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Full Length Research Paper

Effects of different types of water decoctions of fruit of *Melia azedarach* on glucose induced hyperglycemia, liver transaminases, lipid peroxidation and reduced glutathione in normal albino rats

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Four types of extracts were prepared from *Melia azedarach* fruits. These were methanol extract and three types of water decoctions; urine treated water decoction (UE), milk treated water decoction (ME) and plain water decoction (WE). Different doses of these four extracts were tried in normal rats to estimate the hypoglycemic activity of these extracts against glucose induced hyperglycemia. Most potent doses of all the extracts were assessed for their effects on lipid peroxidation, toxic effects on liver and reduced glutathione which is considered as first line of defense against free radicals in normal rats after 10 days of oral administration. All the extracts were found to posses hypoglycemic properties. Methanol extract showed toxic effects on the liver as indicated by high activities of Serum glutamate oxaloacetate (SGOT) and serum glutamate pyruvate tranasaminase (SGPT). It also indicated high rate of lipid peroxidation and depleted reduced glutathione levels of plasma. On the other hand three water decoctions showed very low activities of SGOT, SGPT, very low rates of lipid peroxidation and high levels of reduced glutathione. Extract prepared by the Ayurvedic method reduce blood glucose in glucose induced hyperglycemic rats and impart no toxicity.

Key words: *Melia azedarach,* SGOT, SGPT, reduced glutathione, thiobarbituric acid reactive substances, blood glucose.

INTRODUCTION

Melia azedarach, commonly known as Mosalaosi in Botswana is a perennial tree and belongs to family Meliaceae. The tree is fast growing and long lived. The crown is rounded and reaches 50 cm tall and 20 cm in it's spread. The bark is reddish brown and in mature tree it is fissured. The leaves are bipinnate, flowers are purple, fragrant and star shaped and is produced in spring and early summer. Clusters of spherical yellow fruits are produced soon afterward and persist even after the fall of leaves. *Azadirachta indica* is known as nimb and is used in Ayurvedic system for the management of diabetes. Aqueous extract of this pant has also been reported for its hypoglycemic effects by many scientists (Chattopadhyay, 1999; Halim, 2003) and also for its antioxidant properties (Banwara et al, 2000). *Melia azadirachta* is known as mahanimb in Ayurvedic system of medicine and is used for management of diabetes. Hypoglycemic property of fruit and bark of this tree has been already reported (Chaturvedi et al., 2004). Methanol extract and other solvent extracts show the hypoglycemic effects but impart liver toxicity at the same time (Chaturvedi et al., 2005). Water extracts of *M. azdirachta* have not been tried for its hypoglycemic effects in our laboratory. *A. indica* and *M. azedirachta* belong to same family Meliaceae. Aqueous extract of *A. indica* possess hypoglycemic as well as antioxidant properties. Therefore the present study is aimed at preparation of special water extracts by Ayurvedic methods and their trial in normal rats to evaluate the hypoglycemic as well as on the extent of lipid peroxidation.

MATERIALS AND METHODS

Plant materials: Fruits of *M. azadarach* were collected from trees in the Botswana University campus in the year 2002.

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Animals: Male albino rats each weighing approximately 250 g were used for all the experiments. They had free access to water and food ad libitum.

Chemicals: Blood glucose kit and Serum Glutamate Oxaloacetate Transaminase (SGOT) kits, and other chemicals used to estimate thiobarbituric acid and reduced glutathione were bought from Sigma Chemicals. Plant material and preparation of different types of extracts

Collection

Fruits *M. azedarach* were collected from trees from the Botswana University campus. After collection, the plant materials were dried and crushed to powder.

Preparations of methanol extract (MetE): Powder was extracted with cold percolation method using 70% methanol. The extract was made methanol free by evaporating it in butchi type rotary evaporizer under reduced pressure.

Preparation of extracts by Ayurvedic methods: Special extracts of fruits only, were prepared by the methods described in Ayurvedic text with slight modification (Shobha, 2003). These processes remove the toxicity during processing. Three types of extracts were prepared by these methods;

Extract UE: Powder was soaked with cow's urine for three days. Each day, the powder was washed with water and soaked with fresh urine. After that it was washed well with water. After washing, the powder was boiled with water to get water extract. The ratio of powder and water was 1:4 (v/v) and boiling was stopped when the water volume was reduced to $1/4^{th}$ of the normal volume.

