EFFECT OF POLYHERBAL PREPARATION ON THIOACETAMIDE INDUCED LIVER DAMAGE AND HEPATIC ENCEPHALOPATHY IN RATS

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ABSTRACT

To evaluate the hepatoprotective activity of polyherbal preparation on thioacetamide (TAA) induced liver damage and hepatic encephalopathy in rats. Serum ALT, AST, ALP, GGT, bilirubin and liver histological changes were assessed in control and PHE treated rats. The effect of PHE against hepatic encephalopathy was evaluated by the assessment of blood ammonia level, motor and cognitive activity and brain neurotransmitters such as dopamine, noradrenaline, serotonine and GABA in rats. Pretreatment with PHE significantly reduced liver enzymes level in blood as compared to TAA treated animals. PHE prevents the TAA induced elevation of ammonia level in blood. Pretreatment with PHE had significant protection against TAA induced changes in brain neurotransmitters level and impairment of motor and cognitive function. Histology of the liver sections confirmed that PHE prevented hepatic damage induced by TAA showing the presence of normal hepatocytes, absence of necrosis and fatty infiltration. The behavioral, biochemical and histological results indicated the protective effect of PHE against TAA induced liver damage and hepatic encephalopathy in rats.

Key words: Hepatic encephalopathy, liver damage, thioacetamide, polyherbal preparation, ammonia, memory and cognitive function.

INTRODUCTION

The liver is the largest glandular organ in the body which plays a pivotal role in regulating various physiological processes in the body, such as metabolism, secretion and storage. It has great capacity to removes toxic substances from the blood, including substances that have been generated in the brain and must be eliminated from the body, as well as compounds produced in other tissues that are harmful to the brain’s nerve cells (i.e., are neurotoxic).

When the liver becomes fibrotic and cirrhotic, the number of functional hepatocytes decreases and the liver loses its capacity to remove toxic substances from the blood. Blood that bypasses the liver is not detoxified, and toxic substances including ammonia, manganese and mercaptans are increase and enter the brain and are neurotoxic. Consequently, brain function in patients with severe liver disease is compromised, resulting in a condition known as hepatic encephalopathy. Accumulation of ammonia in the circulation is considered to play an important role in the onset of hepatic encephalopathy. Brain ammonia is metabolised in astrocytes to glutamine, an osmolyte, and increased glutamine accumulation in these cells may contribute to cytotoxic brain edema, which often complicates hepatic encephalopathy.

Hepatic encephalopathy is associated with a shift in the balance between inhibitory and excitatory neurotransmission towards a net increase of inhibitory neurotransmission, as a consequence of at least two factors: (1) Down-regulation of Glutamate receptors resulting in decreased glutamatergic tone. (2) An increase in inhibitory neurotransmission by γ-amino butyric acid (GABA). Changes in serotonin and dopamine and their receptors may contribute to some of the motor manifestations of hepatic encephalopathy. In the absence of a reliable liver protective drug in the modern system of medicine, a number of medicinal preparations in Ayurveda are recommended for the treatment of liver disorders. About 700 commercial herbal formulations with claimed hepatoprotective activity are being sold all over the world. However, only a small proportion of hepatoprotective plants as well as formulations used in traditional medicine are pharmacologically evaluated for their safety and efficacy.

In the present study, polyherbal hepatoprotective preparation contains dried methanolic extracts of five plants. These are Solanum nigrum, boerhaavia diffusa, Indigofera tinctoria, Tephrosia purpurea and operculina turpenthum. The criteria for selection are based on (i) claimed as Ayurvedic medicine, (ii) commercially available, (iii) with known hepatoprotective activity of all above mentioned plants. There is no systematic scientific study has been undertaken to evaluate the activities against liver damage and hepatic encephalopathy for this polyherbal preparation. Based on this, the present study was designed to investigate the effect of polyherbal preparation on thioacetamide induced liver damage and hepatic encephalopathy. Efficacy of this preparation will be studied on biochemical, histopathological and pharmacological parameters in rats.

