DISCUSSIONS
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LD50 STUDIES:

When LD50 with 10% Krishna Tulas suspension in distilled by per oral route of administration with the help of metal intragastric canula was attempted, no mortality was observed in any group with 0.2, 0.4, 0.8 and 1.6 ml volume of the suspension at the end of 24 hours. LD50 was conducted in fresh stock of female mice of the weight range of 28 to 32 g body weight. The animals were observed upto 24 hours.

In the same way intraperitoneal administration of 10% particle free Krishna Tulas solution in male albino mice of the weight range of 31 to 33 g body weight with the doses of 0.2, 0.4, 0.8 and 1.6 ml of the volume of Krishna Tulas suspension. After 24 hours the animals did not show any mortality. From these experiments it was concluded that Krishna Tulas 10% aqueous suspension or particle free solution by oral or intraperitoneal route was safe. LD50 values therefore, could not be calculated.

In the neuropharmacological tests as designed by Irwin, with the dose of 100 mg/Kg i.p. particle free solution of Krishna Tulas injected in the volume of 0.2, 0.4, 0.8 and 1.6 ml per mouse, the concentration being 500 mg/ml. The drug was administered with the
help of 30 gauge needle Krishna Tulas was found to produce alertness, increasing the activity, pointing out the action on Central Nervous System. The mood of the animals did not change. Reactivity (motor activity) and pain responses (motor activity), were improved considerably as compared to the control group.

CNS effects as a form of startle response was also improved as compared to the control group. It was quicker than the normal. Likewise, the Pinna and the corneal reflexes were improved and they were more quick as compared to the control, normal animals.

ANS parameters remained unaffected, when the dose of Krishna Tulas was doubled (200 mg/Kg/1 p.), by intra peritoneal dose. The observations made previously with half the dose, were reproduced with a only difference that they were slightly on the higher side of the scale and with these doses diuresis was noticed which was not seen with 100 mg/Kg i.p. dose. There was not appreciable effects on heart or the circulatory system and the salivation, pilo erection or the body temperature.

SLEEPING TIME:

A sleeping time with the help of Hexabarbital, is a useful tool to assess the ability of the liver to metabolize substances like Hexabarbital which are exclusively handled by the liver cells. Hexabarbital is a ultra short acting CNS depressant (Hypnotic) whose action sets in within few seconds and lasts for about 30 minutes. When the liver cells are damaged the ability to metabolize Hexabarbital decreases with the effect that the sleeping time was
increased up to 150 minutes. When Krishna Tulas is administered as a 10\(^\circ\) volatile particle free decoction then the sleeping time falls to around 100 minutes i.e. by approximately 33\(^\circ\) (50 minutes less). This has happened with both aqueous and alcoholic extracts of Krishna Tulas, 10 or 20\(^\circ\), by intraperitoneal route. Reduction in the duration of sleeping time if compared with untreated blank controls, still shows a considerable increase in the sleeping time (from 30 minutes to about 100 minutes or by 3.25 times approximately) denoting a heavy death of the hepatocytes due to drastic protoplasmic poisoning action of CCl\(_4\).

That the various extracts of the Krishna Tulas were able to reduce the sleeping times points out to the possibility of Krishna Tulas components helping the surviving hepatocytes to metabolize the Hexobarbital faster and bring about the reduction in the duration of sleeping time. It cannot be concluded at this stage how and where this happens. Krishna Tulas contains aromatic principles like camphor, linalool, eugenol which might as well combine with hexabarbital and makes its action ineffective.

It may be so that due to the diuretic action of Krishna Tulas, Hexabarbital gets excreted from the urine either as such or as metabolites which do not possess hypnotic action. It can be said that the extracts of Krishna Tulas has the ability to stimulate Hexabarbital metabolism and reduce the sleeping time.
Laboratory animals under the laboratory conditions have a steady weight gain. The graph, if plotted on day to day basis will appear as a steady increase and a smooth upward straight line. In the present study in control animals such a steady growth and a smooth upward steadily going line graph has been obtained. That distilled water per oral does not affect either weight gained or the percentage weight gained at the end of the 15 day period. Weight after two hour feeding and 22 hours fasting appears to be steady and there was not significant change in the weight gained at the end of the 15th day. Same thing was observed for the percentage weight gained then distilled water injected by intraperitoneal route. Weight before feeding remained almost the same. So also, the weight after feeding. There was not much either weight gained or the percentage weight gained, at the end of the 15 day period. All the four parameters like the weight before and after feeding, weight gained and percentage weight gained were unaffected (2 charts and 2 graphs).