Extract ME: Powder was soaked with warm milk for 8 h. After that the powder was washed and water extract was prepared as above.

Extract WE: The powder was boiled with water to get water extract. The ratio of powder and water was 1:4 (v/v) and boiling was stopped when the water volume was reduced to $1/4^{th}$ of the normal volume.

Biochemical estimations

Blood glucose

The glucose was determined by the glucose oxidase method after enzymatic oxidation in the presence of the glucose oxidase. Glucose is oxidized to gluconic acid and hydrogen peroxide. Hydrogen peroxide reacts with o-dianisidine in the presence of peroxidase to form coloured product. Oxidized o-dianisidine reacts with sulphuric acid to form a more stable product. The intensity of the pink colour measured at 540 nm is proportional to the original glucose concentration (Harold, 1988)

Serum glutamate oxaloacetate tranasaminase and Serum glutamate pyruvate Transaminase (SGOT and SGPT)

Methods described in the kit was followed. Activities of enzymes were expressed as U/L.

Thiobarbituric acid reactive substances (TBARS)

TBARS was estimated by method described by Sushmakumari et al. (1989). It is measured by formation of TBARS such as malonyldialdehye (MDA). MDA formed from the breakdown of fatty acids, serves as a convenient index product of lipid peroxidation that react with thiobarbituric acid to give red species. Ethoxypropane was used as standard.

1.2 ml of TCA-TBA-HCL reagent in the ratio of 1:1:1 (0.37% Thiobarbituric acid, 0.25N hydrochloric acid and 15% Trichloroacetic acid) was added to 0.8 ml of the plasma. The reactants were mixed and allowed to stand for 10 min in boiling water for reaction. After which it was cooled and 2 ml of freshly prepared sodium hydroxide was added. The absorbance was measured at 535 nm

Reduced glutathione (GSH)

Reduced glutathione can be estimated by the method of Ellman GC, 1959. 5, 5- dithiobis 2-nitrobenzoic acid is reduced by free sulphydryl SH group of glutathione to form one mole of 2- nitro-s-mercaptobenzoic acid per mole of –SH. The nitrobenzoic acid anion released has an intense yellow colour and can be used to measure –SH groups, which has an absorption maximum at 412 nm 50 µl of the serum , 950 µ of distilled water and 1.5 ml of the precipitating solution were mixed and then stand for 5 min, from there 1 ml of the mixture was mixed with 4 ml of phosphate solution and 0.5 ml 0f DTNB (5,5-dithiobis-2-nitrobenzoic acid) absorbance was measured at 412 nm

Experimental design

This experiment was performed to assess the effects of three special extracts of *M. azedarach* fruit extracts prepared by Ayurvedic methods. These methods excrete away the toxins parts of the plant parts and preserve the useful components.

Assessment of the effects of UE, ME, WE and MetE on elevated glucose levels after oral glucose load in normal rats

To assess hypoglycemic effect of MetE, UE, ME and WE on glucose induced hyperglycemia 80 rats were used. To assess the hypoglycemic effects of UE, 20 rats were divided into 4 groups with five in each. Group 1 was labeled the control group and group 2, 3 and 4 were the experimental groups (UE1, UE2 UE3). These rats were bled at 0 h. The control group was orally administered with glucose (2 g/kg body weight). Three experimental groups UE1, UE2 and UE3 were administered UE (4 ml, 8 and 16 ml /kg body weight respectively) and glucose (2g/kg body weight glucose). After that the rats were bled at the first, second and third hour to measure the glucose levels. Same experimental protocol was followed for ME and WE. In case of MetE, the extract doses for three experimental groups were 10, 20 and 40 mg/Kg body weight.

Assessment of effects of fruit extracts of Melia azedarachon on Liver transaminases, lipid peroxidation and reduced glutathione

This experiment was carried out to assess the effects of MetE, UE, ME and WE on liver transaminases, lipid peroxidation and antioxidants effects after long term administration. 25 rats were used for this experiment and divided into 5 groups. These are control group (C) and Experimental groups (U, M, W and MET). All the rats were bled on day 0. After that, C was administered distilled water, U was administered UE (16 ml/kg body weight), M was administered ME (16 ml/kg body weight),W was administered WE (16 ml/ kg body weight) and MET was administered MetE (40 mg/kg body weight for 15 days. On day 16, rats were bled to estimate SGOT, SGPT, TBARS and GSH.

