HYPOGLYCEMIC AND SAFETY EVALUATION OF THE AQUEOUS LEAF EXTRACT OF *HYPTIS SUAVEOLENS* IN MICE

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ABSTRACT

The aqueous leaf extract of Hyptis Suaveolens leave was evaluated for possible hypoglycaemic and toxic effects of alloxan induced hyperglyceamia in Swiss albino mice. Haematological and serum biochemical parameters were determined. The acute toxicity result revealed that the H. Suaveolens extract caused no death of the mice even at 5000mg/Kg body weight. Daily oral administration of graded doses of (400- 1200mg/Kg body weight of the extract for 21 days reflected a reduction in the weights of all the treated animals with the highest reduction in the weight of the animals that received the highest dose (1200mg/kg body weight). An increase in weight was however recorded in the control animals. A reduction in the blood glucose level was observed with a significantly (P>0.05) lower level in the animals treated with 1200mg/kg body weight of extract. An elevation in the serum level of glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and alkaline phosphatase was observed, which was dose dependent. The control had lower levels of serum alkaline phosphatase, glutamate pyruvate transaminase and glutamate oxaloacetate. There was no significant (P>0.05) reduction in the packed cell volume in the entire treated group. The total protein levels of the animals that received 1200mg/kg body weight were significantly (P<0.05) higher than that of the other treated groups and the control. The Hyptis Suaveolens plant appear therefore, to have some hypoglycemic activity and also relatively safe at lower doses.

INTRODUCTION

Traditional use of plant drugs popularly known as herbal remedies, in the treatment of a variety of diseases, is widely practiced in the Nigerian communities as well as in other developing countries. Traditional medicines are used by about 60% of the world population both in the developing countries where modern medicines are predominantly used (Mythilypriya *et al.*, 2007). The exclusive use of herbal drugs for the management of certain ailments continues unabated in most developing communities due to easy access affordability and reported efficacy. Plants, therefore, remain the main source of the active drugs from a natural source and are still indispensable in the traditional medicine for treating a number of diseases. (Ogbonna *et al*, 2008).

Herbal remedies mainly used in the traditional medicine contain active organic compounds and are employed in the treatment of diseases of diverse origins (Mythilypriya *et al* 2007). The practitioners most often prepare the recipes from combination of two or more plant products, which could be used in the treatment of more than one disease condition. They are administered in most disease condition over a long period of time without a proper dosage monitoring and consideration for toxic effects that might result from such a prolonged usage. The danger associated with the potential toxicity of such therapy and other herbal therapies used over a long period of time demand that the practitioners be kept abreast of the reported incidence of renal and hepatic toxicity resulting from the ingestion of medicinal herbs. (Tedong *et al*, 2007).

Hyptis suaveolens called *"Efirin"* by the Yorubas in Nigerian is a valuable and cherished medicinal plant and is widely used by the traditional herbalists. The remedies may be prepared with the leaves alone or in combination with other herbs and used locally in the treatment of diabetes and other diseases. The combination therapy is more common, since most traditional herbal practitioners believe that a combination of many plant products would create the desired synergy. The extract of *H. Suaveolens* indicated presence of alkaloids, tannins, phenols, saponin, flavonoids (Edeoga *et al*, 2006). For a plant or herbal preparation containing active organic principles to be identified for use in the traditional medicine, a systemic approach is required for the evaluation of efficacy and safety through experimental and clinical findings (Mythilypriya et al., 2007).

The aim of this study therefore is to evaluate the safety of *Hyptis Suaveolens* leaf extract by determining the indices of renal and hepatic toxicities in albino Swiss mice.

MATERIALS AND METHODS

Plant Collection

The *Hyptis suaveolens* leaves were collected in the month of July in Minna, Nigeria. Plant sample was

authenticated at the Department of Biological Science Federal University of Technology, Minna. The leave was air dried under shade and powdered to coarse particles.

Plant Extraction

50g of powdered *Hyptis suaveolens* was mixed with 400ml of distilled water and refluxed for two hours. The mixture was filtered and evaporated to dryness on a water bath.

Animals

30 albino mice weighing between 20 and 25g were used for this study.

The animals were allowed to acclimatise for two weeks in the animal laboratory of the department of Biochemistry. They were housed in well ventilated cages and 12 hours light / dark cycle. Twenty of the animals were divided into 5 parallel groups consisting of diabetic and non diabetic pair of 4 animals each. The animals were maintained on poultry feed pellets (Vital Feeds, Jos, Plateau State, Nigeria.) and water given ad-libitum.

