Protective Effect of the Aqueous Extract of *Cyamopsis tetragonoloba* Seed against the Paracetamol-induced Toxicity in Female Wistar Albino Rats

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ABSTRACT

**Background:** Paracetamol is also known as acetaminophen which is the most widely and commonly used painkiller drug among the overall human population. On excess usage and high dosage, paracetamol can cause liver injury, gastrointestinal damage and kidney damage. *Cyamopsis tetragonoloba* is the most commonly available plant in India which has weight loss properties as well as used to treat cancer and diabetes.

**Aim:** This study is to estimate the potential effect of *C. tetragonoloba* against paracetamol-induced hepatotoxicity.

**Methodology:** There were seven groups of rats which were divided as follows: Group-1 as Normal control, Group-2 administrated with paracetamol (900mg/kg b.w./day, i.p.), Group-3 is induced with paracetamol (900mg/kg b.w./day, i.p.) and *C. tetragonoloba* (500 mg/kg b.w./day, p.o.), Group-4 is same as Group-3 however *C. tetragonoloba* dosage differs as 1000 mg/kg b.w./day, p.o., Group-5 is given with paracetamol (900 mg/kg b.w./day, i.p.) and silymarin (25 mg/kg b.w./day, p.o.), Group-6 and Group-7 is administrated with *C. tetragonoloba* (500 mg/kg b.w./day, p.o.) and *C. tetragonoloba* (1000 mg/kg b.w./day, p.o.) respectively. After 4 hrs of drug administration, the animals were sacrificed from which blood and organs were collected for the toxicity study. The samples were used for the analysis of liver and kidney enzyme markers, antioxidant assays and histopathological changes.

**Conclusion:** The study has proven that *C. tetragonoloba* has a potential effect on the toxicity-induced groups. On compared to the standard drugs, *C. tetragonoloba* has shown better results.

**Key words:** *Cyamopsis tetragonoloba*, Pharmaceutical drug-induced toxicity, Plant extract, Paracetamol, Wistar albino rats.

INTRODUCTION

The largest organ of the body is the liver, which plays an important role in the maintenance of internal functions of our body by doing multiple functions. It involves metabolic pathways such as lipids, carbohydrates and proteins. Commonly it is involved in the detoxification and excretion process through xenobiotic metabolism by which exogenous and endogenous compounds were released out from the body. When these processes get disturbed, it leads to severe health issues in the affected persons. Hepatotoxicity is an adverse drug effect of the non-steroidal anti-inflammatory drug NSAIDs. On long term uses; it causes liver damages due to oxidative stress, xenobiotic and various drugs. Other effects of NSAIDs include hereditary disorder, bile syndrome and hepatitis A, B and C due to viral disinfection. More than 17,000 death was reported due to liver dysfunction. Acute kidney injury is caused by the overdose of paracetamol. Studies reveal about the drug-induced hepatotoxicity and renal dysfunction due to excess consumption of paracetamol. Kidney is a vital organ of humans involved in the removal of toxicants.
like exogenous and endogenous toxic substances. The kidney also plays a role in body fluid balance and acid-base balance. The injured kidney will not remove detoxification and the excretion process will be damaged that result in nephrotoxicity. Gastrointestinal tract (GI) is the most essential for the stomach and intestine. Gastrotoxicity can also cause mucosal damage due to the ingestion of NSAIDs medications, unhealthy foods, alcohol consumption, smoking and high-level stress. The damage can occur because of the rupture of the stomach and intestine mucosal layer.

Prostaglandin is an inflammation and fever mediator. Paracetamol has the ability to inhibit cyclooxygenase (COX) enzyme activity to prevent biosynthesis of prostaglandins. COX exists in two isoforms such as COX-1 producing prostaglandin that preserves the integrity of the gastric mucosa and COX-2 is a cytokine that induces prostaglandin that is known to cause pain and inflammation. Paracetamol or Acetaminophen (N-acetyl-p-aminophenol; APAP) is the most commonly used drug for analgesic and antipyretic. The person's drug consumption produces alanine derivatives through the process of hydrolysis and later it is converted into hydroxylamine. N-Acetyl-p-benzoquinone imine (NAPQI) is the intermediate compound formed by acetaminophen when cytochrome-p450 causes hepatic damage and tubular necrosis in both human and laboratory animals.

