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. I\textsuperscript{st} group served as control whereas, I\textsuperscript{nd} group was treated with ketamine alone. I\textsuperscript{IInd} & I\textsuperscript{IVth} groups were treated with different concentrations of phytochemical and V\textsuperscript{th} & VI\textsuperscript{th} 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\textsuperscript{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\textsuperscript{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\textsuperscript{3}. 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\textsuperscript{13}. The recreational misuse of ketamine can be physically hazardous and possesses many dangerous side-effects on the users especially at the higher dosage\textsuperscript{22}. Ketamine is noted to induce stress, and can affect the physiological process of liver\textsuperscript{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\textsuperscript{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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life, called phytochemicals\textsuperscript{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 \textit{Citrus limon} against hepatic diseases in rats \textsuperscript{3,6,21}. The work of \textit{Citrus limon} leaves along with ketamine on zebrafish are scarce, therefore in the present investigation we have taken ketamine as sedative & \textit{Citrus limon} leaves extract as phytochemical in zebrafish.

**Materials and Methods**

1. **Collection and maintenance of fishes**
   The fishes \textit{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 \textit{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 \textit{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 \textit{Citrus limon} leaves extract on zebrafishes after 96 hours.

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

   - **Group 1:** Untreated \textit{i.e.} Control
   - **Group 2:** Received sedative (ketamine) in concentration 12.5mg/L
   - **Group 3:** Subjected to phytochemical with concentration 1 ml/L
   - **Group 4:** Also received phytochemical with another concentration \textit{i.e.}, 2ml/L
   - **Group 5:** 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.
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\textsuperscript{16}. Sedative induced stress decreases metabolic rate requiring higher amount of energy and due to which concentration of protein declined in tissues\textsuperscript{15,19}. When treated with 1ml/L of \textit{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 \textit{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 \textit{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\textsuperscript{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 & 0.086 mg/dL ±0.024 in muscle tissues whereas when treated with ketamine the concentration increases drastically i.e., 2.22 mg/dL ±0.927 in liver & 0.665 mg/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\textsuperscript{7}.

When fishes were exposed to phytochemicals alone (1ml/L & 2ml/L of \textit{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\textsuperscript{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\textsuperscript{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\textsuperscript{11,28,30}.

Although very little work is estimated out using \textit{Citrus limon} leaves extract on Creatinine & TPRO but our results are in accordance with the other research works using other phytochemicals in fishes\textsuperscript{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\textsuperscript{3,4}. Similar findings of inverse relationship between total protein & creatinine level were reported by previous researchers\textsuperscript{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.

<table>
<thead>
<tr>
<th>Biological Parameter (Creatinine)</th>
<th>Control</th>
<th>Ketamine 12.5mg/L</th>
<th>Phytochemicals</th>
<th>Ketamine + Phytochemicals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group-I</td>
<td>Group-II</td>
<td>1ml/L Group-III</td>
<td>2ml/L Group-IV</td>
</tr>
<tr>
<td>Liver (mg/dL) S.D±</td>
<td></td>
<td></td>
<td>1ml/L Group-V</td>
<td>2ml/L Group-VI</td>
</tr>
<tr>
<td>0.16 ±0.043</td>
<td>2.22 ±0.927</td>
<td>0.19 ±0.0953</td>
<td>0.23 ±0.0208</td>
<td>0.08 ±0.05</td>
</tr>
<tr>
<td>Muscle (mg/dL) S.D±</td>
<td>0.086 ±0.024</td>
<td>0.665 ±0.185</td>
<td>0.02 ±0.015</td>
<td>0.06 ±0.0305</td>
</tr>
</tbody>
</table>
Potential Efficacy Of Citrus limon On Protein And Creatinine Profile In Ketamine treated Zebra fish, Danio rerio

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 diagnostic tool. Furthermore studies are needed on long-term exposure of ketamine and coalesced of ketamine and Citrus limon leaves extract.

References


