CHAPTER III
FRACTIONATION AND EVALUATION OF
HYPOLIPIDEMIC PRINCIPLES OF RIPE C. PAPAYA
INTRODUCTION

Increasing tendency of having junk food which contain high quantity of cholesterol and triglycerides and frequent increasing trend of alcohol consumption is an issue of great concern of society. These habits may lead to obesity, hyperlipidemia, atherosclerosis and consequential heart diseases. Such food habits may also affect the primary organ liver. The initial alcoholic liver lesion develops to form fatty liver and Cirrhosis which cannot be prevented by nutritionally adequate diet or by feeding lipotropic factors\(^{(1-3)}\). Neither the clofibrate, antioxidant or anabolic steroid are found effective to prevent alcoholic fatty liver. However, onion and garlic have been shown to be one of the dietary method of intervention/prevention/curation of hyperlipidemia and consequential CVD/IHD and chronic alcoholic fatty liver \(^{(4,5)}\).

Chapter - I and II of the present work have revealed the efficacy of ripe papaya fruit pulp in reducing the risk factors of CVD/IHD. To date Hypolipidemic principle in fruit pulp is not isolated and partially fractionated. Therefore, in the present work presence of hypolipidemic principle in aqueous alcoholic and ketonic fractions have been investigated.
MATERIAL AND METHODS
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1. Selection of Animals: Adult male albino rats of wistar strain weighing 200-300 g were taken and aclimatised for 15 days under ambient conditions of animal house. During this period animals had free access to food (Gold Mohar rat feed pellet, Lipton India Ltd.) and water.

2. Groups of animals: Animals recieving above treatment were divided into five groups (each N=6) as Group -I, Group -II, Group -III, Group- IV and V Group.

3. Partial Fractionation of ripe papaya fruit pulp for Hypolipidemic principle: Aqueous, alcoholic and Ketonic fractions of ripe C. papaya fruit pulp were prepared as follows:

(i) Aqueous fractions: Saturated solution of fruit pulp was prepared by mixing and grinding 100g of pulp in water to give 100 ml in a waring blender for 2 to 3 minutes at top speed.

(ii) Alcoholic fractions: 100% w/v solution of fruit pulp was prepared in 95% alcohol (Ethanol) as above.

(iii) Ketonic fractions: 100% w/v Ketonic fractions were prepared in acetone as above.

Above suspensions were squeezed through two folds of muslin cloth and centrifuged to collect clear supernatant. Residue was discarded. The collected clear supernatant of alcoholic and ketonic fractions was evaporated in a incubator at 60°C to dryness. The dried residue was suspended in distilled water to a volume equivalent to aqueous fraction so as to give (1g = 1ml) and designated as alcohol and ketonic fractions of ripe papaya fruit pulp.
EXPERIMENTATION

Animals of all the five groups were fasted for 8 hours and following treatments were given.

**Group - I** : Control group animals received only 3 ml. normal saline intragastrically.

**Group - II** : Animals received 3 ml of 25% ethanol per 100gm body weight and 3 ml normal saline intragastrically.

**Group - III** : Animals were fed 3 ml of 25% ethanol per 100g body weight + 3 ml aqueous fraction intragastrically.

**Group - IV** : Animals recieved similar quantity of ethanol mixed with 3 ml alcoholic fractions as above.

**Group - V** : Rats were given ethanol as above mixed with 3 ml ketonic fractions.

Animals of all the groups were killed by decapitation on the fasting conditions 16 hours later and the blood and liver were collected for experiments. Triacylglycerol and total cholesterol were estimated in serum. Lipids in the liver were extracted and TAG, T-C were estimated from the extracts. Fresh liver tissue was homogenised in 5% TCA and MDA was estimated in it. The results are expressed in SI units in m moles/L or m moles/kg fresh tissue weight. Statistical analysis of the data was done applying student 't' test.
RESULTS AND OBSERVATIONS
TABLE - 1
Effects of feeding different fractions of ripe C. papaya fruit pulp on certain lipid parameters in serum and liver of albino rats