The botanical name for cluster bean or guar is Cyamopsis tetragonoloba. It is a member of the Fabaceae family that grows annually. It is available on the market in the form of guar-gum or gum powder. The origin of a plant is uncertain and is presumed and formed from the Cyamopsis sengalensis species native to Africa. It is a legume plant that is useful in crop rotation and helps to fix nitrogen by symbiosis bacteria such as azotobacterial rhizobium. The plant is mostly cultivated for over a century in India and Pakistan. The plant grows to a height of 2-3 m long. Guar gum has a chemical composition of galactose and mannose sugar. It can withstand temperatures as low as 80°C (176 °F) for five minutes. Studies have shown the supplementation of guar gum as effective in fasting blood glucose and reducing the LDL cholesterol. It also possesses the losing property as it is rich in fiber content.

Milk thistle is a plant from which the medication Silymarin (SLY) is extracted from polyphenolic substances. The product is obtained from the fruit and seeds of the plants. The plant's scientific name is Silybum marianum. The plant has antioxidant, anticancer and anti-inflammatory properties which are the most widely used drug for hepatic and bile disease treatment. It is active in nephrotoxic defense, major neuroprotective and gastroprotective. Interestingly, our previous research on anti-inflammatory activity of Cyamopsis sengalensis at multiple dose levels of 500 and 1000 mg/kg b.w have shown potential against MSU-induced arthritic rats. Thus, here we have used the same dose levels to understand the efficacy in toxicity model. This is the first study to be reported on Cyamopsis tetragonoloba seed as a hepatoprotective drug. Thus, the aim of our research is to identify the beneficial activity of Cyamopsis tetragonoloba aqueous seed extract.

MATERIALS AND METHODS

Chemical’s reagent and drugs

Paracetamol is obtained from pharmaceutical Labs, Pvt, Ltd, Mumbai, Maharashtra, India. SLY drug is obtained from Natural remedies private limited, Bangalore. Diagnostic kits like liver and renal marker enzymes were obtained from Diagnostics Ltd, Surat, Gujarat, India. Using a standard protocol, antioxidant assays were being performed for the kidney, liver, stomach and intestine.

Plant extraction

C. tetragonoloba seed (CTS) powder was purchased from Nature Vit. The powder 10 g was taken and dissolved in 100 ml of distilled water and incubated for 24 hrs at the shaker. After that, using the Whatman filter paper filter, Guar Gum powder dissolved is filtered and used for experimental rats.

Animal Experimental design

Female Wistar albino rats (280-300g) were obtained from the VIT animal house, Vellore Institute of Technology, Vellore, Tamil Nadu, India. Animals were properly acclimatized with the pathogen-free room, fed freely with commercially available standard pellets from Hindustan Lever Ltd, Mumbai, India. The animals were under maintained temperature and laboratory conditions for a 12 hr light / dark cycle per week. The ethical committee, VIT, Vellore, accepted the following experimental protocol, which was carried out according to Indian CPCSEA guidelines (VIT/IAEC/17/ Feb2020/11). Animals were grouped as follows;

Group 1: Normal control

Group 2: Paracetamol (900mg/kg b.w./day, i.p.)

Group 3: Paracetamol (900mg/kg b.w./day, i.p.) + CTS (500 mg/kg b.w./day, p.o.)

Group 4: Paracetamol (900 mg/kg b.w./day, i.p.) + CTS (1000 mg/kg b.w./day, p.o.)

Group 5: Paracetamol (900 mg/kg b.w./day, i.p.) + Silymarin (25 mg/kg b.w./day, p.o.)
Group 6: CTS (500 mg/kg b.w./day, p.o.)
Group 7: CTS (1000 mg/kg b.w./day, p.o.)
Rats were anesthetized after 5 to 6 hrs. and blood was collected to get the serum. After sample collection, blood was centrifuged at 2000 rpm for 10 mins that was used for biochemical analysis. The organs were stored in PBS and 10 percent formalin solution. The PBS solution's organs were homogenized for antioxidant testing and the formalin organs were used for histopathic testing.

