Hepatoprotective Potential of Methanolic Extract of Gymnema sylvestre Leaves on Acetaminophen-Induced Liver Damage in Wistar Strain Albino Rats

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Abstract: Drug-induced liver injury is a potential complication of all medications because the liver has a central role in the metabolism of drugs and toxic substances. This study was designed to evaluate the hepatoprotective activity of the methanolic extract of Gymnema sylvestre leaves on acetaminophen-induced liver damage in wistar rats. The experiment was carried in three phases. In Phase I, the hepatoprotective activities of the methanolic extract of the leaves was determined by assaying for some liver function indices (AST, ALT & ALP) on acetaminophen-induced liver damage in rats. In phase II, the methanolic extract of the leaves was further fractionated with chloroform, ethylacetate and n-butanol to obtained chloroform, ethylacetate, n-butanol and residual extracts respectively; and the effect of these fractions on liver function (AST, ALT & ALP) was determined. Histopathological examinations were also carried out at each phase of the experiment. From the results, treatment with the methanolic extracts (200mg/kg & 400mg/kg) and the fractions (200mg/kg each) significantly (P<0.05) decreased serum levels of ALT and ALP compared with liver damaged wistar rats, while there was inconsistencies in AST levels after treatment. However, out of all the fractions, the residual fraction showed better hepatoprotective activity. Histopathological examination of the liver showed better architecture and well defined nuclei in rats treated with the methanolic extracts and it fractions while liver of untreated acetaminophen-induced rats was evident of cytolysis. GC-MS analysis of the residual fraction showed the presence of flavonoids, phenolics amongst others. In conclusion, this study has demonstrated the hepatoprotective activity of Gymnema sylvestre.

Keywords: Hepatoprotection; Acetaminophen; Liver; Histopathology; GC-MS; Gymnema sylvestre.

INTRODUCTION

The liver often plays a major role in the metabolism of drugs and toxic substances [1]. Hepatotoxicity refers to liver dysfunction or liver damage that is associated with an overload of drugs or xenobiotics [2]. Acetaminophen (Paracetamol, N-acetyl-p-aminophenol or APAP) is a widely used analgesic drug which is safe and effective when taken at therapeutic doses [3]. However, when administered in an acute or cumulative overdose, it can cause severe liver damage or even acute liver failure that can be fatal in humans and experimental animals [4]. Liver disease is now a global problem due to several factors including increased in alcohol consumption, malnutrition, anemia, infection and availability of hepatotoxic drugs like acetaminophen. Furthermore, conventional drugs used in the treatment of these diseases may themselves cause hepatic damage e.g. azathioprine causes cholestatic jaundice and elevation of serum transaminases by interferon and virazole [5].

Hepatoprotective drugs are having potency to prevent liver diseases [6]. Hena, Srivastava & Ghoshal [7] have shown that large number plants possess hepatoprotective activities. The hepatoprotective effects of herbal formulations are usually studied either in vivo or in vitro against drugs/compounds like alcohol CCl4, β-Galactosamine, Thioacetamide, Nimusalide, Isoniazid, Rifampicin and Acetaminophen at different dose and varying duration [4]. These drugs are regarded as toxic as they result in elevation of serum liver enzymes [5]. Hence, detoxification by antioxidants is important in restoring the cofactors and repair altered biomolecules [6]. In the absence of reliable hepatoprotective drugs in medical practices, herbs with antioxidant properties such as Silymarin, a flavonol lignan mixture extracted from the milk thistle (Silybum marianum) has been used in the management of various liver diseases like hepatitis and cirrhosis [7]. Other hepatoprotective plants include Orthosiphon stamineus, Foeniculum vulgare e.t.c [4].
Medicinal plants play a key role in human and animal health care and about 80% of the world’s population rely on traditional medicine, which is predominantly based on plant material [8]. Despite the popularity of these herbal medicines in general and for management of liver diseases in particular, most remain unacceptable treatment modalities for liver diseases due to privation of information on standardization, active ingredient(s)/principles(s) present, results of randomized controlled clinical trials (RCTs) and toxicological effects of the plants [4]. Hence, the management of liver diseases remains a challenge to modern medicine [9]. Thus, the need to develop effective plant-based hepatoprotective drugs. Gymnema sylvestre R.Br is a medicinal herb native to Central and Western, Tropical Africa [10]. The leaves of Gymnema sylvestre has been reported to possess antimicrobial [11], anti-inflammatory activity [12], anti-oxidant activity [13] and antibacterial potentials [14]. Beverly and Sudarsananam [10] also reported anti-diabetic, anti-sweetner and Antihypercholesterolemic activities from the leaves of Gymnema sylvestre. These plant leaves contains some varieties of chemical constituents such as terpenoids, steroids, coumarin, saponin, flavonoids, carbohydrate, glycosides [15] [14]. The leaves also contain resins, albumin, chlorophyll, carbohydrates, tarteric acid, formic acid, butyric acid and anthraquinone derivatives [16]. Thus, the properties of these leaves can be considered a gold mine for discovery as hepatoprotective agents. We therefore launched our study in a trial to investigate the hepatoprotective effect of the leaves of Gymnema sylvestre. on acacetaminophen-induced in wistar rats.

