Histological and Biochemical study on effect of dried fruit extract of *S. nigrum* on Hepatopathy induced by CCL4.


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Abstract

The results of the present study evaluate the efficiency of dried fruits extract on hepatic damage induced by CCL4. The histopathological changes were less obvious in livers related to rats in (G111) which treated with the extract and then with CCL4 comparative to histological exam of livers related to rats in (G11) treated with only CCL4 and control animals (G1).

In this study we assessed some of hepatic enzymes levels such as GOT, GPT, ALP and total bilirubin in blood serum. All enzymes showed high significant reduce (p<0.01) in animals treated with the extract compared to that in animals treated with CCL4 alone and with controls.

The results also described the histopathological changes including damage, necrosis of hepatic cells and central vein congestion in addition to bile ducts hyperplasia in rats livers in (G11) compared with animals in (G1) and (G111).

The rats treated with CCL4 resulted in enlargement of livers while the animals treated with extract showed livers normal with size similar to that in control group.

Introduction

The herbs play a vital role in the management of various liver disorders. In the absence of a reliable liver protective drugs in the modern medicine, a number of medical preparation in a yurvedh are recommened for the treatment of liver disorders (Chartterjee, 2000).

*Solanum nigrum* (Family: Solanaceae) is a shrub found through India, it is traditionally used for inflammation, diuretic and liver disorders (Nadkarni, 1995). It is also used in treatment as antihypertension, antispasmodic and anticancer factor specially with digestive and urinary system carcinoma (Friedman *et al.*, 2003).
S. nigrum contain many essential reactive compounds like glycosidal alkaloids and steroidal alkaloids (Lamb & Buck, 1989). The toxicity effects of S. nigrum related to Solanine and Solasodine alkaloids in addition to its content of nitrate and nitrogen with high rate more than 2.5% (Neilson, 1974). Mather et al. (1987) studied the treatment ability found in most plants extracts including S. nigrum and called Liv (25) on liver damage and blood sugar lowering which caused by beryllium nitrate. The effect of three types extracted from S. nigrum fruits and their ability on healing peptic ulcer induced by aspirin in laboratory rats were described by Jahnu & Devi (2006).

Freidman (2003) identify the effect of steroidal glycoalkaloids on laboratory mice infected with hepatomegaly.

Alcoholic extract of S. nigrum play an important role as antioxidant factor in experimental rats infected with carcinoma induced by N-diethyl nitrosamine through its action as inhibitor to tumour growth factor (Shahjahan et al., 2005).

Ethanolic extract from S. nigrum dried fruits have an action to reduce the cholesterol level in rabbits and this give an indicator to use these fruits in treatment of disease caused by increase fatty level in blood like arteriosclerosis (Odetola et al., 2004).

Materials and Methods

1. Plant material

The fruits of S. nigrum were collected, washed and cut into small pieces, dried and powdered according to (Raju et al., 2003). 20 gm from coarse powder was taken and mixed with 400 ml of 95% v/v ethanol, then subjected to continuous hot extraction in a Soxhlet for 24 hours. The ethanol was removed by the rotary evaporate system. These extract were suspended with gum acacia to study the effect of this material on liver damage induced by CCL4 in experimental rats.

2. Animals

Eighteen male albino rats of Rattus norvigicus weighting (120-130) gm were procured from animal house of Biology department – Science collage. The animals were housed in plastic cages maintained in controlled temperature (20-25) and light cycle (12 h light and 12 h dark). They were feed on commercial diet and waterad ad libitum during experiment period (Al.fartosi, 2004).

The selected animals were divided into three groups of (6 rats) on each and were treated as follows:-

G1: Animals were consider as control group inoculated (0.2ml) of 5% gum acacia daily for (7 days).

G11: Animals inoculated with (0.2 ml) of gum acacia only.

G 111: animals inoculated with ethanolic extract of S. nigrum dried fruits at dose (250
mg \text{kg} \) of body weight suspended with (0.2ml ) of 5\% gum acacia daily for (7 days ).

At seventh day carbon tetrachloride (1.25ml/kg ) peritonealy was administrated (30 min )after the last dose to all rats except rats in (G1) . After (36 h )all animals were sacrificed under chloroform anesthesia ,then the blood was collected in sterile centrifuge tube and allowed to clott . serum was separated by centrifuging and used for the estimation of serum enzymes like GOT,GPT by used the transaminase kit and ALP by used phosphatase Kit in addition to total bilirubin content with photometric test .

After the animals were scarified ,the abdomen was cut opened and the livers removed ,the morphological changes like size and colour was observed , all livers fixed with formalin (10\%) and were processed for paraffin embedding ,five micron section of livers ,stained with haematoxyline –eosin were examined with light microscopy to study the histopathological changes( Luna,1968 ).

Results

1-Biochemical estimations

The biochemical parameters results showed variation in all enzymes concentration levels (GOT ,GPT ,ALP ) and total bilirubin content in all rats treated with CCL4 compared with control rats (Table -1 ).

