INHIBITION OF THIOACETAMIDE –INDUCED LIVER FIBROSIS BY PIPER NIGRUM LINN

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ABSTRACT

Piper nigrum Linn (Kalimirch) belonging to family Piperaceae is well known plant in ayrvedic and unani medicine. In the present study an attempt was made explore The anti fibrotic effect of ethonolic extract of this plant .Liver fibrosis was induced in the rats by thioacetamide orally 1ml/kg for 28 days. The extent of liver fibrosis was assessed by measuring the levels of liver hydroxyl proline (HP) Serum enzymes and total bilurubin (TBL) levels due to deposition of collagen .The liver weight of animals was increased following thioacetamide trated animals. And treated with Ethonolic extract of this plant reduced significantly the HP, Serum enzymes and SGOT, SGPTAnd TBL level and inhibited liver fibrosis induced by thioacetamide.

Key words: Piper nigrum fruit, Liver fibrosis, Hepatoprotective, Thioacetamide
INTRODUCTION:

Liver is the largest organ with wide range of functions including storage of nutrients, maintance of carbohydrates, homeostasis, secretory and excretory functions; one of the important functions of liver fibrosis is detoxification of xenobiotics \(^{(1)}\). Liver diseases have become one of the major cause of morbidity and mortality all over the globe. Conventional medicine does not provide many remedies for hepatitis, cirrhosis, fibrosis and liver damage. \(^{(2)}\) However, plant drugs have been traditionally used in country like India for treatment of such complicated diseases. One such plant is *Piper nigrum* linn.

Liver fibrosis is one of the most prevalent chronic diseases in the world. Liver fibrosis and nodular regeneration leads to cirrhosis. It is serious and generally irreversible diseases and is the tenth leading cause of death. With an age adjusted death rate of 9.2 per 1, 00,000 per year in USA alone.

*Piper nigrum* linn \(^{(3)}\) (piperaceae) known as black pepper is commonly available in the Indian market. Traditionally most of the plant parts are used in the treatment of various diseases. The plant is known to have anti periodic in malarial fever, gonorrhoea, anti chronic bronchitis, diuretics, antifibrotics and aromatic stimulant activities.

In this study, the effect of Ethonolic extract of *Piper nigrum* was evaluated against thioacetamide induced liver damage in albino rats by measuring the levels of Serum glutamate oxalo acetate transaminase (SGOT) Serum glutamate Pyruvate Phosphatase (SGPT) Hydroxyl Proline (HP) and Total Bilirubin level (TBL)

PLANT MATERIAL:

The plant material *Piper nigrum linn* (piperaceae) wasAuthenticated by Dr.C.Venkatramaiah, Reader in Botany, V.R.College, Nellore in Andrapradesh. The specimens were shade dried. *Piper nigrum linn* (Piperaceae) fruits were collected from the Nellore, in Andrapradesh.
PLANT EXTRACTION:

*Piper nigrum linn* (Piperaceae) fruits were finely powdered and extracted with Ethanol by using soxhlet apparatus for 48 hrs. The extracted was filtered and evaporated under reduced pressure\(^{(4)}\) To give a colour (yield 25\%) a Phyto chemical screening of powder revealed the presence of Flavonods, Phytosterols, Phenolic compounds, Proteins and Amino acids\(^{(5)}\).

EXPERIMENT ANIMALS:

Albino wistar rats of either sex, weighing 180 – 200g, were housed under standard environmental conditions of temperature, humidity and were provided with standard rat chow and water adlibitum\(^{(6)}\). The experimental procedures were carried out in strict compliance with the institutional animal Ethical Committee Regulations.

STUDY PROTOCOL:

A pilot study was conducted to study the dose response relation ship of the plant extract, A dose of 100mg/kg per orally was selected and used thorough the study.

INDUCTION OF LIVER FIBROSIS BY THIOACETAMIDE:

Thioacetamide was given to rats orally 1 kg twice a week for 28 days. Three days after the last dose, rats was sacrificed under ether anesthesia and blood and liver samples were collected.

TREATMENT PROTOCOL:

Animals were divided into 4 groups. Each group contains six animals
.CONTROL GROUP (G1)

The animals were given 2ml/kg gum tragacanth (1%w/v) orally daily for 28 days.