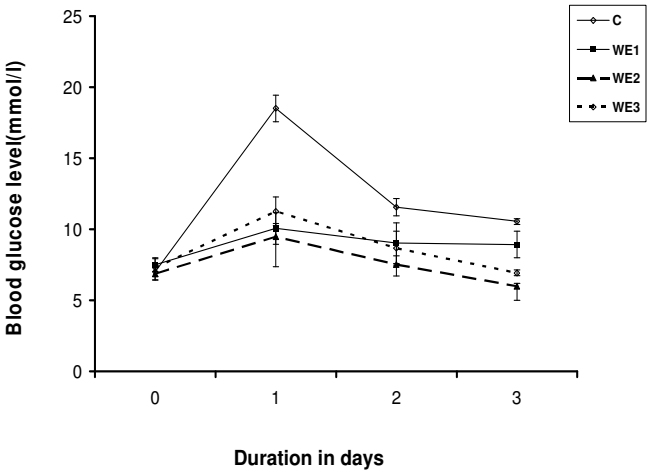

Figure 1. Effects of different doses of WE on glucose induced hyperglycemia. C: Control group administered glucose (2 gm/kg body weight); WE1: Experimental group WE1 administered glucose (2 gm/kg body weight) plus WE (4 ml/kg body weight; WE2: Experimental group WE2 administered glucose (2 gm/kg body weight) plus WE (8 ml/kg body weight; WE3: Experimental group WE3 administered glucose (2 gm/kg body weight) plus WE (16 ml/kg body weight).

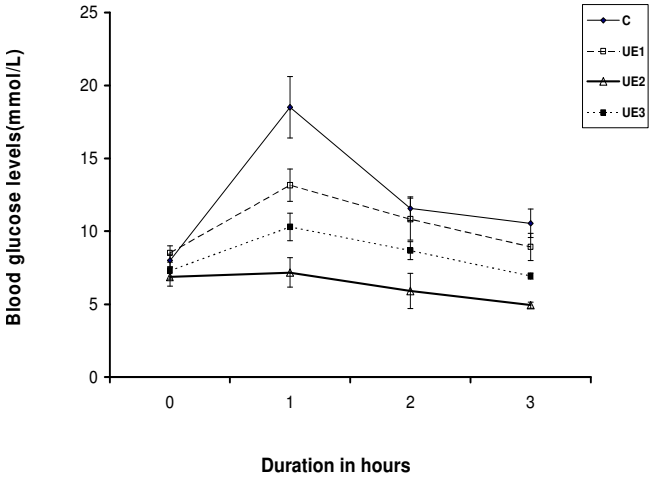

Figure 2. Effects of different doses of UE on glucose induced hyperglycemia. C: Control group administered glucose (2 gm/kg body weight); UE1: Experimental group UE1 administered glucose (2 gm/kg body weight) plus UE (4 ml/kg body weight; UE2: Experimental group UE2 administered glucose (2 gm/kg body weight) plus UE (8 ml/kg body weight); UE3: Experimental group UE3 administered glucose (2 gm/kg body weight) plus UE (16 ml/kg body weight.

RESULTS AND DISCUSSION

Hypoglycemic effects of WE, UE, ME and MetE

The results of effects of WE on glucose induced hyperglycemia are presented in Figure 1. The figure shows that WE at all the tried doses had significant hypoglycemic effects on glucose induced hyperglycemia as compared to the control group. The glucose levels were ranging from 8 to 10 mmol/L in experimental groups and it was around 18 mmol/L for control group. It is obvious from these results that control group in the first hour has glucose level higher than all the experimental groups. The levels are significantly low in all experimental groups when compared with control group ($p<0.05$).