MATERIALS AND METHODS

Drugs and Chemicals

Gratis sample of Thioacetamide was obtained from Chiti chem corporation, vadodara. All other chemicals and reagents use for the study was of AR and laboratory grade.

Plant materials

The plant materials for the polyherbal preparation are Solanum nigrum (leaves), boerhaavia diffusa (root), Indigofera tinctoria (leaves), Tephrosia purpurea (root), Operculina turpenthum (root). Gratis sample of standardized dried methanolic extracts of all above plants was obtained from AMSAR PVT LTD., Indore, India. Polyherbal preparation was formulated according to information mentioned in traditional ayurvedic literature. Dried powdered methanolic extract of all five plants were mixed in equal amounts. Freshly prepared aqueous solution of this mixed extract (PHE) was used for experimental study.

Preliminary phytochemical analysis

The qualitative chemical investigation of polyherbal extract (PHE) was carried out to check the presence of various phytoconstituents.
Animal
Wistar albino male rats (300-400 g) were acclimatized for 7 days under standard husbandry conditions, i.e., room temperature of (26±10) °C, light:dark cycle of 12:12 hrs and had free access to food and water up to the time of experimentation. The experiment conducted during the light period (08.00-16.00 hrs). All the protocols were approved by the Institutional Animal Ethical Committee (IAEC) (Protocol No. CPCSEA/IAEC/ARCP/2011-2012/10) and conducted according to the Committee for the Purpose of the Control and Supervision of Experimental on Animals (CPCSEA) guidelines.

Experimental method
Wistar albino male rats (300-400 g) were randomly divided into four groups of six animals each.

Group 1: (Normal control) Animals were served as normal control and received 1ml/kg volume of vehicle.

Group 2: (Disease control) Two injections of thioacetamide (200mg/kg, i.p.) at 24 hrs interval was administered.

Group 3: (PHE 300) Polyherbal extract (300 mg/kg) was administered orally for 14 days followed by two dose of thioacetamide (200 mg/kg, i.p.) at 24 hrs intervals.

Group 4: (PHE 600) Polyherbal extract (600 mg/kg) was administered orally for 14 days followed by two dose of thioacetamide (200 mg/kg, i.p.) at 24 hrs intervals.

Polyherbal extract was administered for 14 days and on 14th day all the animals were fasted for 12 hrs and all animals except those in group 1, treated with thioacetamide(200mg/kg, i.p.) every 24 hrs for two consecutive days. Supportive therapy by administering 5% dextrose (25 ml/kg body weight) containing 0.45% NaCl and 20 (meq/l) potassium chloride was given to avoid weight loss, hypoglycaemia and renal failure. This supportive therapy was administered every 12 hrs following the first injection of thioacetamide (or saline) via intraperitoneal injection. Motor activity, cognitive function and reflex performance were measured on 18 hrs after the last dose of TAA. Heparinized blood samples were obtained afterwards from the heart for liver function test. For analysing the liver damage and brain neurotransmitter level, rats were sacrificed 18 hrs after second dose, where significant histological changes of liver were observed1.

Behavioral test
The spontaneous motor activity of rat was assessed by an actophotometer3 for 5min. Number of counts was recorded. Cognitive function was assessed by measurement of step down latency in step down passive avoidance test9 and transfer latency in elevated plusmaze test10. Reflex performance test15 was assessed by measurement of righting reflex, withdrawal reflex and headshake reflex tests. In these entire tests, an animal can score up to 4 points. If the animal can perform the behavioral test at 50–75% of the control value, 3 points were given(Stage I); at 25–50% of the control value, 2 points were given(Stage II); at 1–25% of the control value, 1 point was given(Stage III); and for no response, 0 point was given(Stage IV)11.