Biochemical activities in rats
Albumin, Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), Alkaline Amino Transferase (ALP), total protein, total cholesterol level was taken from serum to obtain the hepatoprotective activity of CTS. The nephroprotective activity of CTS is done with renal enzyme markers such as urea, uric acid and creatinine.

Antioxidant activities in rats
The organs which were stored in phosphate buffered saline (PBS) solution is homogenized in 0.1M ice-cold PBS. After tissues get homogenized, antioxidant assays like Superoxide Dismutase (SOD), Catalase (CAT) and Reduced Glutathione (GSH) were performed.

Histopathological work
Organs such as kidney, liver, stomach and intestine obtained from rats were rinsed in PBS buffer and preserved in 10% formalin for histopathological analysis. The analysis is done with tissue staining using haematoxylin and eosin stains and the morphological in the tissue structure is seen under microscope view.

Statistical analysis
The statistical analysis of results was expressed using one-way ANOVA which was followed by Student Newman-keul’s test. The results are denoted as mean ± standard deviation and they were considered as statistically significant at the level of \( P< 0.05 \).

RESULTS

CTS on the liver enzyme markers of paracetamol-induced rats
Figure 1 shows CTS hepatoprotective activity on liver enzyme markers like ALT, AST and ALP. Paracetamol-induced rats have shown a significant elevation (\( P<0.05 \)) in the level of liver enzyme markers. Group 3 and group 4 rats have shown a decrease in the level of liver enzyme markers while comparing with group 2. SLY administrated group has shown a decrease in the level of liver enzyme markers as compared with the CTS administrated rats. CTS alone treated group shown a normal level of ALT, AST and ALP liver enzyme markers compared with the normal group.

Figure 2 shows the significant decrease in the level of albumin and total protein on paracetamol-induced rat's when compared with the normal group. Group 3 and group 4 treated rats has shown a significant increase in the level of liver enzyme marker on compared with group 2. Similar results were obtained in SLY administrated rats on compared with the normal group. On comparison with SLY and CTS administrated rat groups, CTS administrated rats has shown the better result.

Figure 3 and Figure 1 represents the activity on CTS hepatoprotective activity of total cholesterol in paracetamol-induced rats. It has shown that there is a significant increase (\( P<0.05 \)) in the level of total cholesterol on paracetamol-induced rats. Group 3 and group 4 paracetamols induced with CTS shown a decrease in the level of total cholesterol compared with group 2. SLY administrated group has shown a similar decrease in the level of total cholesterol as compared with the CTS administrated rats. CTS alone treated
group shows a decrease in the level of total cholesterol compared with group 2.

**CTS on renal enzyme markers with paracetamol-induced rats**

Figure 4 represents the nephroprotective activity of urea; creatinine and uric acid of CTS treated rats. There was a significant increase in the level of renal enzyme markers in the paracetamol-induced group compared with the normal group. Group 3 and group 4 have shown a decrease in the level of renal markers compared with group 2. Similar results were observed in the SLY treated group. The drug alone group has shown a normal range in the level of renal markers.

**CTS on antioxidant assay with paracetamol-induced rats**

Table 1-3 represent the protective effect of CTS on the liver, kidney, stomach and intestine antioxidants. The antioxidant level has significantly reduced (P<0.05) in the paracetamol-induced rats. CTS and SLY along with paracetamol were able to normalize the antioxidant level of the liver, kidney, stomach and intestine. CTS has shown its ability to normalize the liver, kidney, stomach, intestine antioxidant parameter level. Group 2 shows a decreased antioxidant level due to toxicity. The represented unit of SOD is Units /mg protein. The represented unit of CAT is Units /mg protein. The represented unit of GSH is nmol/mg protein.

**CTS on histopathological changes of paracetamol-induced rats**

Liver histopathology of the normal group has shown the normal liver morphology (Figure 5). The paracetamol-induced rats have shown inflammation in the liver tissues of the portal area. CTS treated rats have shown normal and mild feathery degradation in the liver tissue. SLY treated rats were observed to show distorted forming lymphoid aggregates. The drug alone administrated group has shown normal tissues of the liver.