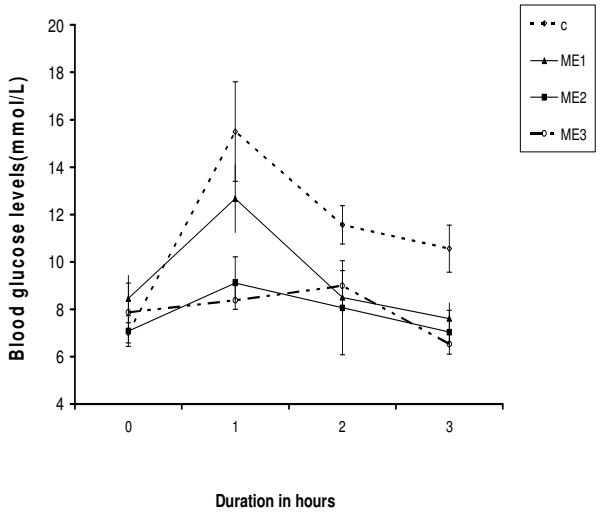

Figure 3. Effects of different doses of ME on glucose induced hyperglycemia. C: Control group administered glucose (2 gm/kg body weight); ME1: Experimental group ME1 administered glucose (2 gm/kg body weight) plus ME (4 ml/kg body weight). ME2: Experimental group ME2 administered glucose (2gm/kg body weight) plus ME (8 ml/kg body weight); ME3: Experimental group ME3 administered glucose (2 gm/kg body weight) plus ME (16 ml/kg body weight).

The Effects of UE on glucose induced hyperglycemia are presented in Figure 2. The figure shows that the blood glucose levels in control group were significantly high at first hour (18.51 mmol/L) as compared to the levels in three experimental groups. At 2nd h the glucose levels were around 12 mmol/L in control group. Group UE1 showed similar trends but the levels were lower than C group although it did not reach the normal levels. Group UE2 and UE3 showed significant reduction in the glucose levels at 1st h as compared to the control group ($p<0.05$). The group UE3 had most significant effect. This dose brought the glucose levels to normal (6.5 mmol/L) at first hour and these levels were maintained up to 3rd h.

The results of effects of ME extract on glucose induced hyperglycemia are presented in Figure 3. The figure shows that there was a significant hike in the levels of glucose in control group (15 mmol/L) as compared to the levels in three experimental groups. Blood glucose levels did not hike in experimental groups ME2 and ME3 and it ranged between 9 to 9.5 mmol/L. At the end of 2nd h, blood glucose levels came to normal for all three groups. The results of effects of MetE on glucose induced hyperglycemia are presented in Figure 4. It shows that extract

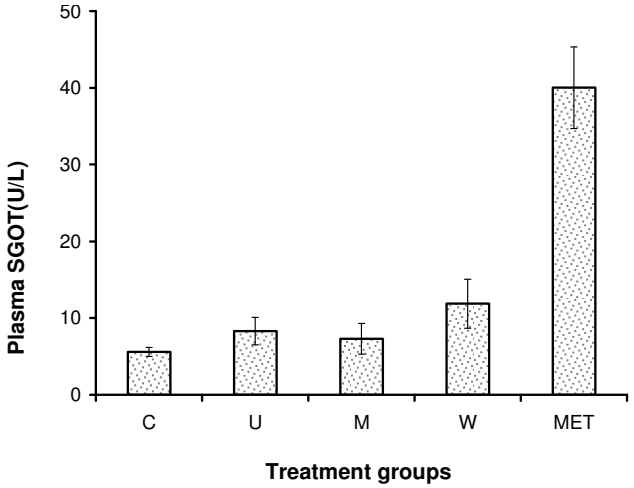

Figure 4. Effects of different extracts on plasma SGOT in experimental rats after 15 days of administration. C: Control group, administered distilled water, U: Administered UE (16 ml/kg body weight), M: Administered ME extract (16 ml/kg body weight), W: Administered WE (16 ml/kg body weight) and MET: Administered MetE (40 mg/kg body weight) P<0.05 when MET compared with C, U, M and W.

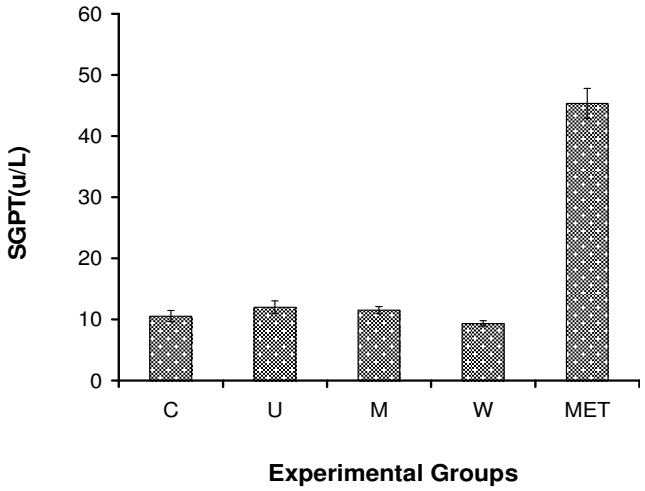

Figure 5. Effects of different extracts on plasma SGOT in experimental rats after 15 days of administration. C: Control group, administered distilled water, U: Administered UE (16 ml/kg body weight), M: Administered ME extract (16 ml/kg body weight), W: Administered WE (16 ml/kg body weight) and MET: Administered MetE (40 mg/kg body weight) P<0.05 when MET compared with C, U, M and W.

begins to reduce the glucose level after 1 h of its administration and continued up to 3 h. The maximum reduction is observed at 3rd h with the doses 20 and 40 mg/kg.

