ABSTRACT:
Traditional use of herbs and plants is centuries old and they have shown therapeutic properties. But scientific studies are not done to confirm their efficacy and margin of safety. Acacia was collected from UQU, Makkah. Leaves were separated from shoots and aqueous extract of *Acacia tortilis* was prepared at the concentration of 100mg/ml. *Acacia* aqueous extract was administered at the dose of 800mg/kg in treated rats for seven days. The various parameters studied include blood glucose levels, serum cholesterol levels, HDL, LDL, AST, ALT and Urea. All parameters were estimated using diagnostic kits spectrophotometrically. At the end of study autopsy also performed. Administration of *Acacia* aqueous extract (800mg/kg) for seven consecutive days caused significant (*P* < 0.05) decrease in blood glucose, serum total cholesterol, and LDL level while an increase in serum HDL-cholesterol was observed as compared to their control rats. However a statistically significant (*P* < 0.05) decrease in body weight was also observed. Autopsy did not show any sign of abnormality and all rats were comparable with their control rats. The findings of the present study confirm that above-mentioned plant validates our claim. In the light of our pharmacological and toxicological studies, *Acacia tortilis* appears to be a valuable plant, which can be useful, at least as an adjunct, in the therapy of diabetes and hypercholesterolemia or in a condition in which hyperglycemia and dyslipidemia coexist quite often. Further the toxicological, hematological and autopsy results further confirm the wide marginal safety of *Acacia tortilis* leaves aqueous extract.

Keywords: *Acacia tortilis* leaves aqueous extract, Hypoglycemia, Hypcholesterolemia, Flavanoids.

INTRODUCTION

Hypercholesterolemia and Diabetes mellitus (DM) are the commonest disorder that affects more than 100 million people worldwide (6% of the population) (WHO/Acadia, 1992). A previous population-based national epidemiological study conducted among adult Saudi (age 15 years) reported that the risk of developing hyper-cholesterolemia increased by 2% for each year (Al-Nuaim, 1997). Hypercholesterolemia, high levels of serum triglycerides (TG) are strong, independent predictors for CHD (Hokanson & Austin, 1996 and Haim *et al.*, 1999). Hypercholesterolemia is an important risk factor for atherosclerosis, myocardial infarction or stroke (Morozova *et al.*, 2005). While DM is found to damage many of the body systems, particularly the blood vessels and nerves (Nagappa *et al.*, 2003). For its therapy, various clinical practice guidelines have addressed the treatment of hypercholesterolemia. The American College of Physicians has addressed hypercholesterolemia in patients with, diabetes (Snow *et al.*, 2004). However, along with the synthetic drugs, a change in diet (Tang *et al.*, 1998), weight management, physical activity and many agents of the plant origin are also in use. The National Center for Complementary and Alternative Medicine focused on use of complementary and alternative medicine...
(CAM) suggest that CAM was used to treat hypercholesterolemia by 1.1% of U.S. adults during 2002 (Durrington, 2003). Plants are always an exemplary source of drugs; in fact many of the currently available drugs were derived either directly or indirectly from them. According to world ethnobotanical information reports, almost 800 plants may possess antidiabetic and hypercholesterolemic activity (Alarcon-Aguilara et al., 1998; Iman et al., 2003; Metwally et al., 2009). Now in recent years there has been a growing trend to evaluate the plant constituents on pharmacological basis and screening of more effective and safe hypoglycemic and hypocholesterolemic agents has continued to be an important area.

Therefore the present work have been planned to study indigenous plants (Acacia tortilis) of Makkah using new and sensitive technologies and statistical analysis. Acacia is a genus of shrubs and trees belonging to the subfamily Mimosoideae of the family Fabaceae, first described from Africa by Linnaeus in 1773. Acacia is one of the plant that has been frequently used as medicine like Acacia Bark has been used in treatment of haemoptysis, gonorrhea (Wallis, 1967), leprosy and headaches (Stacey and Michael Chillemi, 2007). Acacia Catechu has been used for treating fever, diarrhoea, leucorrhoea, piles, erysipelas (Kirtikar, 1935), throat infection (Usher, 1984). It is reported that Acacia catechu has hypoglycaemic activity (Singh et al., 1976) hepatoprotective, antipyretic and digestive properties (Chopra et al., 1956; Jayasekhar et al., 1997). Considering the above facts, the present study was undertaken to evaluate the hypocholesterolemic and hypoglycaemic activities of Acacia in experimental animal models.

MATERIALS AND METHOD

**Plant Material:**

Acacia was collected from Umm-Al-Qura University (UQU) in Alawali Makkah, Saudi Arabia in May 2010 and the leaves were separated from shoots and wood. The plant was authenticated by plant taxonomist Dr. Kadry Abdel Khalik, Associate Professor of Plant Taxonomy, UQU, Faculty of Science, Biology Department, Makkah, Saudi Arabia.