MATERIALS AND METHODS

Plant Material and Authentication

Fresh leaves of the Gymnema sylvestre were collected from Filin Shagari, Shira L.G.A. of Bauchi State Nigeria between September and October (rainy season), 2017. The Plant was identified and authenticated by a botanist at the department of Biological Sciences, Bayero University, Kano with a voucher specimen (BS.BUK 109950). The leaves of Gymnema sylvestre were then washed with water, cut into pieces and air-dried at room temperature. The dried leaves were pulverized into coarse powder with a grinding machine.

Drugs and Chemicals

The ALT, AST, ALP, SOD reagents kits were from Randox laboratories Limited, (Antrim, United Kingdom) and elabscience Spectrum Diagnostics (Cario, Egypt), Acetaminophen (Emzor Pharmaceuticals, Lagos, Nigeria), Silymirin (Micro Pharmaceuticals, India). Methanol, chloroform, ethylacetate, Butanol (JHD, China) and all other chemicals used in this research were of analytical grade.

Preparation of the leaf Extracts of Gymnema sylvestre

Extraction of the leaves was carried out as described by Warashina & Noro [16], with some modification. Briefly, leaves of Gymnema sylvestre were washed with water, cut into pieces and air-dried at room temperature. The dried leaves were pulverized into coarse powder in a laboratory pestle and mortal. The ground leaves (1kg) was extracted with 60% methanol (5litres) and the slurry allowed standing at room temperature for 24 hours with occasional stirring. The solvent containing extract was then decanted and filtered. This was repeated using the same solvent, twice with 50% methanol. The filtrates from each extraction were pooled and the solvent was evaporated under reduced pressure using a rotary evaporator at 40°C to obtain crude extract. This was kept in an airtight container until needed.

The methanolic extract of Gymnema sylvestre was fractionated as described by Abdul et al. [17]. Briefly, about 60g of the extract was dissolved in 100ml of methanol (90%). The prepared solution was then partitioned using solvents of increasing polarity: chloroform (100ml), ethyl acetate (100ml) and n-butanol (100ml) to obtain chloroform, ethyl acetate and n-butanol fractions. All the fractions including the residual fraction were evaporated to dryness using a rotary evaporator at 39°C. All fractions were labelled appropriately and kept in air tight containers until further analysis.

Experimental Animals

A total of sixty (60) albino wistar rats of either sex weighing between 110 and 165g were used in the study. They were obtained from National Veterinary Research Institute (NVRI), Vom, Jos, Plateau State of Nigeria. The rats were kept at the animal house at the department of Biological Science, Bayero University Kano, Nigeria. They were fed with commercial grower feeds (Vital Feed Finisher) and given water ad libitum. All the animals were acclimatized to the laboratory conditions for two weeks before commencement of the experiment. The Experiments animals have been examined and approved by the appropriate ethics committee. "Principles of laboratory animal care” (NIH publication No. 85-23, revised 1985) will be followed during the experiment.

Acute Toxicity Studies

As reported by Potawale et al. [15] and Damini et al. [10], no human toxicity of this plant have been reported. A 52-weeks study of oral-repeated-dose toxicity for the extraction powder of Gymnema sylvestre in wistar rats was performed by Damini et al. [16] showed no toxic effect. In an acute toxicity study of the Gymnema sylvestre in mice carried out by Potawale et al. [15], showed no gross behavioral, or neurologic effects. The acute LD<sub>50</sub> was 3990 mg/kg. The safety ratio (LD<sub>50</sub>/ED<sub>50</sub>) was 11 and 16 in normal
and diabetic rats respectively. Therefore, doses of drugs and extracts given to the animals in this experiment were done following the OECD’s (Organization of Economic Corporation and Development’s) guidelines [18].