Kidney histopathology of normal group possessed the normal tissue of the kidney (Figure 6). Paracetamol-induced group has shown the inflammation in the tubules and acute tumor necrosis (ATN) were also observed. CTS administrated with paracetamol-induced
Table 2: Protective effect of CTS on liver, kidney, stomach and intestine of antioxidant CAT.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
<th>Group 6</th>
<th>Group 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>53.58±0.15</td>
<td>28.91±0.38</td>
<td>52.04±0.15</td>
<td>57.40±0.17</td>
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<td>a<em>b</em>c*e</td>
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<td>Kidney</td>
<td>62.76±0.41</td>
<td>28.40±0.22</td>
<td>55.1±0.18</td>
<td>63.57±0.17</td>
<td>60.19±0.18</td>
<td>65.34±0.22</td>
<td>68.20±0.19</td>
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<td></td>
<td>a*</td>
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<td>a<em>b</em>c</td>
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<td>a<em>b</em>c*</td>
<td>a<em>b</em>c</td>
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<tr>
<td>Stomach</td>
<td>55.21±0.77</td>
<td>20.00±0.70</td>
<td>54.94±0.76</td>
<td>55.36±0.99</td>
<td>54.06±0.99</td>
<td>58.71±0.63</td>
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<td>b<em>c</em></td>
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<td>b<em>c</em></td>
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<tr>
<td>Intestine</td>
<td>56.72±0.82</td>
<td>20.83±1.29</td>
<td>55.36±0.65</td>
<td>58.57±1.01</td>
<td>55.79±1.66</td>
<td>57.88±1.09</td>
<td>58.38±0.941</td>
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</table>

Each value shows the mean ± SD of the above-mentioned groups. Comparison were made as follows: a) Group- 1 vs Group- 2, 3, 4, 5, 6, 7; b) Group- 2 vs Group- 3, 4, 5, 6, 7; c) Group- 3 vs Group- 4, 5, 6, 7; d) Group- 4 vs Group- 5, 6, 7; e) Group- 5 vs Group- 6, 7; f) Group- 6 vs Group- 7. The symbols represent statistical significance at *P < 0.05. Statistical analysis was calculated by one-way ANOVA followed by the Student Newman-Keul’s test.

Table 3: Protective effect of CTS on liver, kidney, stomach and intestine of antioxidant GSH.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
<th>Group 6</th>
<th>Group 7</th>
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</thead>
<tbody>
<tr>
<td>Liver</td>
<td>24.34±0.22</td>
<td>14.70±0.30</td>
<td>23.40±0.22</td>
<td>24.61±1.13</td>
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<td>19.86±0.13</td>
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<td>b*</td>
<td>b*</td>
<td>b*</td>
<td>a<em>b</em>e</td>
</tr>
<tr>
<td>Kidney</td>
<td>27.83±0.15</td>
<td>11.34±0.14</td>
<td>25.27±0.20</td>
<td>28.33±0.13</td>
<td>26.49±0.18</td>
<td>25.33±0.13</td>
<td>26.61±0.13</td>
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<td>a<em>b</em>c</td>
<td>a<em>b</em>c</td>
<td>a<em>b</em>c*</td>
<td>a<em>b</em>c</td>
</tr>
<tr>
<td>Stomach</td>
<td>25.67±0.84</td>
<td>10.70±0.82</td>
<td>21.62±1.07</td>
<td>24.61±1.13</td>
<td>23.76±1.01</td>
<td>23.82±1.01</td>
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<td>b*</td>
<td>b<em>c</em></td>
</tr>
<tr>
<td>Intestine</td>
<td>27.52±0.98</td>
<td>11.77±0.71</td>
<td>25.59±0.95</td>
<td>28.63±1.00</td>
<td>26.53±1.09</td>
<td>25.58±0.97</td>
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Figure 5: CTS effect on liver Histopathology.
Histopathology of liver using (H and E:400x staining) a) Group 1: normal liver morphology. b) Group 2: inflammation in the portal area. c) Group 3: normal liver morphology d) Group 4: mild feathery degradation in liver tissue. e) Group 5: distorted forming lymphoid tissues of the liver f) Group 6: normal liver morphology g) Group 7: normal morphology of the liver.