Evaluated parameters
Biochemical analysis
Blood was withdrawn through the heart and collected into heparinized tube for liver function tests. The collected blood sample were sent to clinical diagnosis laboratory (Dr. Sanjay Laboratory, Anand) to determine alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphate levels(ALP), γ-Glutamyl transferase(GGT), total, direct and indirect bilirubin and ammonia using an autoanalyzer.

Measurement of body and relative liver weight
The liver was weighed and liver:body weight ratio (liver index) was calculated.

Histological evaluation of the liver
After draining the blood, liver samples were excised, washed with normal saline, and processed separately for histological observations. Initially, the material was fixed in 10% buffered neutral formalin. The sections will cut in 5 mm thickness, stained with hematoxyline and eosin, and examined microscopically for histopathological changes12.

Measurement of neurotransmitter
After the drug treatment, rat was sacrifice and brain was isolated. The level of 5-HT was estimated in whole rat brain following the method of Jacobowitz and Richardson(1977)13. The level of noradrenaline and dopamine were measured by the method of Ghos and Bhattacharya(2008)14 and the brain gamma amino butyric acid (GABA) content was estimated according to the method of Lowe et al., (1958)15.

Statistical analysis
Results of biochemical estimations have been indicated in terms of mean ± SEM. Data has been analyzed by one way ANOVA followed by Tukey’s test. A P value of less than 0.05 was considered as statistically significant.

RESULTS
Phytochemical Screening of polyherbal extract (PHE)
The PHE showed the presence of Alkaloids, Flavanoid, Starch, Proteins and amino acids, Steroids, Carbohydrates, Lipids and Glucose, sucrose and fructose.

Liver:Body weight ratio
The body weight (BW) increase was smaller in TAA treated rats than in control group while liver weight (LW) increase was more in TAA treated rats than normal groups. As shown in Table 1, the liver:body weight ratio for disease control group was higher than normal control group. In PHE treated animals at the dose of 300 mg/kg and 600 mg/kg, liver:body weight ratio was less increased than disease control group (Table 1).

Behavioral tests
As shown in Table 2, significant (p<0.001) reduction in the locomotor activity and step down latency were observed in disease control group when compared with normal control group. The 14 days pretreatment with PHE at the doses of 300 mg/kg and 600 mg/kg, significantly (P<0.001) prevented the TAA induced fall in locomotor activity as compared to the TAA treated group. Rats pretreated with PHE at the doses of 300 mg/kg showed significantly (p<0.05) less reduction of step down latency than TAA treated rats indicating prevention of memory impairment while PHE at the dose of 600 mg/kg not showed significantly prolongation of TAA induced reduction of SDL. As shown in Table 2, significant (p<0.001) increased in the TL was observed in TAA treated group as compared to normal control group. Rats pretreated with PHE at the doses of TAA 300 mg/kg and 600 mg/kg, showed significantly (P<0.001 and p<0.05, respectively) less prolongation of transfer latency than TAA treated rats indicating prevention of memory impairment.

Reflex performance test
As shown in Table 3, significant (p<0.001) increased in the time for righting reflex, latency for withdrawal reflex and headshake reflex were observed in disease control group as compared to normal control group. The 14 days pretreatment with PHE at the doses of 300 mg/kg and 600 mg/kg, significantly (P<0.001) prevented the TAA induced increase in time for regain of righting reflex and latency for withdrawal reflex as compared to the TAA treated group.
while PHE showed no significant effect on TAA induced increase in latency for headshake reflex.

Rat showed performance in normal control group considered as 100% performance. All behavior and performance of the TAA treated rat were observed in between 1-25% as compared to normal control. Hence, stage III of hepatic encephalopathy produced in TAA treated rats.

**Measurement of neurotransmitter**

As shown in Table 4, concentration of dopamine and noradrenaline were significantly (p<0.001) decreased while concentration of 5-HT and GABA were increased in disease control group as compared to normal control group. 14 days pretreatment with PHE at the dose of 300 mg/kg and 600 mg/kg, significantly (p<0.05) prevented the TAA induced fall in brain noradrenaline level while PHE at the dose of 600 mg/kg showed significant (p<0.05) preventive effect on TAA induced decrease in brain dopamine level. TAA induced rise in 5-HT and GABA concentration in brain were significantly (p<0.05) prevented in PHE treatment animals at the dose of 300 mg/kg and 600 mg/kg for 14 days as compared to the TAA treated group.