Effects of *Melia azedarach* fruit extracts on transaminases (SGOT and SGPT), TBARS and GSH

Effects of Melia azedarach fruit extracts on SGOT & SGPT

Results of chronic administration of different extracts of *M. azedarach* fruit on SGOT and SGPT are presented in Figures 5 and 6. The Figure 5 shows that there is marked elevation in the activity of SGOT in the group that received methanol extract as compared to other experimental groups that received UE, ME and WE extracts. The activity was very low in control group. It was 4.5 U/L in control group and 40 U/L in MetE treated experimental group. Figure 6 also shows the similar trend for SGPT

Effects of *Melia azedarach* fruit extracts on TBARS

Results of chronic administration of different extracts of *M. azedarach* fruit on SGOT are presented in Figure 7.The figure shows that there is marked elevation in the levels of TBARS in the group that received MetE than control group (p<0.05) and other experimental groups. In the experimental groups, the level was very low in the group that received UE.

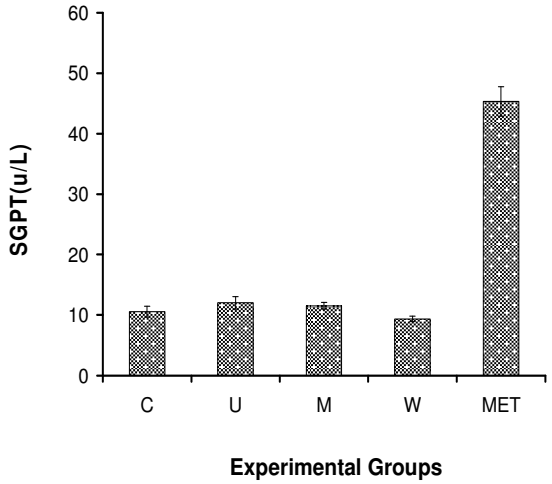

Figure 6. Effects of different extracts on plasma SGOT in experimental rats after 15 days of administration. C: Control group, administered distilled water, U:Administered UE (16 ml/kg body weight), M: Administered ME extract (16 ml/kg body weight), W: Administered WE (16 ml/kg body weight) and MET: Administered MetE (40 mg/kg body weight) P<0.05 when MET compared with C, U, M and W,

Effects of *Melia azedarach* fruit extracts on GSH

Results of chronic administration of different extracts of *M. azedarach* fruit on SGOT are presented in Figure 8.The figure shows that there is marked reduction in levels of reduced glutathione in the group that received MetE. The levels were very high in the group that received water extract.

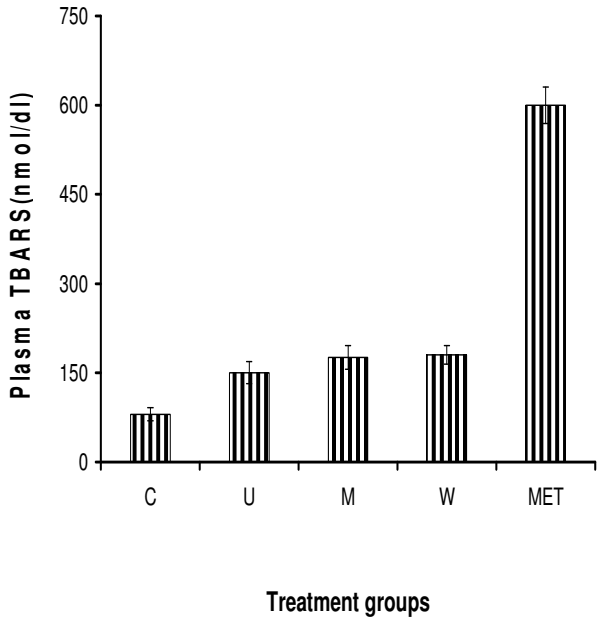

Figure 7. Effects of different extracts on plasma TBARS in experi-mental rats after 15 days of administration. C: Control group, administered distilled water; U: Administered UE (16 ml/kg body weight), M: Administered ME extract (16 ml/kg body weight); W: Administered WE (16 ml/kg body weight) and MET: Administered MetE (40 mg/kg body weight) P<0.05 when MET compared with C, U, M and W.