Acute Toxicity Test

The acute toxicity study was carried out using twelve females and males Swiss albino mice weighing 25-40g each. The animals were randomly distributed into five groups containing three animals per group and were deprived of food over night. From a stock solution of 1g of extract in 10ml of distilled water, the animals in groups 1, 2, 3, 4 and 5 were orally dosed with 400mg/kg, 800mg/kg, 1200mg/kg , 2500mg/kg and 5000mg/kg respectively. The acute toxicity test was terminated after 48 hours (Shal., *et al*, 1997; Borger., *et al*, 2005).

Sub-chronic Toxicity Test

Using a modified method of Cruz *et al*. (2006) the mice were divided at random into four groups of three mice per group. The experimental groups orally received the *H.suaveolens* aqueous leaf extract at the doses of 400, 800 and 1200 mg/kg respectively, while the control group received normal saline (0.85% of NaCl in distilled water). The animals were observed and weighed weekly for three weeks.

Determination of Blood Glucose

Prior to diabetes induction the mice were subjected to 12 hours fast and blood samples were collected from the tail vein of the mice before and after diabetes was induced with alloxan, the glucose levels were determined using a One Touch glucometer (Lifescan, Inc. Milpas, California, USA). Three days after 00 treatment with alloxan, diabetes was confirmed in the alloxan treated mice with a fasting blood glucose concentration above 200 mg/dl. The blood glucose levels were determined after an oral administration of the aqueous leaf extract of *H.suaveolens*, normal saline and standard diabetes drug (chlorpropamide) twice daily (6am and 6pm). The experiment lasted for 21 days.

Determination of Packed Cell Volume (PCV).

The heamatocrit (PCV) of each sample was determined according to the method of Dacie and Lewis, (1991). Heparinised capillary tube was filled 2/3 of the length with whole blood. One end of the tube was sealed with plastacine, placed in heamatocrit centrifuge and spurn for 5 minutes at 12000 rpm. The PCV was read with the micro heamatocrit gauge.

Determination of Serum Enzymes

The animals were anaesthetized under chloroform vapour. Blood samples were collected by cardiac puncture into clean non-heparinised bottle the serum was separated from the clot, centrifuged and stored in clean sample bottle for biochemical analysis.

Aspartate and alanine transaminase activities were determined with Randox Diagnostic kits using the method of Reitman and Frankel (1957) while alkaline phosphatase activity was determined by the method of Heins (1995).

Statistical Analysis.

Using SPSS 15.0 software, 2006 version, the data obtained from the analysis were subjected to analysis of variance (ANOVA) to ascertain any significant differences amongst the sample groups for each parameter. Duncan multiple range test was used to separate the means that were significantly different.

RESULTS AND DISCUSSION

Table 1 shows the acute toxicity of the aqueous leaf extract of *hyptis suaveolens* in mice. No death was recorded in all doses administered, also no toxic changes were observed for the 400, 800 and 1200 mg/kg doses. The mice treated with 2,500 and 5,000mg/kg body weight however, showed slight dullness and inactivity (morbidity) within the first 6h, but the animal's behaviour later returned to normal.

Acute toxicity test give clues on the range of doses that could be toxic to the animal, it could also be used to estimate the therapeutic index (LD_{50}/ED_{50}) of drugs and xenobiotics (Rang *et al*, 2001). The acute toxicity study in mice showed that at a dose of 5000mg/kg body weight, the plant is safe for consumption and medicinal uses. The absence of mortality in the

different treated groups suggest the plant extract is safe and is supported by the reports of Schorderet (1992) and Iwueke *et al,* 2009), who stated that substances with LD_{50} values greater than 5000mg/kg body weight are considered to show low toxicity. The acute toxicity test therefore show the extract is safe for consumption, since the highest dose (50000mg/kg) body weight did not kill the animals even after 72h. Furthermore, since (50000mg/kg) body weight is the maximum allowable dose by the organization for economic cooperation and development (OECD) guidelines for the testing of chemicals (OECD, 2000). It was not necessary to administer higher doses.