**Biochemical tests**

A significant(p<0.001) increase in serum ALT, AST, ALP, bilirubin and ammonia levels was measured in TAA treated group as compared to normal control group. Serum ALT, AST, ALP, bilirubin and ammonia levels increased by TAA treatment were prevented significantly (p<0.001) by pretreatment of animals with PHE (300 mg/kg and 600 mg/kg) for 14 days (Table 5,6). Blood GGT concentration was not altered significantly by any of the above treatments (Table 5).

**Histopathology of liver**

In histopathology study of liver, normal group showed normal architecture. The liver of TAA treated rats showed necrosis and focal mild fatty changes in the hepatic cells. The rats treated with PHE (300 mg/kg and 600 mg/kg) showed less damage of hepatocytes indicating protection against TAA induced liver damage. Magnification was set at 200X and the method of staining was hematoxylin and eosin (Figure 1).

**DISCUSSION**

Liver is an organ of paramount importance, which plays a pivotal role in regulating various physiological processes in the body, such as metabolism, excretion and storage. It is responsible for detoxifying poisonous substances by transforming and removing toxins and wastes. These toxins and wastes are converted to less harmful substances by the liver and then eliminated from the body. Liver damage may be caused by viral infection, tissue immune mediated damage, toxic agents, obstructive jaundice, gene abnormalities or alcohol and non-alcohol steatohepatitis. Hepatotoxic agents like Thioacetamide(TAA), CCl₄, high dose of paracetamol, isoniazid, diclofenac and ibuprofen are used for induction of liver damage in experimental animals. TAA induced hepatotoxicity and hepatic encephalopathy is well established animal model for studying the hepatoprotective property.

TAA is metabolized to thioacetamide-S-oxide by cytochrome-P450 enzyme system in liver. Thioacetamide-S-oxide is responsible for the change in cell permeability, increase in intracellular Ca²⁺ concentration, increases in nuclear volume, enlargement of nucleoli and inhibition of mitochondrial activity which leads to cell death. TAA reduces the number of viable hepatocytes as well as rate of oxygen consumption. Injury to hepatocyte and bile duct cells lead to accumulation of bile acid inside the liver, which promotes further liver damage. The nucleolar changes induced by TAA have led to the hypothesis that it interferes with the movement of RNA from the nucleus to the cytoplasm which may cause membrane injury and possibly leading to necrosis of liver cells.

Rats treated with TAA are reported significant reduction in body weight due to loss of skeletal muscles and adipose tissue. Reduction in body weight of the animals in our study may correlate with TAA treatment. Increase in liver weight and liver:body weight ratio (liver index) were reported with TAA treatment. In the present study, 14 days treatment with PHE showed protective effect against TAA induced rise of liver index.

Liver contains large amount of marker enzymes. Elevated levels of liver enzymes in serum are indicative of cellular leakage and loss of functional integrity of cell membrane in liver. Liver enzymes like ALT, AST, ALP and bilirubin are leaked in to serum resulting in elevation of their serum concentration. In the assessment of liver damage by TAA, the determination of enzyme levels such as ALT and AST are largely used. Hepatocellular necrosis produced rise in serum ALT and AST level. However, ALT is a better index of injury, as its activity represents 90% of total enzymes present in the body. Therefore, ALT is more specific to the liver, and is thus a better parameter for detecting liver injury. TAA is known to increase serum ALT and AST level in rats. In our study, the rise in serum ALT and AST levels induced by thioacetamide was significantly reduced by 14 days treatment with PHE suggesting that its hepatoprotective activity via its effect against cellular leakage, loss of functional integrity of the cell membrane and the loss of stabilization of plasma membrane.

