafish when treated with ketamine for 96 hours are presented in Table-2. Creatinine controlled value is estimated to be 0.16 mg/dL ±0.043 in liver & 0.086mg/dL±0.024 in muscle tissues whereas when treated with ketamine the concentration increases drastically *i.e.,* 2.22mg/dL ±0.927 in liver & 0.665mg/dL ±0.185 in muscle tissues.

It can be postulated that the stress passed through by the fish due to treatment of ketamine is related to the impairment of kidney functioning. High creatinine level implied that many waste products in the fish blood stream would not be cleared, indicating that the Kidneys were not functioning properly[7].

When fishes were exposed to phytochemicals alone (1ml/L & 2ml/L of *Citrus limon*) the creatinine level was closed to the control fish due to its positive impact on renal functioning. On combination therapy of Ketamine with Phytochemicals treated groups there was a decrease in the creatinine concentration when compared to II nd group in liver and muscle tissues. Both the values are closer to control value. Here, Phytochemical showed a positive impact on the reduction of the effect of ketamine by lowering the creatinine concentration. Phytochemicals present in green tea extract have protective activity against renal injury in lead exposed rats through ROS scavenging activity[27]. Phytochemicals have important effect on renal physiology and possesses diuretic and natriuretic properties, as well as exerting renoprotective effects in acute and chronic kidney disease[11,28,30.]

Although very little work is estimated out using *Citrus limon* leaves extract on Creatinine & TPRO but our results are in accordance with the other research works using other phytochemicals in fishes[23,29]. The alteration in TPRO & Creatinine content may also be due to the cell necrosis of different tissues caused by ketamine exposure as the cell damage was observed in many histological studies due to pharmaceuticals, heavy metals and pesticides[3,4]. Similar findings of inverse relationship between total protein & creatinine level were reported by previous researchers[9,25].

Here, we also assessed sub-anaesthetic concentration of ketamine acutely modulate aggression in adult Zebrafish. Our findings showed that 12.5mg/L ketamine increases aggression towards glass jar ,

TABLE-2 : Estimation of Creatinine in Liver & Muscle tissues of zebrafish following 96 hours of exposure.

Biological Parameter (Creatinine)	Control	Ketamine 12.5mg/L	Phytochemicals		Ketamine + Phytochemicals	
			1ml/L	2ml/L	1ml/L	2ml/L
	Group-I	Group-II	Group-III	Group-IV	Group-V	Group-VI
Liver (mg/dL) S.D±	0.16 ±0.043	2.22 ±0.927	0.19 ±0.0953	0.23 ±0.0208	0.08 ±0.05	0.05 ±0.01
Muscle (mg/dL) S.D±	0.086 ±0.024	0.665 ±0.185	0.02 ±0.015	0.06 ±0.0305	0.055 ±0.005	0.05 ±0.0152

increases locomotion and circulation behaviour , which may impair overall swimming patterns and exploratory activity of fish. Changes in jumping behaviour, equilibrium loss and colour changes were also observed[14,18]. This may be due to inactivation of central nervous system of treated fishes which may lead to failure of latero-acoustic system of fishes because inhibition occurred in the activity of acetylcholinesterase[17,20].

Conclusion

The study demonstrated that phytochemicals have important effects on renal and liver physiology. As 77% genome of zebrafish are similar to that of humans, so our findings suggest that in monitoring ketamine toxicity, TPRO and CREATININE activity can be a potent diagnoslic tool. Furthermore studies are needed on long-term exposure of ketamine and coalesced of ketamine and *Citrus limon* leaves extract.

References

1. Adewusi EA, Afolayan AJ. A review of natural products with hepatoprotective activity. *J Med Plants Res.* 2010; **4** : 1318-1334.
2. Ahsan MR, Islam KM, Bulbul IJ. Hepatoprotective activity of Methanol Extract of some medicinal plants against carbon tetrachloride-induced hepatotoxicity in rats. *Global J Pharma-col.* 2009; **3**: 116-122.
3. Bekkouch O, Dalli M, Harnafi M, Touiss I, Mokhtari I, Assri SE, Amrani S. Ginger (*Zingiber officinale* Roscoe), Lemon (*Citrus limon* L.) Juices as Preventive Agents from Chronic Liver Damage Induced by CCl4: A Biochemical and Histological Study. *Antioxidants*. 2022; **11**(2) : 390.
4. Bhuvaneshwari R, Padmanaban K, Babu Rajendran R. Histopathological alterations in muscle, liver and gill tissues of zebra fish *Danio rerio* due to environmentally relevant concentrations of organochlorine pesticides (OCPs) and heavy metals. *International Journal of Environmental Research*. 2015; **9**(4) : 1365-1372.
5. Chattopadhyay RR. Possible mechanism of hepatoprotective activity of *Azadirachta indica* leaf extract: part II. *J Ethno-pharmacol.* 2003; **89**: 217-219.
6. Chaudhari SY, Ruknuddin G, Prajapati P. Ethno medicinal values of *Citrus* genus: A review. *Medical Journal of Dr. DY Patil University*. 2016; **9**(5) : 560.
7. Chu, Peggy Sau Kwan, et al. "The destruction of the lower urinary tract by ketamine abuse: a new syndrome?." *BJU international.* 2008; **102**(11) : 1616-1622.
8. Deshwal N, Sharma AK, Sharma P. Review on hepatopro-tective plants. *Int J Pharm Sci Rev Res.* 2011; **7**: 15-26.
9. Guo Z. The modification of natural products for medical use. *Acta Pharmaceutica Sinica B*. 2017; **7**(2) : 119-136.
10. Huang, Zhongdi, et al. "Icariin protects rats against 5/6 nephrectomy-induced chronic kidney failure by increasing the number of renal stem cells." *BMC Complementary and Alternative Medicine.* 2015; **15 (**1) : 1-8.
11. Kaneez Zahra and Akanksha Singh . Antioxidant effects of green tea extract on biochemical parameters of *Heteropneustes fossilsis* poisoned by cypermenthrin. *Indo american Journal of Pharmaceutical science,* 2018; **5**(5) : 4908-4913.
12. Kumar A. Dogra S. and Prakash A. Protective effect of naringin, a citrus flavonoid, against colchicine-induced cognitive dysfunction and oxidative damage in rats. *Journal of medicinal food*. 2010; **13**(4) : 976-984.
13. Kurdi Madhuri S, Kaushic A, Theerth, Radhika S Deva. "Ketamine: current applications in anesthesia, pain, and critical care." *Anesthesia, essays and researches.* 2014; **8** (3) : 283.
14. Michelotti P, Quadros VA, Pereira ME, Rosemberg DB. Ketamine modulates aggressive behavior in adult zebrafish. *Neuroscience letters*. 2018; **684** : 164-168.
15. Miyake Y, Yamamoto K, Tsujihara N, Osawa T. Protective effects of lemon flavonoids on oxidative stress in diabetic rats. *Lipids.* 1998; **33**(7) : 689-695.