Figure 6: CTS effect on kidney Histopathology.

rats has shown mild ATN. Paracetamol with SLY administrated rats was observed to show moderate interstitium Inflammation changes of kidney tissues. The drug alone administrated group possesses normal tissue of the kidney.

Stomach histopathology of Group 1 possesses the normal mucosal layer of the stomach (Figure 7). Rats that are administrated with paracetamol possessed the inflammation in the mucosal layer of the stomach.

Paracetamol with CTS treated group shown normal mucosa layer of the stomach with gastric glands. Paracetamol with SLY treated group possesses the mild changes in the mucosal layer. The drug alone treated group has possessed the normal mucosal layer of the stomach of tissues as similar to the normal group.
toxicity. In the recent decades, 900 mg/kg b.w of paracetamol treatment for one day was standardized to cause various toxicity in pre-clinical research. Since the followed standardized protocol has single dose effectiveness within a day, the current study rivals that CTS has the ability to normalize paracetamol-induced toxicity, which has been examined by indicators of the liver and renal enzymes, antioxidants and improvements in histopathology. In the toxicity group caused by paracetamol, the liver enzyme markers such as ALP, AST and ALT have been increased due to hepatocellular dysfunction that leads to liver damage. Albumin is a plasma protein formed in hepatic cells which has a role to play in maintaining intravascular osmotic pressure. The albumin level in the toxicity community caused by paracetamol is reduced due to liver damage, which decreases the plasma protein in the serum. The alteration in serum total protein level is the sign for the development of various diseases. The total protein level in the toxicity community caused by paracetamol is decreased which shows liver damage. Total cholesterol is the prominent part of liver metabolism, bile metabolism and thyroid gland metabolism. In the toxicity group caused by paracetamol, the total cholesterol level is increased due to liver metabolism failure. Administrations of CTS have normalized the liver enzyme markers, albumin, total protein and total cholesterol this is evident that our drug has hepatoprotective property.

The markers of renal enzymes such as urea, creatinine and uric acid are used to determine kidney disorders and their metabolism. In our study, the level of urea, creatinine and uric acid get increased in the paracetamol-induced toxicity group which shows the presence of kidney disease. After ammonia production, it gets transported into the bloodstream and excreted through kidney. Creatinine is found in the muscle that aids in energy storage. When there is a need for energy from the creatinine muscles, it is transported with plasma throughout the blood and is purified in the kidney and excreted as urine. Uric acid plays a role in our circulatory system’s microbial degradation. It is synthesized by purine metabolism. Uricase is the enzyme that assists in the conversion of uric acid which is excreted through the kidney. CTS drug has shown the decrease in renal enzyme markers than the elevated level of paracetamol-induced toxicity groups and also proved that drug has the nephroprotective activity.

Antioxidants play a part in preventing cell damage from active compounds. The active free radicals are metabolized by antioxidant enzymes such as SOD, catalase and GST. Excessive reactive oxygen
species cause oxidative stress, which has contributed to cellular damage in the body.\textsuperscript{29,30} Likewise previous reports show bisphenol A toxicity in rats found to have a similar effect.\textsuperscript{32,33} Thereby present study shows CTS has restored the antioxidant levels which proven the potent free radicals scavenging activity. Naturally in the antioxidants are found in vegetables and fruits of our diet. Vitamin C and E are mostly the essential antioxidants on daily coverage.\textsuperscript{34} Due to oxidative stress, the amount of antioxidants in our sample decreases in the paracetamol-induced group compared to the normal control group.\textsuperscript{35} Our study shows that the CTS has the ability to manage the amount of antioxidants and it demonstrates defensive activity against oxidative stress triggered by community induced by paracetamol. The major organs are liver, kidney, brain, stomach, intestine and heart. Once affected by illness there is a structural change in the tissues of the organs. The structural changes such as a vascular tumour necrosis in kidney tissue, portal inflammation in liver tissue, mucosal inflammation in the stomach and ulceration in the intestine are seen in the paracetamol-induced toxicity groups. Histopathological research also found that CTS has the capacity to normalize changes in tissue in the liver, kidney, stomach and intestine. The CTS interpreted the toxicity caused by paracetamol as being close to that of the normal control group. Then the SLY treated group were also possessed similar architecture as compared with the normal group.