Alkaline phosphatase (ALP) is an enzyme in the cells lining the biliary ducts of the liver. ALP activity is related to the functioning of hepatocytes and increase in its activity is due to increased synthesis, in the presence of increasing biliary pressure. ALP levels in serum are raised with large bile duct obstruction, intrahepatic cholestasis or infiltrative diseases of the liver. In present study, TAA treated rats showed significantly rise in serum ALP level as compared to control rats. Here, 14 days PHE treatment in rats showed significant protection against TAA induced change in ALP level. This indicated that obstruction of biliary duct and increasing biliary pressure by TAA may be protected by PHE.

Bilirubin, an endogenous organic anion and is a breakdown product of heme. The liver is responsible for clearing bilirubin from the blood. Bilirubin binds reversibly to albumin and transported to the liver where it is conjugated with glucuronic acid and secreted in bile through intestine. Total bilirubin is elevated in the cases of jaundice, hemolytic anemia, internal hemorrhage, deficiencies in bilirubin metabolism or excretion. Hepatobiliary disease or bile duct obstruction by gallstones should be suspected in this condition. Excess of unconjugated bilirubin (indirect bilirubin) indicated the location of the problem is upstream of bilirubin excretion.

TAA treated rats showed significant rise in total, direct and indirect serum bilirubin level as compared to normal control. 14 days treatment of PHE was reduced the total, direct and indirect serum bilirubin elevated by TAA. This suggested that PHE may prevent the TAA induced impairment of metabolism and excretion of bilirubin and the obstruction of bile duct.
GGT (γ-Glutamyl transferase) catalyses the transfer of a γ-glutamyl group from one peptide to another. It is rise in hepatobiliary disease, cholestasis⁶. In our study, there was no significant change in GGT level in TAA treated rats.

TAA treatment decreased the number of function hepatocytes and caused liver damage. This liver loses its capacity to remove toxic substances such as ammonia from the blood. Hence, level of toxic substance ammonia is increased in blood. Excess of ammonia in blood enters in the brain which is responsible for hepatic encephalopathy⁷. Blood ammonia crosses the blood brain barrier and enters in brain astrocytes. Brain ammonia is metabolized in astrocytes to glutamine, an osmolyte, and increased glutamine accumulation in these cells may contribute to astrocyte swelling, cytotoxic brain edema, which often complicates hepatic encephalopathy (HE). In present study, blood ammonia level was significantly increased in TAA treated rats as compared to control rats. 14 days PHE treatment prevented the TAA induced rise in blood ammonia level. This indicates that PHE have protective effect in TAA induced hepatic encephalopathy in rats.

The clinical features of hepatic encephalopathy are memory impairment⁸, motor and sensory abnormalities⁹ because of changing in neurotransmitter level. It is also reported that increased activity of metabolizing enzymes (MAO-A and MAO-B) and subsequent modification of monoaminergic synaptic function could contribute to pathogenesis of HE¹⁰. Impairment in brain neurotransmitter level with TAA is responsible for HE. TAA is reported to decrease brain dopamine¹¹ and noradrenaline¹² level and increase brain GABA¹³ and 5-HT¹⁴ level. In the present study, TAA produced fall in brain dopamine and noradrenaline level in rats while it caused rise in brain GABA and 5-HT level in rats as compared to control animals.

A loss of striatal dopamine D₂ receptors and increased activities of the dopamine metabolizing enzymes MAO-A and MAO-B, have been reported in brain tissue of cirrhotic patients of HE¹⁵. These finding reported that a decrease in dopaminergic tone may contribute to motor disturbances in HE. 14 days treatment of PHE reversed the TAA induced decrease of dopamine and noradrenaline and offered protective effect against hepatic encephalopathy. Serotonicergic mechanisms contribute to behavioral manifestation of hepatic encephalopathy induced by TAA. Increased in serotonergic inhibitory tone may contribute to fall down of locomotor activity in TAA treated rats. Improvement of locomotor activity was reported with wide spectrum serotonin antagonist methysergide¹⁶. TAA induced hepatic encephalopathy in rats is associated with down regulation of 5-HT₂A receptors¹⁷. In the present study, TAA caused rise in brain 5-HT level. Further, 14 days treatment of PHE prevents TAA induced changes in brain 5-HT and improved motor performance in rats.