The extract did not enhance weight gain (Figure 1) in the treated animals as there was decrease in weight of all treated animals, there was a progressive weight increase in the control which was however not significant. The decrease in weight was more remarkable in the test animals that received 1200mg/kg dose of the extract. The weight changes of animals during the period of observation was more visible at higher doses, this suggest the presence of anti-nutrients especially tannins and other phenolics which are thought to interfere with absorption of nutrients making them unavailable and thereby reducing feed intake (Kumar and Singh, 1984). The changes in body weight have been used as an indicator of adverse effects of drugs and chemicals (Theo *et al,* 2002). Also some plant metabolites have been reported to control the desire for food under diabetic conditions, (Kim *et al,* 2006). Animal studies suggest that the use of extracts of *H. suaveolens* in high doses may be accompanied with weight loss and toxic effects on the liver (Attawish, *et al,* 2005). A study of water extract of *H. suaveolens* for 6 months chronic toxicity in Wistar rats at five treatment doses failed to produce any dose related changes or significant toxic effects based on heamatologic, biochemical and histopathologic parameters (Attawish, *et al,* 2005).

There was no significant different in the level of PCV (Figure 2), though the level in control was higher than in test animals. The study therefore showed that the plant may induce anaemia if the animals are exposed to this plant for a long period of time (Kachmar and Grant 1992).

Figure 3 shows the level of glucose in both test and control. The extract had a decreasing effect on the blood glucose level, especially at the highest dose in the treated mice indicating the possible presence of hypoglycaemic components in the extract (Sushruta *et al,* 2006). This observation gives credence to the use of *Hyptis Suaveolens* herbal preparation as a hypoglycaemic agent.

TABLE 1: Acute toxicity of aqueous leaf extract of *hyptis suaveolens after 48h*

Group	Dose mg/kg	*T/D	Signs of Toxicity Observed
1	400	0/3	No toxic changes observed
2	800	0/3	No toxic changes observed
3	1200	0/3	No toxic changes observed
4	2500	0/3	Slight dullness was observed but the animals recovered from it.
5	5000	0/3	Slight dullness was observed but the animals later became normal.

*T/D: Number of mice treated against number of death

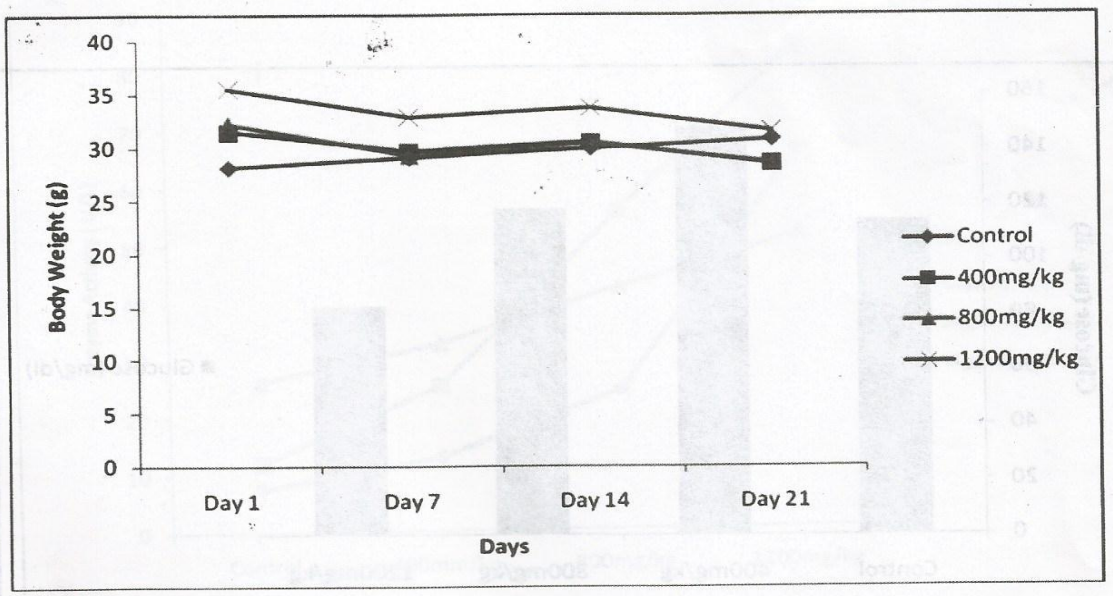

Figure 1: Effects of aqueous leaf extract of *H. suaveolens* on body weight in alloxan induced hyperglyceamia in mice.