When the animals were given the 10% aqueous decoction of Krishna Tulas, by oral route in the doses of 0.5 ml per mouse (approximately 15 ml / Kg / P.O), then there was still increase in weight gained both before and after food. These changes were found to be significant.

Calculation total weight gained shows that the weight gained starts from the 3rd day onwards, attains its maximum of the 5th or the 6th day, and remain at that level even after 15th day. These changes are significant. When these doses were doubled i.e. 1 ml per mouse per oral, or 30 ml / Kg / P.O then all the weights increased significantly. The total
weight gained and the percentage weight gained were conspicuous on the 2nd day itself and attains its maximum on 3rd and 4th day. In this group it was observed that weight gained or percentage weight gained dropped almost to half on 13th day onwards upto 15th day, which is not explicable. It could be because of gastric irritation or an anorexigenic effect.

When Krishna Tulas aqueous decoction was administered intraperitonea in a dose of 0.2 ml per mouse (approximately 6 ml/Kg/i.p) it was found that there was increase in weight both before and after the food as well as there was a total gain in weight and percentage weight gain. These changes were noticeable on their 2nd day. Peak effect was observed on 4th day. The effect did not appear to be regular. When the intraperitoneal dose was doubled both the total weight gained and the percentage weight gained were conspicuous from the 2nd day and remained conspicuous till the end of the trial i.e. up to the 15th day.

Krishna Tulas is praised in Ayurvedic texts as an appetizer (Deepan). It appears from the present study that increase in appetite vis-a-vis food consumption, and increase in weight is not dose dependent, and after increasing the dose to double there was no change in the increase in weight gained or the percentage weight gained. The appetizer action appears to be a central action on the feeding center and the satiety center. Krishna Tulas must be acting on these centers situated in the thalamic region of the brain. In other words the Krishna Tulas aqueous or the alcoholic extracts have the ability to cross the blood brain barrier and reach these centers. The effect on weight gained is also irrespective of the route of drug administration and it is almost equal. Since there were no signs of gastric irritation or inflammation, the point that the Krishna Tulas must be acting on these centers
is strengthened. The 10 and 20 percent aqueous and alcoholic extracts either by the oral
route or the intraperitoneal route show the same effect of weight gained and the
percentage weight gained. These effects are not dose dependent. But the periodic weight
gain in waves or weight gained to a certain level only point out to the fact that the satiety
and the feeding centers become unresponsive to the higher doses of the Krishna Tulas
extracts either aqueous or alcoholic.
When the Krishna Tulas leaves were extracted in 50 ° of ethanol ,and a 10 ° solution
was prepared and administered by oral route at a dose level of 0.5 ml per mouse
(approximately 15 ml / Kg / P O. Then the total weight gained and percentage weight
gained was more conspicuous. The weight gained became conspicuous on the next day
and a steady increase in weight till the end of the observation period of `5 days. In this
period it shows 3 peaks of maximum weight gained on 4 th, 10 and 15 th day . The difference
in between weight efore and after feeding remained the same for all the groups of aqueous
extract and the all the groups of alcoholic extract. This shows that even though when the
quantity of food given and eaten to animals remained the same the ability to convert it into
body parts improved considerably more so with alcoholic extracts.
When the experiment was repeated with 20 ° of alcoholic extract the results were the
same except their magnitude increased slightly.
Fresh liver weights were studied in comparison to the control and challenge i.e. CCl4. It
was found that Krishna Tulas both the extracts are able to check the weigh gained after
poisoning the liver with carbon tetrachloride. Carbon tetrachloride is a specific poison for
the liver and in the beginning, initially it will disrupt the intracellular elements, induce
inflammation, retain water, so that there will be accumulation of water (water logging) and
increase in the liver weight. In the present study in the control group the average liver weight was 7.93 g, the SD being 0.74. When the liver was poisoned the liver weight increases to 9.3 g, SD being 0.77. This increase in weight of 1.37 g is nothing but water logging and inflammatory fluids. This increase in weight amounts to 17.28 per cent. On the other hand when the various extracts of Krishna Tulas were administered by various routes, then the liver weight does not increase. On the other hand it falls slightly. This indicates the effective protection against known liver poisons like CCl4. When Krishna Tulas 10% extract was administered orally along with CCl4 it was found that the liver weight is reduced as compared to control group by 1.13 g or in other words by 14.24 per cent. When the concentration was increased to 20 per cent then the liver weight was 7.12 g, the difference in between the weights of control and 20% Krishna Tulas extract treatment being 0.81 and the percent reduction in weight being 10.22 percent. This observation was reproducible with Alcoholic extracts, both 10 and 20 per cent concentrations. With 10% alcoholic extract when given by intraperitoneal route then the liver weight was reduced by 1.01 g, the percent reduction being 12.6. Again, when the concentration was increased to 20% then the liver weight was found to be 7.21 %. i.e. the reduction in liver weight was found to be 0.72 or in other words there was a 9.07 percent reduction in weight. These findings suggest prevention of inflammatory processes and prevention of water logging in the liver parenchyma. One of the reasons for water logging in the liver substance is the retention of Na++ ions which are known to hold the water molecules along with them. Krishna Tulas extracts might be interfering with this process and also may be effectively either dispersing the water elsewhere in the body or the same is excreted through the agency of kidneys via urination. Krishna Tulas has a mild diuretic
action which was noted in the behavioral studies. In this process water more than normal might get expelled with the result that liver weight is found to be below normal. One cannot expect an exact return to normal of the liver weights and the tilted negative water balance might be restored later on.