Ammonia may indirectly increase GABA-ergic neurotransmission and also inhibit the function of CNS by synergistic activity with natural benzodiazepine receptor ligands¹⁸. Presynaptic GABA_B activation inhibits depolarization induced GABA release. Loss of GABA_B receptors was observed with TAA which probably raise brain GABA release. In the present study, brain GABA level was increased in TAA treated rats as compared to control rats. It is also responsible for sedative and depressant effects in rats. 14 days treatment with PHE improved the locomotor activity and reflex performance by preventing TAA induced elevation of GABA level in rat brain.

Impairment in cognitive function can be assessed by elevated plusmaze and step down passive avoidance test. Alterations in the levels of various neurotransmitters (such as acetylcholine, epinephrine, dopamine, GABA, glutamate etc.) have been found to play a crucial role in the pathogenesis of impaired memory of laboratory animals¹⁹. 14 days treatment of PHE prevented the cognitive impairment induced by TAA in elevated plusmaze test and step down passive avoidance test.

Histopathological studies showed that TAA produced fatty changes and necrosis in hepatocytes of rat liver. Pretreatment with PHE protected against TAA induced necrotic changes in rat liver.

Taken together, the finding in the current study showed that PHE (300 mg/kg and 600 mg/kg) showed protective effect against TAA induced liver damage and hepatic encephalopathy. The efficacy of most herbal remedies is attributed to various active principles in combination. All five plants in the preparation have individually reported hepatoprotective activity in various experimental models.

Antioxidant activity of all five plants of PHE has also been reported. The hepatoprotective activity of PHE observed in the present study against TAA induced liver damage is also mediated through augmentation of antioxidant defenses. Hence, protective action observed in our study against TAA induced liver damage and hepatic encephalopathy may be due to combined actions of all plants.

AKNOWLEDGEMENT

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REFERENCES


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Table 1  Effect of polyherbal extract (PHE) on liver:body weight ratio of Thioacetamide treated rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose, Route</th>
<th>Body weight (g)</th>
<th>Liver weight (g)</th>
<th>Liver:Body weight ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal control</td>
<td>1 ml/kg, p.o</td>
<td>372 ± 27.28</td>
<td>10.27 ± 0.45</td>
<td>2.791 ± 0.12</td>
</tr>
<tr>
<td>2</td>
<td>Disease control (TAA)</td>
<td>200 mg/kg, i.p.</td>
<td>382 ± 13.19</td>
<td>11.38 ± 0.29</td>
<td>2.987 ± 0.09</td>
</tr>
<tr>
<td>3</td>
<td>PHE 300</td>
<td>300 mg/kg, p.o</td>
<td>310 ± 8.94</td>
<td>8.95 ± 0.19</td>
<td>2.892 ± 0.06</td>
</tr>
<tr>
<td>4</td>
<td>PHE 600</td>
<td>600 mg/kg, p.o</td>
<td>340 ± 20.74</td>
<td>9.70 ± 0.37</td>
<td>2.873 ± 0.08</td>
</tr>
</tbody>
</table>

Each value expressed as mean ± SEM, (n=6) for each group. Data were analyzed by one way ANOVA followed by Tukey’s test.

Table 2  Effect of polyherbal extract (PHE) on locomotor activity, Step down latency and transfer latency of Thioacetamide treated rats.