**Experimental Design**

The hepatoprotective effect of the leaves of *Gymnema sylvestre* was carried out in three phases. In Phase I, the effect of the methanolic leaf extract of *Gymnema sylvestreon* liver function was done as described by Adebiyi and Abatan [4].

Twenty five (25) apparently healthy wistar rats were divided into five (5) groups of five (5) rats each. Animals in II to V were pretreated with distilled water, 100mg/kg of the standard drug (Silymarin) and the methanolic extract at 200 and 400 mg/kg, respectively for 7 days. On the 8th day, liver damage was induced by a single oral dose of 800 mg/kg acetaminophen. After 24 hours of induction, the animals were humanely sacrificed and their blood collected in a plain containers. The blood samples were allowed to coagulate and serum obtained by centrifuging at 3000rpm for 20 min and was used for liver function analysis (ALT, AST & ALP). Histopathological examination of liver tissues of the experimental animals was also determined. Following the hepatoprotective results of phase I, the methanolic extract was then partitioned using three (3) solvents to obtain chloroform, ethlyacetate and n-butanol and the residual fractions. Hepaprotective effect of these fractions was carried using the method [4].

Thirty five (35) rats were then divided into seven groups of five (5) rats each:

Group-I: (normal control) received neither the plant extract nor acetaminophen for 8 days.

Group-II: (negative control group): The animals received distilled water for 7 days.

Group-III: (positive control group) – pre-treatment with silymarin (100mg/kg) for 7 days (orally).

Group-IV: Pre-treatment with 200mg/kg chloroform extract, orally for 7 days.

Group-V: Pre-treatment with 200mg/kg ethylacetate extract for 7 days, orally.

Group-VI: Pre-treatment with 200mg/kg n-butanol extract for 7 days (orally).

Group-VII: Pre-treatment with 200mg/kg residual extract, orally for 7 days.

Liver damage was also induced by a single oral dose of acetaminophen (800 mg/kg) in group II to VII. The serum obtained from all the groups, twenty four (24) hours after induction of liver injury was used for liver function analysis(ALT, AST & ALP) and histopathological examination of liver tissues of the experimental animals were also determined. Following the results in the second phase, the compounds in the fraction with the best hepatoprotective was identified using GC-MS.

**Serum Biochemical Analyses**

Liver function in both phases test was determined according to the manual described by RANDOX Laboratory reagent kits (RANDOX® Laboratories Ltd., Ardmore, United Kingdom). Serum biochemical parameters analyzed were: Aspartate aminotransferase (AST) [19], alanine aminotransferase (ALT) [19] and alkaline phosphatase (ALP) [20].

**Histopathological Examination**

Histopathological examination of the liver was carried out in animals within both phases of the experimental. A 2g portion of the liver tissues were collected and placed in 10% formaldehyde solution for histopathological study. The pieces of liver were then processed and embedded in paraffin wax and sections were made about 4-6 μm in thickness. After staining with haematoxylin and eosin (H&E), slides were then examined under microscope (Olympus, Japan) for histopatological changes.

**Gas chromatography mass spectrometry Analysis (GC-MS)**

Agilent – 7890B GC-MS (company name) was used to identify of the most active compounds in the fractions. The GC-MS was equipped with a split injector and an ion – trap mass spectrometer detector together with a fused – capillary column (Agilent Hp.5ms ultra inert) having a thickness of 3μm, 250μm, x 0.25μm and temperature limits of 60°C to 325°C. The column temperature was programmed between 60°C and 250°C and flow rate at a rate of 3.0ml/min, pressure; 4.4867psi. The temperature of the injector and detector were at 250°C and 200°C respectively. The split ratio was 20:1 Split flow at 14ml/min. Helium gas was used as a carrier gas, methanol was used as the solvents used to dissolve the sample.