The enzymes levels didn’t show any significant differences (p<0.01) in (G1) rats before and after inoculation with gum acacia while high significant difference (p<0.01) in blood enzymes levels and total bilirubin observed in rats related to (G11) .

A significant (p<0.01) reduction was observed in GOT ,GPT ,ALP and total bilirubin content in the group treated with ethanolic extract of dried fruits of S .nigrum (G111) comparison to group (G1) and (G11) ( Table -1 ).

2—Effect of S .nigrum extraction and CCL4 on livers weight

All the livers related to animals in group (G11) which treated with gum acacia and then CCL4 appeared enlargement that were pale reddish brown in colour and showed high significant difference (p<0.01) in their weight compare to control animals .(Table 2) .

The group (G111) which treated with ethanol extract of S.nigrum showed livers size which was similar to control group and the livers with dark-red colour .However a significant (p<0.01 ) restoration in their weight found in this group treated with extract (Table -2 ).
Table (1) Effect of *S. nigrum* extract on some biochemical parameter

<table>
<thead>
<tr>
<th>Biochemical parameters</th>
<th>Test time</th>
<th>Group</th>
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<tbody>
<tr>
<td>Total bill (mg%) mean ±SD</td>
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<tr>
<td>0.606±0.076</td>
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<td>G1 (control treated with gum acacia)</td>
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<td>0.620±0.073</td>
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<tr>
<td>0.626±0.066</td>
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<td>G11 treated with gum acacia then CCL4</td>
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<tr>
<td>1.050 **±0.084</td>
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<td>After</td>
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<tr>
<td>0.6833±0.060</td>
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<td>G111 treated with dried fruits and gum acacia then CCL4</td>
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<tr>
<td>0.8333±0.033</td>
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<td>After</td>
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<tr>
<td>ALP (kau/ml) mean ±SD</td>
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<tr>
<td>11.967±0.128</td>
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<tr>
<td>12.467±0.334</td>
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<tr>
<td>11.967±0.102</td>
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<tr>
<td>20.817 **±0.542</td>
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<td>11.700±0.204</td>
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<td>16.817 *±0.912</td>
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<td>24.700±0.774</td>
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<td>25.217±0.651</td>
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<td>23.967±0.771</td>
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<td>36.688 **±2.337</td>
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<td>24.283±0.454</td>
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<td>28.890 *±0.364</td>
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<td>2.200±0.073</td>
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<td>Before</td>
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<td>2.501±0.201</td>
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<td>2.38±0.121</td>
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<td>5.150 **±0.150</td>
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<td>2.183±0.047</td>
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<td>3.825 *±0.395</td>
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**High significant difference on (P<0.01) in (R.L.S.D)test

*Low significant difference on (P<0.01) in (R.L.S.D) test

Table (2) Effect of dried fruits extracts on rats livers weight (gm)

<table>
<thead>
<tr>
<th>(G1)(control)treated with gum acacia only</th>
<th>G11 treated with gum acacia then with CCL4</th>
<th>G111 treated with dried fruits extract suspended with gum acacia and then with CCL4</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.966±0.064</td>
<td>6.450±*0.381</td>
<td>4.725±0.212</td>
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</table>

*High significant difference on (P<0.01) test

3-Histopathological study

*S. nigrum* dried fruits were resulted in this study.

The histopathological changes in experimental rats caused by CCL4 induced liver damage and effect of ethanol extract of Histopathological examination of the livers related to animals treated with gum acacia didn’t show any changes and the hepatocytes
appear normal size with central one or two nuclei, the cells arranged in hepatic cords concentrically around central vein and these cords separated by vascular spaces called the hepatic sinusoids (Fig. 1).

The livers of CCL4–treated rats revealed severe pathological changes, they showed central vein congestion, dense an inflammatory cells proliferation specially around the bile ducts and prenecrotic changes at some places distant from the central vein (Fig – 2), in addition hepatocytes hypertrophy with vacuolated cytoplasm and hemorrhage also observed (Fig – 3).

Light microscopic examination of sections from rats treated with extract suspended with gum acacia then CCL4 showed significant regeneration, minor inflammatory cells around central vein with kupffer cells through sinusoides (Fig -4).

Fig. (1) Liver of control rats showing the central vien (CV) Hepatocytes (——) and sinusoids (►) (500X H&E)
Fig (2) Section in liver of rats treated with gum acacia then with CCL4 showing congestion in central vein (CV), infiltration of an inflammatory cells (→) and bile ducts hyperplasia (●)(500x H & E)

Fig.(3) Section in liver of treated rats showing complete degeneration of hepatocyte (→) bile ducts hyperplasia (▲) and proliferation of inflammatory cells (●) 500x H&E

H & E 500x
Fig. (4) Photomicrograph of liver related to rats treated with the extracts and then CCL4 showing few inflammatory cells ( ), partial degeneration of hepatocytes ( ) and Kupfer cells ( ).