<table>
<thead>
<tr>
<th>G</th>
<th>Treatment</th>
<th>Dose, Route</th>
<th>Number of counts</th>
<th>Step down latency (sec)</th>
<th>Transfer Latency (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal control</td>
<td>1 ml/kg, p.o</td>
<td>406.5 ± 8.97</td>
<td>161.0 ± 6.80</td>
<td>Before TAA (learning) 33.67 ± 2.33 After TAA (memory) 34.67 ± 1.49</td>
</tr>
<tr>
<td>2</td>
<td>Disease control (TAA)</td>
<td>200 mg/kg, i.p.</td>
<td>45.0 ± 5.20</td>
<td>95.0 ± 4.35</td>
<td>36.33 ± 1.43 91.83 ± 6.00</td>
</tr>
<tr>
<td>3</td>
<td>PHE 300</td>
<td>300 mg/kg, p.o</td>
<td>178.3 ± 6.21</td>
<td>125.7 ± 6.38</td>
<td>32.17 ± 3.52 65.33 ± 3.90</td>
</tr>
<tr>
<td>4</td>
<td>PHE 600</td>
<td>600 mg/kg, p.o</td>
<td>134.7 ± 10.39</td>
<td>115.7 ± 3.52</td>
<td>34.33 ± 4.23 72.17 ± 3.85</td>
</tr>
</tbody>
</table>

Each value expressed as mean ± SEM, (n=6) for each group. Data were analyzed by one way ANOVA followed by Tukey’s test. *P<0.001 when compared with normal control. **P<0.001 when compared with disease control. ***P<0.001 when compared with before treatment.
Table 3 Effect of polyherbal extract (PHE) on righting reflex, withdrawal reflex and headshake reflex of Thioacetamide treated rats.

<table>
<thead>
<tr>
<th>G</th>
<th>Treatment</th>
<th>Dose, Route</th>
<th>Time for regain of righting reflex (ms)</th>
<th>Latency for withdrawal reflex (ms)</th>
<th>Latency for headshake reflex (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal control</td>
<td>1 ml/kg, p.o</td>
<td>29.33 ± 2.41</td>
<td>38.60 ± 1.20</td>
<td>17.50 ± 0.92</td>
</tr>
<tr>
<td>2</td>
<td>Disease control(TAA)</td>
<td>200 mg/kg, i.p.</td>
<td>53.17 ± 1.88</td>
<td>84.17 ± 2.21</td>
<td>31.66 ± 1.05</td>
</tr>
<tr>
<td>3</td>
<td>PHE 300</td>
<td>300 mg/kg, p.o.</td>
<td>36.16 ± 2.58^*</td>
<td>62.33 ± 2.49^</td>
<td>26.50 ± 0.99</td>
</tr>
<tr>
<td>4</td>
<td>PHE 600</td>
<td>600 mg/kg, p.o.</td>
<td>37.00 ± 2.35^*</td>
<td>68.33 ± 2.82^</td>
<td>26.17 ± 0.87</td>
</tr>
</tbody>
</table>

Each value expressed as mean ± SEM, (n=6) for each group. Data were analyzed by one way ANOVA followed by Tukey’s test; *P<0.001 when compared with normal control.

Table 4 Effect of polyherbal extract (PHE) on brain neurotransmitter level in Thioacetamide treated rats.

<table>
<thead>
<tr>
<th>G</th>
<th>Treatment</th>
<th>Dose, Route</th>
<th>Concentration of neurotransmitters (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Noradrenaline</td>
</tr>
<tr>
<td>1</td>
<td>Normal control</td>
<td>1 ml/kg, p.o</td>
<td>426.5 ± 11.03</td>
</tr>
<tr>
<td>2</td>
<td>Disease control(TAA)</td>
<td>200 mg/kg, i.p.</td>
<td>303.2 ± 11.81</td>
</tr>
<tr>
<td>3</td>
<td>PHE 300</td>
<td>300 mg/kg, p.o.</td>
<td>351.6 ± 6.55^*</td>
</tr>
<tr>
<td>4</td>
<td>PHE 600</td>
<td>600 mg/kg, p.o.</td>
<td>352.8 ± 5.61^*</td>
</tr>
</tbody>
</table>

Each value expressed as mean ± SEM, (n=6) for each group. Data were analyzed by one way ANOVA followed by Tukey’s test; *P<0.001 when compared with normal control.