Real-time analysis and post-run analysis were evaluated using MSD (Masshunter) matching of the unknown spectra with spectra’s of known compounds from the library of Spectra from the National Institute of Standards (NIST14.L), Washington, USA. The fragmentation patterns of the identified compounds were examined for consistency with known data from literature. The name, molecular weight, mass to charge ratio, retention time and relative percentage composition of the test materials were determined.

STATISTICS ANALYSIS

The results were expressed as Mean ± SEM and analyzed using SPSS 22. The significance among groups were determined by one-way analysis of variance (ANOVA) and LSD Post Hoc test was applied for multiple comparisons. Significance was reported at P<0.05.

RESULTS

The effect of methanolic extract of Gymnema sylvestre leaves on liver function of acetaminophen-induced hepatotoxic wistar strain albino rats is presented on table 4.5. From the results, there was significance (p<0.05) difference in ALT levels of acetaminophen-induced wistar strain rats (39.99± 4.20 U/L) compared to the normal control rats (32.02 ± 1.11). However, ALT levels also decreased significantly (P<0.05) in rats given the extracts at the higher dose of 400mg/kg (22.60 ± 2.92 U/L) compared to the acetaminophen-induced wistar rats (41.00 ± 9.20 U/L).

For AST, there was significant decrease (P<0.05) in the levels of the rats treated with 400mg/kg (25.16 ± 7.49 U/L) when compared with the normal control (50.49 ± 11.26 U/L).

The ALP values showed a significant (p<0.05) decrease in rats treated with the standard drug (22.13 ± 2.25 U/L) and those treated with 400mg/kg (56.20 ± 21.65 U/L) extract compared with the acetaminophen-induced wistar rats (118.93 ± 17.64 U/L). Also, there was significance decrease in the standard control levels (24.03 ± 2.15 U/L) and normal control (113.18 ± 27.15 U/L).

The effect of the fractions of the methanolic extract of Gymnema sylvestre leaves is presented on Table 2. From the results, there was a significance (P<0.05) decrease in ALT levels of rats given 200mg/kg residual fraction (29.53 ± 9.93 U/L) compared with the normal control (58.49 ± 13.06 U/L).

Results of ALP showed a significant (P<0.05) decrease in serum levels of rats given 100mg/kg of standard drug (24.03 ± 2.159 U/L), 200mg/kg of chloroform fraction (39.54 ± 22.68 U/L), 200 mg/kg of ethylacetate fraction (61.63 ± 20.81 U/L), 200 mg/kg of n-butanol fraction (38.37 ± 4.66 U/L) and 200 mg/kg of residual fraction (31.01 ± 8.45 U/L) compared with the negative control (120.93 ± 15.64 U/L) and the normal control (113.18 ± 27.15 U/L).

Histopathological examination of the liver tissues of rats administered with methanolic extract of Gymnema sylvestre are presented on Figure 1. Liver sections of normal rats (Plate I) showed remarkable liver tissues, normal hepatic cells with intact cytoplasm and nucleus, while that of the untreated group showed severe cytolysis and the loss of cellular boundaries (Plate II). Rats treated with 200 mg/kg of the extract (Plate IV), showed proliferative hepatocytes with mild degenerative changes and increased inter cellular space, while those given 400 mg/kg (Plate V) of the extract and the standard drug, silymarin (Plate III) showed normal architecture and well defined nuclei when compared with the normal control group. Consequently, histopathological features of liver tissues of rats administered with the different fractions of methanolic extracts of Gymnema sylvestre are presented in Figure 2. Centrilobular hepatic necrosis, cell degeneration and infiltrating lymphocytes were well displayed in acetaminophen induced rats (Plate II). The sections of livers of rats treated with standard drug, silymarin (Plate III) and those treated Chloroform (Plate V), n-butanol (Plate V) and residual fraction (Plate about VII) were similar, showing normal architectures. Ethylacetate fraction showed mild cytolysis.

From the result of phase II, residual fraction of the methanolic leaf extract is the most active hepatoprotective agent and the identification of it compounds were determined by GC-MS as presented in Table 3.