500X H&E

Fig. (5) Section in liver of rats treated with gum accacia and then CCL4 illustrate hemorrhage in central vein (CV ), hepatocytes hypertrophy with cytoplasm degeneration ( ) and infiltration of inflammatory cells (L ). (500X H&E)
Discussion
The present study focus on the liver damage induced by CCL\textsubscript{4} and the efficacy of \textit{S.nigrum} dried fruits extracts as hepatoprotective material.

1- Effect of dried fruits extract on biochemical parameters

The present study showed that some of liver enzymes like (GOT ,GPT ,ALP ) in addition to total bilirubin content were significantly higher (p<0.01) in (G11) animals compared with control (G1) ,while the enzymes concentration showed significantly decrease in (G111) animals.

The variation in these enzymes concentration and total bilirubin content in (G111) animals which treated with CCL\textsubscript{4} may be caused by the ability of this compound to induced hepatic cells degeneration ,damage of supplied blood vessels and then disturbed the metabolic activity of liver.

The study agreed with Anderson (1980) who identified the important of liver in synthesis and storage of glycogen ,lipids and some vitamins ,also the liver role in synthesis of plasma proteins and its main organ with detoxification activity for many drugs and plant extractions.

Our finding identified that the enzymes concentration was significantly decreased in G11 animals which treated with dried fruits extraction and this may be related to unknown cellular mechanical protection for hepatic cells and then restored normal structure compare with liver of animals treated with CCL\textsubscript{4} , This finding agreed with Raju \textit{et al}., (2003) who confirmed the role of \textit{S.nigrum} dried fruits extract on some liver enzymes levels.

Venkumar &bathes (2000) confirmed that ethanolic extract from \textit{Curculigo orchiods} rhizomes has an effect on reduced liver enzymes levels in animals infected with damage.

Our observation showed significant increase (p<0.01) in livers weight of animals in group G11 also these livers looks enlargement and pale colour comparative with livers in G1 and G111 animals which didn’t showed any significant difference.

The study in agreement with Raju \textit{et al}.,(2003) who related the main cause in hepatomegaly and colour change to CCL\textsubscript{4} while the animals treated with dried fruits extract restored to normal structure similar to that in normal rats. This suggest the maintenance of structural features of hepatocytes or regeneration of damage cells by the effect of extract.

On the light of histologic observation the CCL\textsubscript{4} was hepatotoxic caused liver damage and liver section of rats treated with it showed necrosis ,hyperplasia in bile ducts walls and dense infiltration of inflammatory cells while the rats treated with the extract and then CCL\textsubscript{4} showed evident of protection as formation of normal hepatic cords and
absence of necrosis and other histopathological changes.

These results agreed with Lindberg et al (1997) who indicated that the damage cells release some compound like prostoglandine (E1) which have chemotactic ability to an inflammatory cells.

Macsween & Whaley (1997) confirmed that the neutrophils cells migrate to damage tissues and secrete more chemicals mediators to attract another cells to the area in addition to release protein caused by cells degeneration Raju et al. (2003) established the toxic effect of CCL4 which induced experimentally liver damage due to free radical formation which caused lipid peroxidation of cellular membrane leading to hepatocytes necrosis and then dysfunction which improved by significant increase (p<0.01) in some enzymes level, also bile ducts hyperplasia which lead to variation in blood serum total biliruin content. The efficacy of dried fruits extract as hepatoprotective material against CCL4 induced liver damage was improved and further study recommended to identify the cellular mechanisms that may be involved.

References


دراسة نسجية وكيميائية حيوية حول تأثير مستخلص الثمار الجافة في نبات عنب الذيب

CCL4 على تلف الكبد المستحت بالS. solanum

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المستخلص

أكدت نتائج الدراسة الحالية الفعالية الإيجابية لمستخلص الثمار الجافة على تلف الكبد المستحت باستعمال رابع كلوريد الكربون (CCL4) على أكياس الجرذان من مجموعة G11 التي عُّمِلت بالمختصر ومن ثم بالمركب CCL4 مقارنة مع الفحص النسجى لأكياس الجرذان ضمن المجموعة G11 التي عُمِلت بالCCL4 فقط. ومع حيوانات السيطرة ضمن المجموعة G1

تم في هذه الدراسة قياس مستوى بعض الأنزيمات الكبد مثل ALP وGPT والGOT في مصل الدم. أظهرت جميع الأنزيمات انخفاضاً معنويًا عند مستوى احتمالية (P<0.01) في الحيوانات المعالمة بالمستخلص مقارنة مع تلك المعالمة بالCCL4 فقط وحيوانات السيطرة. أوضحت نتائج التغييرات النسجية التي تضمنت التلف النسيجي والذبخر والخلايا الكبدية واحتقان الأوردة المركزية فضلا عن فرط النسيج اللاففي الضفري في أكياس عنب الذيب من مجموعة G11 مقارنة مع الحيوانات في المجموعة G1 و(G11). أظهرت الجرذان التي عُمِلت بالمستخلص كانت أكياسها اعتدادية مع حجم طبيعي مشابه لأكياس الحيوانات ضمن مجموعة السيطرة.