Table 5 Effect of polyherbal extract (PHE) on blood ALT, AST, ALP and GGT level in Thioacetamide treated rats.

<table>
<thead>
<tr>
<th>G</th>
<th>Treatment</th>
<th>Dose, Route</th>
<th>Blood ALT level (IU/L)</th>
<th>Blood AST level (IU/L)</th>
<th>Blood ALP level (IU/L)</th>
<th>Blood GGT level (µg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal control</td>
<td>1 ml/kg, p.o</td>
<td>58 ± 3.76</td>
<td>171.5 ± 9.80</td>
<td>189.3 ± 9.71</td>
<td>9.71 ± 0.75</td>
</tr>
<tr>
<td>2</td>
<td>Disease control(TAA)</td>
<td>200 mg/kg, i.p.</td>
<td>127.8 ± 56.70^</td>
<td>1555 ± 90.82</td>
<td>419.0 ± 16.52</td>
<td>12.25 ± 0.85</td>
</tr>
<tr>
<td>3</td>
<td>PHE 300</td>
<td>300 mg/kg, p.o.</td>
<td>631.5 ± 14.99^</td>
<td>800 ± 30.22^</td>
<td>259.3 ± 11.43^</td>
<td>11.00 ± 0.91</td>
</tr>
<tr>
<td>4</td>
<td>PHE 600</td>
<td>600 mg/kg, p.o.</td>
<td>744.8 ± 22.03^</td>
<td>965.8 ± 27.55^</td>
<td>294.5 ± 9.579^</td>
<td>10.50 ± 0.64</td>
</tr>
</tbody>
</table>

Each value expressed as mean ± SEM, (n=6) for each group. Data were analyzed by one way ANOVA followed by Tukey’s test; *P<0.001 when compared with normal control.

Table 6 Effect of polyherbal extract (PHE) on blood bilirubin and ammonia level in Thioacetamide treated rats.

<table>
<thead>
<tr>
<th>G</th>
<th>Treatment</th>
<th>Dose, Route</th>
<th>Bilirubin level in blood (mg/dl)</th>
<th>Blood ammonia level (µg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>Direct</td>
</tr>
<tr>
<td>1</td>
<td>Normal control</td>
<td>1 ml/kg, p.o</td>
<td>0.660 ± 0.02</td>
<td>0.3225 ±0.01</td>
</tr>
<tr>
<td>2</td>
<td>Disease control(TAA)</td>
<td>200 mg/kg, i.p.</td>
<td>2.450 ± 0.09^</td>
<td>1.575 ± 0.10^</td>
</tr>
<tr>
<td>3</td>
<td>PHE 300</td>
<td>300 mg/kg, p.o.</td>
<td>1.135 ± 0.06^</td>
<td>0.665 ± 0.04^</td>
</tr>
<tr>
<td>4</td>
<td>PHE 600</td>
<td>600 mg/kg, p.o.</td>
<td>1.498 ± 0.06^</td>
<td>0.825 ± 0.02^</td>
</tr>
</tbody>
</table>

Each value expressed as mean ± SEM, (n=6) for each group. Data were analyzed by one way ANOVA followed by Tukey’s test; *P<0.001 when compared with normal control. ^P<0.001 when compared with disease control. **P<0.05 when compared with disease control.
Figure 1 Effect of PHE on liver histopathology in TAA induced liver damage in rats.

A) Normal group showing normal architecture.
B) TAA treated group showing necrosis and focal mild fatty changes in the hepatic cells.
C) PHE 300 group showing protection of hepatocytes.
D) PHE 600 group showing protection of hepatocytes. Magnification was set at 200X and the method of staining was hematoxylin and eosin.

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