16. Muting DIETER, HORST Reikowski. “Protein metabolism in liver disease.” *Progress in Liver Diseases.* 1965; **2** : 84.

17. Qayoom I, Shah FA, Mukhtar M, Balkhi MH, Bhat FA, Bhat BA. Dimethoate induced behavioural changes in juveniles of *Cyprinus carpio var.* communis under temperate conditions of Kashmir, India. *The Scientific World Journal*. 2016.

18. R Riehl, E Kyzar, A Allain, J Green, M Hook, L Monnig, K Rhymes, A Roth, M Pham, R Razavi, J Dileo, S Gaikwad, P Hart, AV Kalueff. Behavioral and physiological effects of acute ketamine exposure in adult zebrafish, Neurotoxicol. *Teratol.* 2011; **33** : 658–667.

19. Schricker T, Klubien K, Carli F. The independent effect of propofol anesthesia on whole body protein metabolism in humans. *The Journal of the American Society of Anesthesiologists.* 1999; **90**(6) : 1636-1642.

20. Shao B, Zhu L, Dong M, Wang J, Wang J, Xie H, Zhu S. DNA damage and oxidative stress induced by endosulfan exposure in zebrafish *(Danio rerio)*. *Ecotoxicology*. 2012; **21**(5) : 1533-1540.

21. Shefalee K Bhavsar, Paulomi Joshi, Mamta B Shah, DD Santani. Investigation into Hepatoprotective Activity of *Citrus limon*., *Pharmaceutical Biology.* 2007; **45** (4) : 303-311.

22. Short Brooke, et al. “Side-effects associated with ketamine use in depression: a systematic review.” *The Lancet Psychiatry.* 2018; **5**(1) : 65-78.

23. Singh A, Zahra K. Effect of garlic extract on mortality and biochemical parameters of fresh water fishes *Heteropneustes fossilis* against Cypermethrin. JDDT. 2019; **9**(2):14-9.

24. Tirkey N, Pilkhwal S, Kuhad A, Chopra K. Hesperidin, a citrus bioflavonoid, decreases the oxidative stress produced by carbon tetrachloride in rat liver and kidney. *BMC pharmacology*. 2005; **5**(1) : 1-8.

25. Valtonen MH, Uusi-Rauva A, Eriksson L. The effect of protein deprivation on the validity of creatinine and urea in evaluation of renal function. An experimental study in the goat. *Scand J Clin Lab Invest.* 1982; **42**(6):507-12.

26. Wai MSM, et al. “Long-term ketamine and ketamine plus alcohol treatments produced damages in liver and kidney.” *Human & experimental toxicology.* 2012; **31**(9) : 877-886.

27. Wang, Haidong, et al. “Protective effects of green tea polyphenol against renal injury through ROS-mediated JNK-MAPK pathway in lead exposed rats.” *Molecules and cells.* 2016; **39**(6) : 508.

28. Xin, Shao-bin, et al. “Protective effects of luteolin on lipopolysaccharide-induced acute renal injury in mice.” Medical Science Monitor: *International Medical Journal of Experimental and Clinical Research.* 2016; 22 : 5173.

29. Yadav Vineeta, Ahmad Shadab, Zahra Kaneez. Imidacloprid Toxicity And Its Attenuation By Aqueous Extract of *Moringa oleifera* Leaf In Zebrafish, *Danio rerio. International Journal of Current Pharmaceutical Research.* 2020; 32(38) : 10.

30. Ye, Jian-Hong, et al. “Chemical profiles and protective effect of *Hedyotis diffusa* Willd in lipopolysaccharide-induced renal inflammation mice.” *International Journal of Molecular Sciences.* 2015; **16**(11) : 27252-27269.