To the whole CTS has shown to have a better antioxidant property to neutralize the oxidative stress to protect liver, kidney and gastrointestinal tract with the minimum dose of 500 mg/kg b.w. Where at the same dosage level of \textit{Cyamopsis tetragonoloba} also shows better anti-inflammatory property in arthritic rats.\textsuperscript{19} Aziz Eftekhari \textit{et al.} reported that restoring antioxidant level from Thoridazine-induced hepatotoxicity results in protected mitochondrial polarization.\textsuperscript{36} At molecular level drug-induced oxidative stress in HepG2 cell-line found to be NF-\textit{xB}-insighted cell death. NF-\textit{xB} was increased due to ROS formation in the cell.\textsuperscript{37} Standard drug SLY has shown to have almost recovered the toxicity but the studies like Ali Mandegary \textit{et al.}\textsuperscript{38} has also proved that it has played major role in modulating influence of TNF-\textit{z} cytokine genetic polymorphism. Thus, it’s not advisable to take SLY. These details suggest the further importance of molecular study to be understand.

**CONCLUSION**

The current study has proven the potential effect of \textit{Cyamopsis tetragonoloba} against paracetamol-induced toxicity. The results from the liver enzyme markers, kidney enzyme markers, antioxidant assays and histopathological studies had demonstrated the hepato, renal, gastro protective activity of \textit{Cyamopsis tetragonoloba}. Where our drug at two different dosage level shows similar activity in normalizing the toxicity. Thus, it has been proven that \textit{Cyamopsis tetragonoloba} at dose level of 500 mg/kg b.w. has equal potential to 1000 mg/kg b.w. Moreover, molecular studies can be done further to know the bioactive compounds of \textit{Cyamopsis tetragonoloba} that involved in treating toxicity and mechanism action.

**ACKNOWLEDGEMENT**

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**CONFLICT OF INTEREST**

Authors declare that they do not have any conflicts of interests.

**ABBREVIATIONS**

**NSAIDs:** Non-steroidal anti-inflammatory drugs; **GI:** Gastrointestinal tract; **COX:** Cyclooxygenase; **NAPQI:** N-Acetyl-p-benzoquinone imine; **LDL:** Low-density lipoproteins; **SLY:** Silymarin; **CTS:** \textit{Cyamopsis tetragonoloba} Seed; **CPCSEA:** Committee for the Purpose of Control and Supervision of Experiments on Animals; **PBS:** Phosphate Buffered Saline; **ALT:** Alanine Amino Transferase; **AST:** Aspartate Amino Transferase; **ALP:** Alkaline Amino Transferase; **SOD:** Superoxide Dismutase; **CAT:** Catalase; **GSH:** Reduced Glutathione; **ANOVA:** Analysis of variance; **ATN:** Acute Tumor Necrosis.

**REFERENCES**

**PICTORIAL ABSTRACT**

-Cyamopsis tetragonoloba is the most commonly available plant in India which has weight loss properties as well as used to treat cancer and diabetes. This study is to estimate the potential effect of *C. tetragonoloba* against paracetamol-induced toxicity. Compared to the standard drugs, the study has shown better results. The study has proven that the plant has a potential effect on the toxicity-induced groups.

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**SUMMARY**

*Cyamopsis tetragonoloba* is the most commonly available plant in India which has weight loss properties as well as used to treat cancer and diabetes. This study is to estimate the potential effect of *C. tetragonoloba* against paracetamol-induced toxicity. Compared to the standard drugs, the study has shown better results. The study has proven that the plant has a potential effect on the toxicity-induced groups.

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Dr. Jerine Peter Simon: Completed his doctorate from the Department of Biomedical Sciences, VIT, Vellore, India. His area of research field was Applied toxicology and pharmacology that involves discovering new drugs for Liver damage, Kidney damage and Gastric damages focusing on biochemical, antioxidant, pathological and genetical alteration.

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