.EXTRACT CONTROL GROUP-(G2)

The animals were given orally 100mg/kg of ethonolic extract of dried unripe fruit of *Piper nigrum linn* daily (EXTRACT ALONE) suspended in 1%W/v gum Tragacanth for 28 days.

.FIBROTIC CONTROL GROUP-(G3)

The animals were given Thioacetamide twice a week for 28 days. (1ml /kg thioacetamide mixed with equal quantity of liquid paraffin) the reaming days they received saline.

.TREATMENT GROUP-(G4)

The animals were given thiacetamide twice a week for 28 days (1ml /kg Thioacetamide mixed with equal quantity of liquid paraffin) the animals also received 100mg/kg body weight of ethonolic extract dialy, suspended in 1%w/v of gum Tragacanth orally. After 28 days the animals were left free for 3 days and then the animals were sacrificed.

.METHOD:

Albino Wister rats were divided into IV groups. They were treated as the treatment protocol after the experimental period. The animals were made to fast overnight. They were anesthetized with chloroform and the blood samples were collected by carotid artery bleeding. Blood samples were kept for 30 minutes.
with out any disturbance in the test tubes. Then the supernatant layer was centrifuged for 10 minutes at 3000 rpm to separate the serum.

**HISTOPATHOLOGICAL STUDIES:**

The liver were excised quickly and fixed in 10% Formalin section fixed in Paraffin were prepared and stained with Haematoxylin and Eosin and then observed under microscope for degeneration fatty changes, Necrotic changes and evidence of fibrosis and evaluated by determine the levels of Serum glutamate Oxalo Acetate Transaminase (SGOT) Serum Glutamate Pyruvate Phosphatase (SGPT) (7) Hydroxyl Proline (HP) and total Bilirubin level (TBL) level and lipid peroxides by using Auto analyzer.

**STATISTICAL ANALYSIS:**

The results were expressed as mean statistical investigation was carried out with analysis of variance test (ANOVA) using DUNNETTS TEST. P<0.05 was regarded as significant.

**RESULTS AND DISCUSSION: Statistical Analysis**

The investigation of biochemical parameters proved that all the parameters SGOT, SGPT, ALP, HP, and TBL levels increased with thioacetamide. The increased parameters observed in group III were reduced markedly by treatment with 100mg extract per kg body weight of rat. The group IV showed that SGOT, SGPT were near to normal levels by 100mg extract per kg treatment. From the histopathological studies the sections of liver from treatment groups showed improvements while compared with liver damage of fibrotic control group.

Hydroxyproline content is good marker of fibrosis as the extract has reduced. The same, it is concluded that the drug antifibrotic activity.
CONCLUSION:

Hydroxyproline content is good marker of fibrosis as the extract has reduced. The same, it is concluded that the drug antifibrotic activity

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<table>
<thead>
<tr>
<th>GROUPS</th>
<th>AST (IU/I)</th>
<th>ALT (IU/I)</th>
<th>ALP (IU/I)</th>
<th>TBL (IU/I)</th>
<th>HP (ug/dl)</th>
<th>LIVER WEIGHT gms</th>
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<tbody>
<tr>
<td>CONTROL(G1)</td>
<td>45.08±1.225</td>
<td>41.58±0.780</td>
<td>214.36±1.326</td>
<td>0.69±0.040</td>
<td>53.72±0.834</td>
<td>3.438±0.05</td>
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<td>EXTRACT CONTROL(G2)</td>
<td>47.75±1.371</td>
<td>39.35±0.439</td>
<td>233.8±16.55</td>
<td>0.59±0.042</td>
<td>44.19±0.839</td>
<td>3.44±0.06</td>
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<tr>
<td>FIBROTIC CONTROL(G3)</td>
<td>70.53±3.335</td>
<td>67.73±2.213</td>
<td>404.75±24.16</td>
<td>1.35±0.102</td>
<td>135.91±0.710</td>
<td>4.36±0.1</td>
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<tr>
<td>TREATMENT GROUP(G4)</td>
<td>56.43±1.784</td>
<td>55.46±2.93</td>
<td>256.19±30.19</td>
<td>0.89±0.041</td>
<td>87.45±0.608</td>
<td>3.52±0.03</td>
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