When 20 per cent Krishna Tulas aqueous extract was administered per se to animals then the liver weight falls down to 0.56 g, the difference being 1.37 g and the 20% alcoholic extract was given then the difference was only 0.39 g. From these observations it could be deduced that the aqueous extract affects the water balance in the substance of the liver more than the alcoholic extract. It appears that the action of alcoholic extract is milder than the aqueous extract as far as the liver weights are concerned.
Normal SGPT levels in the mice blood were found to be around 53 international units. When the liver is poisoned with carbontetrachloride, then the SGPT values shot up to 274 international units. When 10% aqueous extract of Krishna Tulas is administered side by side with carbontetrachloride, then the rise in the SGPT levels is checked to 254 international units. Doubling the concentration of Krishna Tulas to 20% of the aqueous extract brings down these levels to 231 international units. It appears that the extraction of Krishna Tulas with the help of alcohol and administering it as 10 or 20% alcoholic extract does not have an added advantage because the rise of SGPT is checked at 264 and 234 international units respectively. If one considers that the reduction in SGPT values against a very strong hepatic poison like carbontetrachloride is very small, it is worth considering the same as the active principles of Krishna Tulas are active against it. Principles of Krishna Tulas may not be adequately effective against such a strong poison, but still they show that they are able to arrest the damage. Similar observations were made with SGOT. The normal SGOT levels are around 80 international units and with carbontetrachloride, it shoots up to 168 international units. When Krishna Tulas was administered along with carbontetrachloride, simultaneously, then the 10 and 20% aqueous extracts of Krishna Tulas brought the SGOT values down to 145 and 137 international units. Extraction of Krishna Tulas in alcohol and administering 10 and 20% solution simultaneously along with carbontetrachloride does not give any added advantage as the values are checked at 140 and 137 international units levels respectively. 66, 67
LDH levels normally in the blank control albino mice were found to be 1047 international units. After poisoning the liver with carbon tetrachloride it shot up to 1088 international units. Administering 10% aqueous or 10% alcoholic extract of Krishna Tulas brought down the levels to 1058 and 1059 international units respectively. 20% of the aqueous extract or 20% alcoholic extract brought these levels to 1054 and 1054 respectively. In fact there was hardly any difference in the values either with 10% or 20% or with the aqueous or alcoholic solvents. From all these experiments, one fact is clear that, in some way or the other, Krishna Tulas extract has either the ability to prevent leakage of these enzymes in general circulation. The enzymes can leak in the general circulation only damage or the death of hepatocytes. It may be so that the extracts of Krishna Tulas either prevent the damage or protect the hepatocytes against carbon tetrachloride and not allowing them to die.

In the present project it was aimed at to find out if either the aqueous or the simple alcoholic extract of Krishna Tulas has hepato-protective effect in albino mice. The present experimental set up shows that the Krishna Tulas extracts have the ability to protect against a hepatotoxin like carbon tetrachloride and improve the function of the surviving hepatocytes. Since carbon tetrachloride is a general protoplasmic poison, it is difficult to say with certainty at what organelle levels Krishna Tulas various extract offer the protection. It is possible that Krishna Tulas extracts might be toughening the cell walls of the hepatocytes at least some of the them. So that carbon tetrachloride is unable to damage it and to enter inside the hepatocytes.