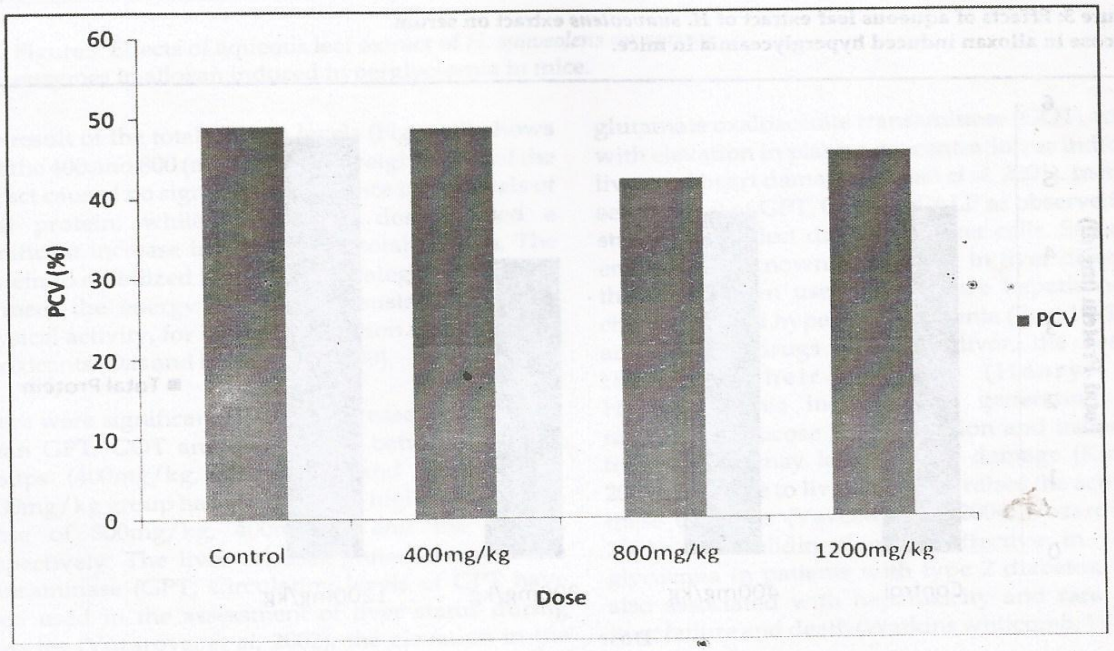

Figure2: Effects of aqueous leaf extract of *H. suaveolens* on PCV in alloxan induced hyperglyceamia in mice.