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<tr>
<td>1. Serum TAG</td>
<td>2.51 ± 0.11</td>
<td>3.82 ± 0.31</td>
<td>2.89 ± 0.12</td>
<td>2.98 ± 0.15</td>
<td>3.0 ± 0.14</td>
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<td>2. Serum T-C</td>
<td>2.20 ± 0.21</td>
<td>3.98 ± 0.23</td>
<td>2.97 ± 0.19</td>
<td>3.0 ± 0.18</td>
<td>3.0 ± 0.19</td>
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<td>3. Liver TAG</td>
<td>12.05 ±0.98</td>
<td>18.86 ± 1.20</td>
<td>14.20 ±1.25</td>
<td>14.90 ± 1.40</td>
<td>15.0 ± 1.50</td>
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<td>4. Liver T-C</td>
<td>18.00± 1.00</td>
<td>30.10 ± 1.90</td>
<td>19.90 ± 1.98</td>
<td>22.10 ± 2.0</td>
<td>23.14± 2.10</td>
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<tr>
<td>5. Liver MDA</td>
<td>3.60± 0.20</td>
<td>2.70 ± 0.18</td>
<td>2.0 ± 0.25</td>
<td>2.18 ± 0.21</td>
<td>2.20 ± 0.25</td>
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All results are expressed as mean ± SD m moles/L or m moles/kg fresh tissue weight.

* p<0.001 and ** p< 0.01 between groups -II and III, IV and V respectively and between group-I and II.
Effects of feeding different fractions of ripe C. Papaya fruit pulp on serum triacylglycerol levels in albino rats.
Effects of feeding different fractions of ripe C. Papaya fruit pulp on serum cholesterol levels in albino rats.

Fig. 2

(Serum Total Cholesterol in mole/L ± Mean ± SD)

Groups

- Group - I
- Group - II
- Group - III
- Group - IV
- Group - V
Effects of feeding different fractions of ripe C. Papaya fruit pulp on liver triacylglycerol in albino rats.
Effects of feeding different fractions of ripe C. Papaya fruit pulp on liver cholesterol in albino rats.
Effects of feeding different fractions of ripe C. Papaya fruit pulp on liver MDA levels in albino rats.
DISCUSSION
RESULTS AND DISCUSSION

The TAG and T-C levels in serum and liver were significantly raised in ethanol fed rats as compared to saline fed and ethanol mixed aqueous, alcoholic, and ketonic fractions of ripe C. papaya fruit pulp. Highly significant low levels of various parameters were observed with Group - III ethanol, aqueous fraction fed animals as compared to much significant low levels in group-IV and V rats. Liver MDA levels were also found to be low in rats after 16 hours feeding of ethanol fed Group - II and ethanol mixed aqueous, alcoholic and ketogenic fruit pulp fractions. The extent of lowering in MDA levels were more in aqueous fraction group-III. (TABLE - 1 Fig. 1-5).

Alcohol feeding have been shown to increase the biosynthesis and decrease the catabolism of fatty acids and cholesterol which may cause their accumulation in liver and consequently hyperlipidemia. Acetaldehyde, the principal metabolite of ethanol in liver is often implicated in development of fatty liver and hyperlipidemia probably forming stable adducts with the hepatic proteins, a probable cause for fatty liver and drainage of hepatic proteins.

Observation suggested the presence of hypolipidemic principle in all the three fractions. The most potent principle(s) seems to be present in the aqueous fractions as compared to alcoholic and ketogenic. The lipid lowering effects exhibited by these fractions strongly suggests the occurrence of both water soluble and lipid soluble hypolipidemic principles in more than one numbers which needs further investigation and which could not be done in the present study.

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Low MDA levels in the investigated fraction rule out their lipid lowering effects through increasing TAG and T-C catabolism. It suggests some other mode of action in lipid lowering which also needs to be investigated.

However, in the present study, alcohol induced/increased biosynthesis of TAG and T-C in group-II with low MDA level in it and highly significant low levels of these parameters in groups -III, IV and V suggested some possibility of reduced TAG and T-C biosynthesis in the presence of the partially fractionated hypolipidemic principles. Fractionation, isolation and characterisation of these hypolipidemic principles and their role in TAG & T-C biosynthetic pathway is further needed.

Ripe papaya fruit pulp have been shown to possess strong protein anabolic effect, helps in the availability of aminoacids for absorption through gut as well as strong hepatoprotective and tissue protective role in CCl₄ induced hepatotoxic albino rabbits. Probably, the maintenance of integrity of tissues and proteins anabolism by the fruit pulp helps to restore the normal TAG and T-C catabolism of liver thus lowering the liver and serum lipid levels. As well as the drainage of hepaticproteins by forming stable acetaldehyde protein adducts may be substantiated by the protein anabolic effects of the ripe C. papaya fruit pulp. The excessive excretion/secretion of bile acids in presence of fruit pulp through increased enterohepatoc circulation may also be a possible cause in the depletion of blood & liver cholesterol.
REFERENCES