Table-I: Effect of Methanolic extracts of Gymnema sylvestre on some liver function test parameters in acetaminophen-induced albino wistar rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALT(U/L)</th>
<th>AST(U/L)</th>
<th>ALP(U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>32.02 ± 1.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.49 ± 11.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>113.18 ± 27.15&lt;sup&gt;2,n&lt;/sup&gt;</td>
</tr>
<tr>
<td>II</td>
<td>39.99± 4.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>49.50 ± 13.28</td>
<td>118.93 ± 17.64&lt;sup&gt;c,d&lt;/sup&gt;</td>
</tr>
<tr>
<td>III</td>
<td>35.20 ± 1.57</td>
<td>44.82 ± 1.79</td>
<td>22.13 ± 2.25&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>IV</td>
<td>25.06 ± 5.82</td>
<td>36.08 ± 9.19</td>
<td>102.71 ± 15.31&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>V</td>
<td>22.60 ± 2.92&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.16 ± 7.49&lt;sup&gt;n&lt;/sup&gt;</td>
<td>56.20 ± 21.65&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n= 5 rats/ group).

Values with the same superscripts within the same column are significantly different at P < 0.05.

I: Normal control, II: Negative control (800 mg/mg of PCM), III: Standard control (100 mg/kg of silymarin), IV: 200 mg/kg methanolic extract, V: 400 mg/kg methanolic extract

Available Online: [http://scholarsmepub.com/sjbr/](http://scholarsmepub.com/sjbr/)
Table-2: Effect of methanolic fractions of Gymnema sylvestre on some liver function test parameters in acetaminophen-induced albino wistar rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALT(U/L)</th>
<th>AST(U/L)</th>
<th>ALP(U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>35.02 ± 1.17\textsuperscript{a}</td>
<td>58.49 ± 13.06\textsuperscript{a}</td>
<td>113.18 ± 27.15\textsuperscript{a,b,c,d}</td>
</tr>
<tr>
<td>II</td>
<td>41.23 ± 9.86\textsuperscript{a,b}</td>
<td>45.50 ± 13.28</td>
<td>120.93 ± 15.64\textsuperscript{a,b,c}</td>
</tr>
<tr>
<td>III</td>
<td>34.20 ± 0.57</td>
<td>44.82 ± 1.79</td>
<td>24.03 ± 2.15\textsuperscript{a}</td>
</tr>
<tr>
<td>IV</td>
<td>44.20 ± 18.78</td>
<td>50.00 ± 7.71</td>
<td>39.54 ± 22.68\textsuperscript{a,b}</td>
</tr>
<tr>
<td>V</td>
<td>35.33 ± 5.33</td>
<td>35.85 ± 4.84</td>
<td>61.63 ± 20.81\textsuperscript{f}</td>
</tr>
<tr>
<td>VI</td>
<td>28.13 ± 3.90</td>
<td>29.53 ± 9.93\textsuperscript{a}</td>
<td>38.37 ± 4.66\textsuperscript{a}</td>
</tr>
<tr>
<td>VII</td>
<td>27.42 ± 3.13\textsuperscript{a}</td>
<td>42.17 ± 6.03</td>
<td>31.01 ± 8.43\textsuperscript{a}</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n= 5 rats/group).
Values with same superscript within the same column are significantly different at p<0.05

I: Normal control, II: Negative control (800kg/mg of PCM), III Standard control (100mg/kg of silymarin); IV: 200mg/kg chloroform extract V: 200mg/kg ethylacetate extract VI: 200 mg/kg n-butanol extract VIII: 200 mg/kg residual extract

Fig-1: Histopathological features of liver tissues of acetaminophen-induced rats administered with methanolic extracts of Gymnema sylvestre leaves

I: Normal control (H&E x 100), II: Negative control; 800kg/mg of PCM (H&E x 400), III: Standard control; 100mg/kg of Silymarin (H&E x 100). IV: 200mg/kg MEOH extract (H&E x 400), V: 400mg/kg MEOH extract. (H&E, mag x 100). A: Site of cytolysis

Fig-2: Histopathological features of liver tissues of acetaminophen-induced rats administered with methanolic fractions of Gymnema sylvestre leaves

I: Normal control (H&E x 100), II: Negative control; 800kg/mg of PCM (H&E x 400), III: Standard control; 100mg/kg of Silymarin (H&E x 100), IV: 200mg/kg chloroform fraction (H&E x 400), V: 200mg/kg ethylacetate fraction; (H&E x 100), VI: 200mg/kg n-butanol fraction; (H&E x 100), VII: 200mg/kg residual fraction; (H&E, mag x 100). A: Site of cytolysis
DISCUSSION

Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase are the most common biochemical markers used to evaluate liver injury [21]. Elevation of these liver enzymes is associated with cell necrosis of many tissues especially the liver [22]. This was confirmed in the present study, as the ALT and ALP enzymes levels were elevated in untreated acetaminophen induced rats. The increase in ALP and ALT concentration following acetaminophen administration is in line with existing literature that synthesis of these enzymes is increased by cells lining bile canaliculi usually in response to cholestasis and increased biliary pressure [22]. The decrease in activities of these enzymes in rats by pre-treated with the methanolic extracts and its fractions indicates that may offer some protection and maintain the functional integrity of hepatic cells. This protective effect has been attributed to its ability to stabilize plasma membrane thereby preserving the structural integrity of cell as well as the repair of hepatic tissue damage caused by acetaminophen [3]. Following the fractionation of the methanolic extract of Gymnema sylvestre to determine which of the fraction offers the highest hepatoprotection. The result showed that the residual fraction significantly (p<0.05) reduced ALT (27.42 ± 12.00 U/L) and ALP (31.01 ± 8.45 U/L) levels than other fractions when compared with negative control rats. The inconsistences in the values of AST when compared with negative control rats were compared with treated rats may be due to the fact the enzyme is not specific to the liver. It is found in other organs like heart, muscle, brain and kidney. Although, AST is useful in detecting liver injury it is considered a less specific biomarker for hepatocellular injury as it can also signify abnormalities in heart, muscle, brain or kidney [23].

The hepatoprotective nature of the methanolic extracts and its fractions of Gymnema sylvestre was also seen during cross examination of the histopathological features of the liver sections among the different groups of the rats. Normal control group showed remarkable liver tissues with normal hepatic cells, cytoplasm and nucleus. The negative control rats showed severe cytolysis with degrees of fatty degeneration and the loss of cellular boundaries as the nucleus were seen in blue stains exposed to the cytoplasm following disruption of the nuclear membrane. Group of rats treated with 200 mg/kg of the methanolic extract showed proliferative hepatocytes with mild degenerative changes and increased inter cellular space. But as the dose increases (400 mg/kg), the liver showed normal architecture and well defined nuclei compared with the normal control group and that of the standard drug, which showed normal architecture with well-defined nucleus as well. For the fractions, the groups of rats treated with ethylacetate, n-butanol and residual fractions showed remarkable liver tissues with define nuclei as compared with the normal control rats. Thus, histopathological changes of these groups pre-treated with these extract fractions showed significant hepatoprotection. These finding suggests that there was an inhibition of elevated hepatic damage as supported by Olamide and Matthew [3]. The hepatoprotection by these extracts and fractions may be due to antioxidant property of the phytochemicals present in them. Also, other compounds with anti-inflammatory and analgesic properties in the extract and fractions may played a role in the hepatoprotection [24]. Although, necrotic changes were still evident in the liver of rat pre-treated with the chloroform extract, the severity of the damage was less intense significant. The possible mechanism responsible for the protection of the liver by the methanolic extract and the fractions of Gymnema sylvestre leaves following acetaminophen overdose may be as a result of the extract and the fractions acting as scavenger by intercepting the radicals generated during the overdose of acetaminophen. The phytochemicals like flavonoids, phenols and fatty acids present in these extracts offers the hepatoprotection against the hepatotoxins [3].

Results from the GC-MS analysis of residual fraction from the methanolic extract of the Gymnema

### Table 3: Phytoconstituents having Hepatoprotective activity in Residual Fraction of Methanolic Extract of Gymnema sylvestre Leaves Identified by GC-MS