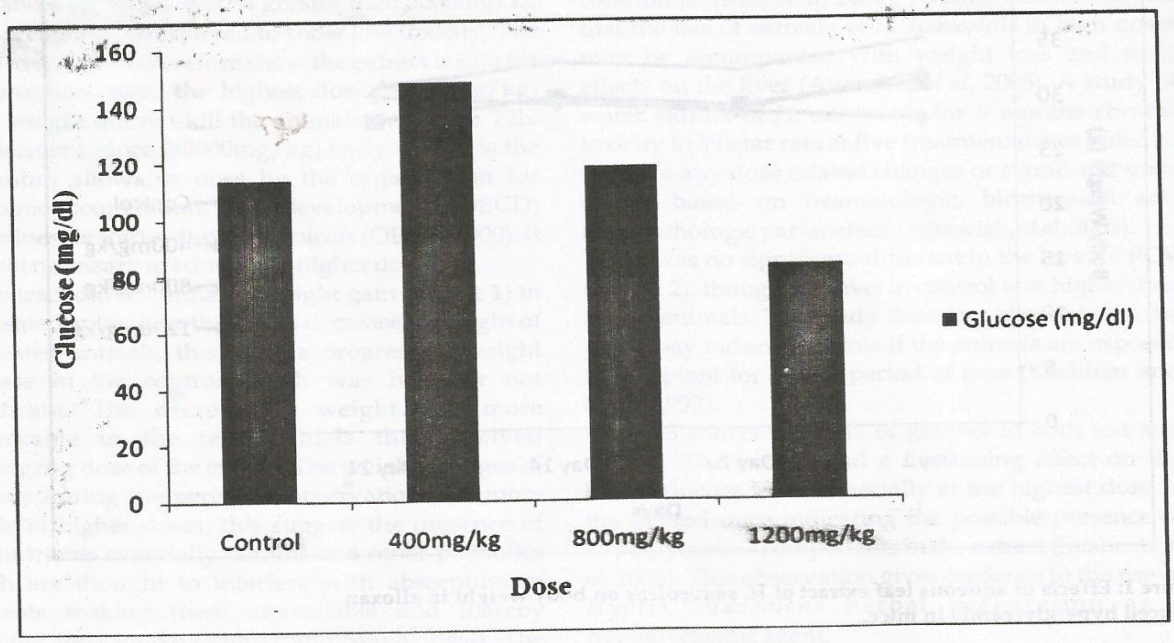

Figure 3: Effects of aqueous leaf extract of *H. suaveolens* extract on serum glucose in alloxan induced hyperglyceamia in mice.

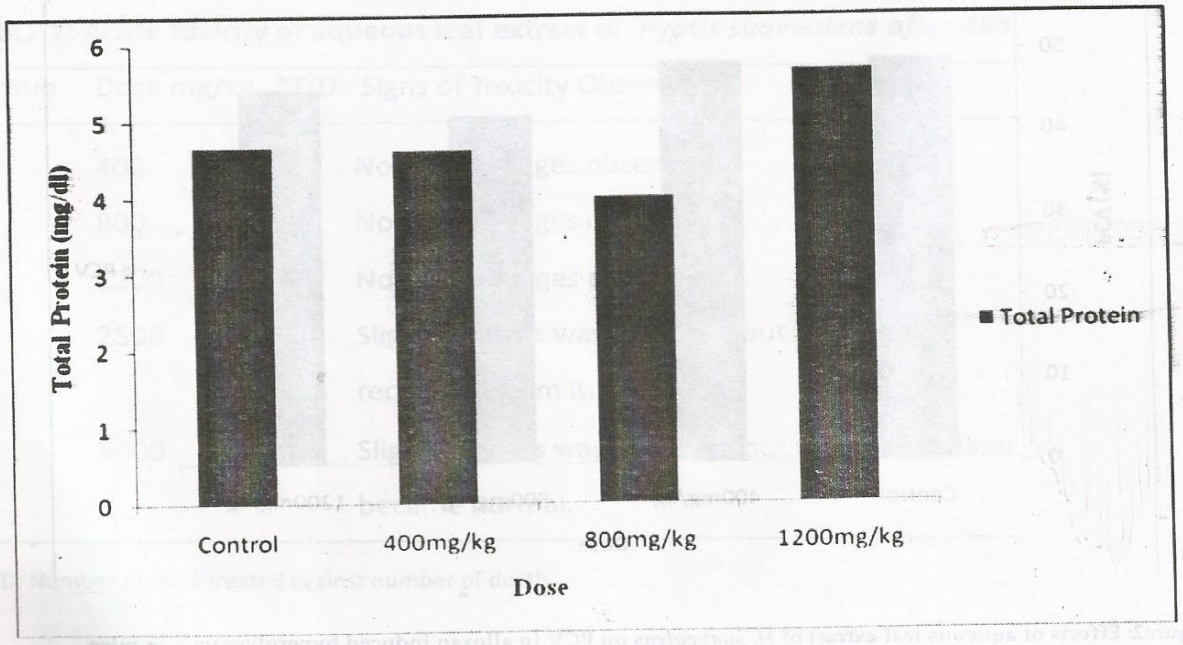

Figure 4: Effects of aqueous leaf extract of *H. suaveolens* on serum total protein in alloxan induced hyperglyceamia in mice.

