Summary & Conclusions
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➢ On the basis of results obtained we conclude that acrylamide cause disturbances in the oxidative status and the effect was pronounced with the high doses, indicated enhanced liver damage risk during exposure to acrylamide.

➢ Liver protein levels in rat were suppressed significantly due to the damage caused by the acrylamide. This was observed in all acrylamide treated groups. But this is reversed in both Rhinacanthus in combination with acrylamide treated, and also Rhinacanthus alone treated groups.

➢ Lipids levels were decreased in all acrylamide treated groups, this was due to damages caused by free radicals generated by acrylamide. Hence the byproducts of lipid peroxidation MDA levels significantly increased. But in all Rhinacanthus extract treated groups showed significantly reduced levels of MDA, this shows us the protective role of Rhinacanthus extract from free radicals.

➢ In rat liver GSH depletion leads to increased production of superoxide, hydroxyl radicals, and also H₂O₂. The intracellular GSH pool is important for limiting oxidative stress-induced hepatic injury and suppression of cell proliferation and cell differentiation.

➢ Because of reduction of glutathione and vitamin-C, the free radical scavenger’s role suppressed in acrylamide treated groups. But in groups treated with both acrylamide and Rhinacanthus methanolic extract, and in groups treated with only Rhinacanthus extract we can observe significant elevation in antioxidant levels than to only AC treated groups. The groups which are treated with only Rhinacanthus methanolic extract were found to have elevated levels of reduced glutathione and Vitamin-C when compared to untreated control (I group). This can convey us that Rhinacanthus could be a better choice for the preparation of medicine to treat many liver diseases.

➢ The preliminary phytochemical screening of the four extracts (ethanol, ethyl acetate, methanol and water) revealed the presence of alkaloids, polyphenols flavonoids, cardiac glycoside, steroids anthroquinones and triterpenes in leaf
extract of *Rhinacanthus nasutus*. The presence of constituents changed from one solvent to other. Among the all solvents methanol showed the highest secondary metabolites due to its high polar nature.

- *Rhinacanthus* leaf powder possess minerals like iron, calcium, sodium, copper, magnesium, molybdenum, boron, which essential for enzymes as cofactors. And also some minerals like calcium, magnesium, molybdenum, copper, iron are chelators of free radicals, there by decreases the oxidative stress.

- In DPPH and $\text{H}_2\text{O}_2$ radical scavenging properties of methanol showed highest activity compared to other solvents like ethanol, ethylacetate, water.

- Methanol shows the highest concentration of phenols and poly phenols, flavanoids than the aqueous extract which showed the lowest phenols, phenolics and flavanoids, probably this might be the reason for highest antioxidant property.

- In all acrylamide treated groups it was observed that all the antioxidant enzymes like catalase, superoxide dismutase, glutathione peroxidase, glutathione-S-transferase, glutathione reductase activities were reduced significantly in dose dependent manner due to the damage of liver proteins, and also due to the suppression in antioxidant levels (vitamin-C, GSH). But in all *Rhinacanthus* treated groups the activities of all these enzymes are regulated to the normal. This is due to the *Rhinacanthus* extract, one way by the possession of minerals which are essential cofactors of antioxidant enzymes activities. On the other way due to the possession of polyphenols, flavanoids and anthraquinones that could eliminate the free radicals.

- Liver function markers like AST, ALT, ALP activities were increased significantly in all acrylamide treated groups which is significance of liver damage, and liver cirrhosis. But in groups which were treated with *Rhinacanthus* leaf methanolic extract, the levels of all these enzymes were suppressed and regulated to the normal. This is due to hepato protective role of *Rhinacanthus* extract, by suppressing oxidative stress, minimized the liver damage.
In liver histology it was investigated that due to the oxidative stress caused by acrylamide, all the biomolecules proteins, lipids, and antioxidants were damaged and hence normal structural integrity of liver and its cells was changed. But *Rhinacanthus* treated groups liver attained normal structural integrity, recovery of liver tissue damage was due to the protection of proteins, lipids, DNA levels.

This study convey us that *Rhinacanthus* is the best choice for the preparation of medications to treat liver diseases. Exact active principle is to be isolated in future studies.