<table>
<thead>
<tr>
<th>No</th>
<th>RT</th>
<th>Name of Compound</th>
<th>MF</th>
<th>MW (g/mol)</th>
<th>Area %</th>
<th>m/z</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.196</td>
<td>3-Hexanol, 2-methyl-</td>
<td>C₆H₁₄O</td>
<td>55.0</td>
<td>1.59</td>
<td>55</td>
</tr>
<tr>
<td>2</td>
<td>6.718</td>
<td>1,2-Cyclopentanediene</td>
<td>C₃H₄O₂</td>
<td>98.101</td>
<td>1.74</td>
<td>98</td>
</tr>
<tr>
<td>3</td>
<td>14.087</td>
<td>Benzoic acid</td>
<td>C₇H₆O₂</td>
<td>122.0</td>
<td>6.68</td>
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<tr>
<td>4</td>
<td>18.610</td>
<td>2-Methoxy-4-vinylphenol</td>
<td>C₇H₁₀O₂</td>
<td>151.177</td>
<td>2.83</td>
<td>150</td>
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<tr>
<td>5</td>
<td>21.657</td>
<td>trans-Cinnamic acid</td>
<td>C₆H₈O</td>
<td>147.1</td>
<td>9.29</td>
<td>147</td>
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<tr>
<td>6</td>
<td>22.391</td>
<td>1,2,3,4-Cyclohexanetetrol</td>
<td>C₃H₁₂O₂</td>
<td>86.0</td>
<td>5.02</td>
<td>86</td>
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<td>7</td>
<td>30.841</td>
<td>Myo-Inositol,4-C-methyl-</td>
<td>C₆H₁₀O₂</td>
<td>73.0</td>
<td>11.38</td>
<td>73</td>
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<tr>
<td>8</td>
<td>43.075</td>
<td>Quercetin 3'-methyl ether</td>
<td>C₁₆H₁₂O₇</td>
<td>316.265</td>
<td>22.16</td>
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<td>9</td>
<td>46.608</td>
<td>Oleylalcohol, trifluoroacetate</td>
<td>C₃₀H₆F₅O₂</td>
<td>57.100</td>
<td>7.51</td>
<td>55</td>
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<td>10</td>
<td>46.642</td>
<td>1-Octadecene</td>
<td>C₁₉H₃₆</td>
<td>252.486</td>
<td>1.01</td>
<td>57</td>
</tr>
<tr>
<td>11</td>
<td>48.644</td>
<td>alpha-Tocopherol</td>
<td>C₃₀H₄₂O₂</td>
<td>430.71</td>
<td>9.60</td>
<td>423</td>
</tr>
</tbody>
</table>

RT: Retention Time, MF: Molecular formula, MW: Molecular weight, m/z: mass to charge.

**Gymnema sylvestre** leaves from the library of National Institute of Standards (NIST14.L) (Table 5) showed about 11 compounds possessing hepatoprotective potentials owing to it antioxidant properties. These include; 3-Hexanol, 2-methyl-, 1-Octadecene, 1,2,3,4-Cyclohexanetetrol [25], Benzoic acid, 2-Methoxy-4-vinylphenol, Oleyl alcohol, trifluoroacetate, [26], 1, 2-Cyclopentanediene [27], trans-Cinnamic acid, Quercetin 3'-methyl ether, alpha-tocopherol [28], Myo-Inositol, 4-C-methyl- (Lamininol) [26] [29].

It is possible that these compounds act synergistically to protect the liver from damage attempted liver damage [30], by either scavenging these free radicals such as superoxide anion radical (O2·−), hydroperoxy radical (HO2·), hydrogen peroxide (H2O2), hydroxyl radical (OH·), Lipid peroxide radical (ROO·) c.t.c or inhibiting the production of these radicals. Most of these compounds do this by donating their electrons, and their ability to donate their electrons lies in their structural orientations and their functional groups. Compounds like 1, 2,3,4-Cyclohexanetetrol, Myo-Inositol, 4-C-methyl-, alpha-Tocopherol, Quercetin 3'-methyl ether, 2-Methoxy-4-vinylphenol have hydroxyl groups (−OH) as part of their functional groups and helps in quenching the free radicals by donating it electron as hydrogen atom. Compounds like 1, 2-Cyclopentanediene have carbonyl group (C=O) as part of their functional groups. It's donates electrons donating it electron as hydrogen atom. C=C donates its electrons, and their ability to donate their electrons lies in their structural orientations and their functional groups. Compounds like 1, 2,3,4-Cyclohexanetetrol have carbonyl group (C=O) as part of their functional groups.

**CONCLUSION**

Based on the resulted presented in these study, it could be concluded that the leaves of *Gymnema sylvestre* possess hepatoprotective activity against acetaminophen-induced liver injury in rats, with the residual fractions being the most active.

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**Competing interests**

Authors have declared that no competing interests exist.

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