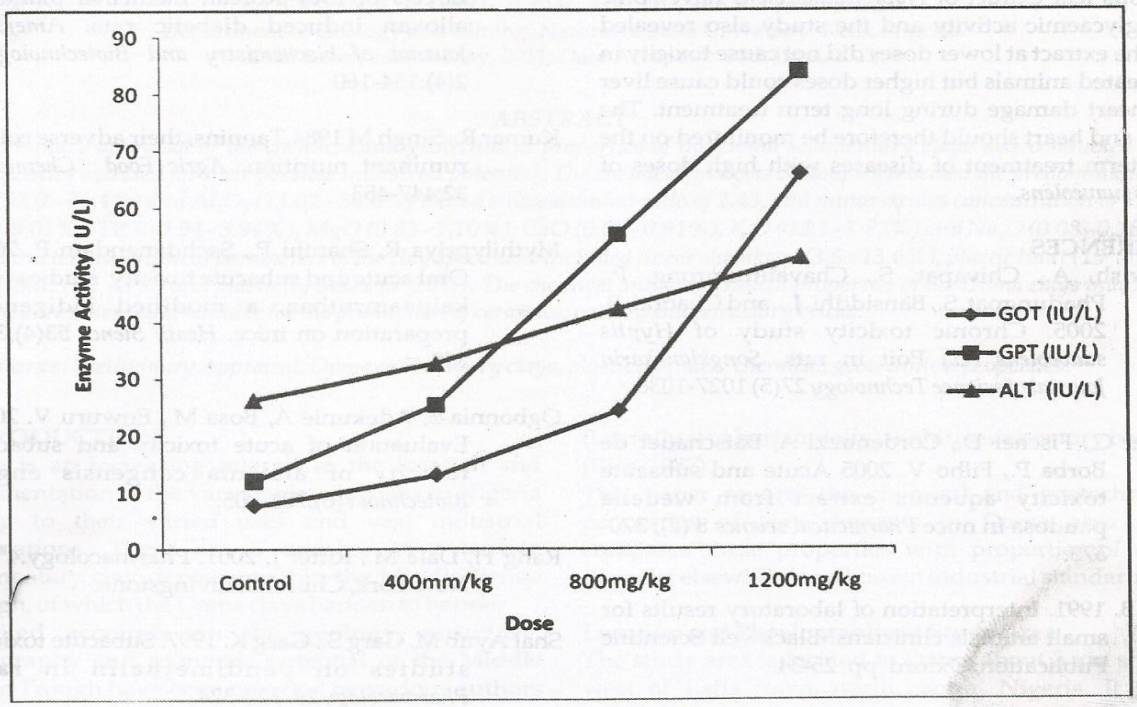

Figure5: Effects of aqueous leaf extract of *H. suaveolens* on serum enzymes in alloxan induced hyperglyceamia in mice.

The result of the total protein levels (Figure 4) shows that the 400 and 800 (mg/kg) body weight doses of the extract caused no significant difference in the levels of total protein, while 1200mg/kg dose caused a significant increase in the level of total protein. The protein so mobilized is one of the strategies employed to meet the energy required to sustain increased physical activity, for biotransformation and excretion of toxicants (Das and Mukherjee, 2000).

There were significant ($P<0.05$) increase in the serum mean GPT, GOT and ALP levels between the test groups (400mg/kg, 800mg/kg, and 1200mg/kg), 1200mg/kg group had significantly higher levels than those of 800mg/kg, 400mg/kg and the control respectively. The liver releases glutamate pyruvate transaminase (GPT. Circulating levels of GPT have been used in the assessment of liver status during diabetes (Vozarova, *et al*, 2002), the elevation in the plasma concentration indicating liver damage in on-set of type 2 diabetes and in experimental diabetes (Naveed *et al*, 2004). The liver and heart release glutamate oxaloacetate transaminase (GOT) and GPT with elevation in plasma concentration as indicator of liver and heart damage (Wasan *et al*, 2001). Increase in serum level of GPT, GOT and ALP as observed in this study may reflect damage of liver cells. Serum liver enzymes are known to increase in liver disease and they have been used to measure hepatic necrosis, cholestasis and hyper-bilirubinemia (Bus, 1991). Most anti-diabetic drugs affect the liver, the heart and therefore their enzymes (Henry, 1997). Hyperglyceamia increases the generation of free radicals by glucose auto-oxidation and increment of free radicals may lead to liver damage (Kim, *et al*, 2006). Damage to liver enzymes raises the activities of these enzymes (Vaveed *et al*, 2004; Vozarova *et al*, 2002). Thiazolidinedione is effective in reducing glycaemia in patients with type 2 diabetes, but was also associated with hepatoxicity and rare cases of liver failure and death (Watkins whitcomb, 1998).

CONCLUSION

From our results in this study we conclude that the aqueous leaf extract of *Hyptis Suaveolens* have some hypoglycaemic activity and the study also revealed that the extract at lower doses did not cause toxicity in the treated animals but higher doses could cause liver and heart damage during long term treatment. The Liver and heart should therefore be monitored on the long term treatment of diseases with high doses of *hyptis suaveolens.*

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