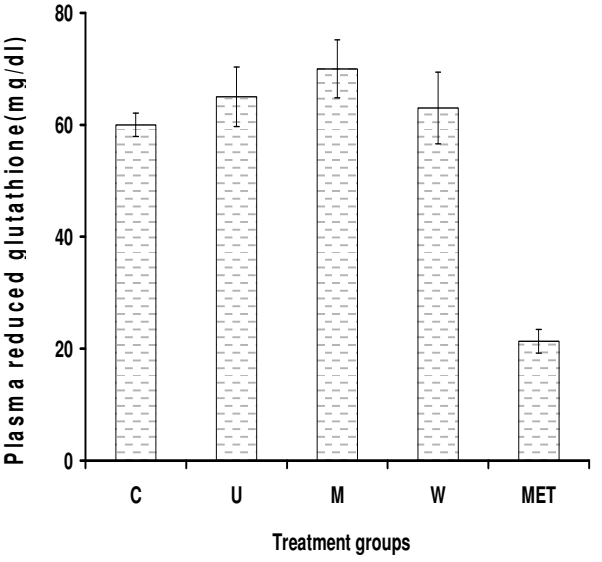

Figure 8. Effects of different extracts on plasma on reduced glutathione in experimental rats after 15 days of administration. C: Control group, administered distilled water, U: Administered UE (16 ml/kg body weight), M: Administered ME extract (16 ml/kg body weight), W: Administered WE (16 ml/kg body weight) and MET: Administered MetE (40 mg/kg body weight) P<0.05 when MET compared with C, U, M and W.

Analysis of the results of three extracts prepared by Ayurvedic methods reveals that all the four extracts show hypoglycemic effects at the doses 8 and 16 mmol/L at 1st h. There could be two possibilities of low glucose levels at 1st h. Low levels of glucose might be due to quick absorption and transport of glucose from the blood into the cells or possibility of slow absorption of glucose from the intestine into the blood.

Fifteen days administration of MetE indicates a signify-cant increase in SGOT levels (40U/L) as compared to the control group ($p<0.05$). Administration of UE, ME and WE to experimental rats for 15 days does not enhance the activities of SGOT as compared to MetE. SGOT is an enzyme, takes part in transamination reaction of liver. This enzyme is localized in hepatic cells and their levels go up in the circulation when the hepatic cells are damaged. Therefore high levels of this enzyme indicate liver damage (Himmerich et al., 2001). High levels of this enzyme in experimental group received MetE indicates liver damage. Low activities of this enzyme in other experimental groups suggest an intact liver. Cell damage is caused by initiation of lipid peroxidation due to free radicals. TBARS are substances produced during lipid peroxidation. Therefore high levels of TBARS indicate cell membrane damage (Janero et al., 1990; Knight et al., 2003). Low levels of TBARS in experimental groups that received UE, ME and WE indicates the inhibition of lipid peroxidation in these groups. Hepatic cell damage in the experimental group that received MetE is due to lipid peroxidation as indicated by high levels of TBARS. Reduced glutathione scavenges free radicals and renders protection against lipid peroxidation caused by free radicals. In the three experimental groups (U, M and W), TBARS levels and SGOT levels are low while GSH levels are high. Glutathione scavenges free radicals generated in the body and renders protection against lipid peroxidation and cell membrane damage (Kurleyet et al., 1998; Nicotera et al., 1986). It appears that there is inhibition of lipid peroxidation in these groups because of scavenging of free radicals by reduced glutathione and/or by extracts. In the group where rats were administered MetE extract, the levels of GSH are very low while activities of SGOT are very high together with high levels of TBARS in circulation. When the lipid peroxidation rate is very high, the store of GSH gets depleted because high rate of scavenging and anti oxidant system loses its control over free radicals. This leads to disruption of cell membranes and finally the cell death. In this case, low levels of GSH indicates high rate of lipid peroxidation.

This is also supported by high levels of TBARS and high levels of SGOT.

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