**Extraction:**

Fifty grams of leaves of Acacia were washed with 1000 ml of distilled water and rinsed twice with 500 ml of distilled water. To prepare the water extract, 50g of leaves were milled using the blender in 500 ml of distilled water to obtain 100mg/ml. The extract mixture was allowed to mix on shaking water bath at 40°C for half an hour and filtered. The resultant solution (500 ml) or filtrate was then refrigerated. This Acacia tortilis leaves aqueous extract (AAq) used on each day of the experiment. The extract solution was administered orally post esophagus in a dose of 800mg/1000 g of animal concentration of extract 100mg/ml (Meena et al., 2006).

**Animals:**

Twenty Adult Sprague dawley rats (200-250g) of either gender were obtained from animal house of UQU and were housed in groups of 3 per cage for seven days prior to experimentation with free access to food and tap water ad libitum and kept on a 12h light/dark cycle. Each experimental group consisted of ten animals. All animals were housed in an air-conditioned room at 23±1 ºC during the quarantine period.

**Toxicological studies in rats:**

Two groups of Sprague dawley rats (200-250 g) containing ten animals in each group were used in this study. All animals were treated orally p.o. once daily for seven consecutive days.

Group I (5 males and 5 females), was treated with Acacia tortilis leaves aqueous extract (AAq) (800 mg/kg), while Group II (5 males and 5 females) served as control received distilled water only.
Estimation of different biochemical Parameters and Autopsy:

At the end of 7th day all survived rats were anaesthetized with ether and autopsied (Mohamed et al., 2002). The blood samples approximately (4 to 8ml) were withdrawn from cardiac puncture before dissecting the animals with sterile disposable syringe (HI Med, Korea) from all treated and control rats, were left at room temperature for 20 minutes. Then incubated at 37°C for 30 minutes and centrifuged separately at the speed of 3000 rpm for 20 minutes. Supernatants (Serum) were separated out and the residue was discarded. Serum obtained (1-3 ml) was subjected for the study of following parameters. Cholesterol, Glucose, Lactate dehydrogenase (LDH), High density lipoprotein (HDL), ALT/GPT, AST/GOT and Urea.

All these kits were purchased from Spinreact, S.A. Ctra. Santa Coloma, 7 E-17176 SANT ESTEVE DE BAS (GI) SPAIN. Spectrophotometer (APEL, PD 303UV) and Water bath (Gesellschaft fur Labortechnik m.b.H and co D3006 Burgwedel 1) were used to measure the absorbance of light and for incubation.

Statistical analysis:

Changes in serum biochemical levels were compared using student’s t-test. P<0.05 was used as level of significance.

RESULTS

Hypoglycemic activity of Acacia (800mg/kg) in rats:

The observations of present study showed that the oral administration of AAq at the dose of 800mg/kg showed a significant (p<0.05) hypoglycemic effect (Table 1) in normoglycaemic rats that’s was 39% in comparison with their control as shown in Fig 1.

Hypocholesterolemic activity of Acacia (800mg/kg) in rats:

Oral administration of AAq (800mg/kg) showed a fall in the serum cholesterol level (17%) in treated rats, when compared to their control values as shown in Fig 1. The reduction in serum cholesterol level was statistically significant (p<0.05) in comparison with their respective controls. It prevented the elevation of lipid profile significantly (Table 1).

After the administration of AAq (800

Fig. 1. Effect of AAq (800mg/kg) on glucose and lipid profile.
Data are expressed as Percent mean (%) ± SEM. Significant at *P < 0.05
mg/kg) a decrease was observed in serum LDL level (31%) as shown in Fig 1. However, an increase was observed in serum HDL level that was 75% when compared to its control as shown in Fig 1.

**Effect of Acacia on body weight:**
Fig. 2 showed the effect of AAq (800mg/kg) on body weight of rats. During the study the changes in body weight were recorded and compared with their respective control. The results showed that AAq was found to decrease body weight in both male and female rats by 10% (Fig. 2). This decrease in body weight after 7th days treatment was statistically significant (P<0.05). The body weights of the rats before (on ‘0’ day) and after (on 7th day) drug administration were compared using Student’s t’ test (Paired).

**Toxicological studies in rats:**
Toxicological studies of Acacia were carried out in rats. Oral administration of Acacia (800 mg/kg) did not show any change in physical behavior of rats. None of the groups examined showed any mortality. None of these animals showed any sign of toxicity and change physical behavior after first 2 hours.

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**Fig. 2. Effect of AAq on body weight**

Values are expressed as mean± SE (n).

* Show P<0.05 indicate significantly different from control

**Table-1**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>65.63±4.50 (10)</td>
<td>55.49±4.62 (10)*</td>
</tr>
<tr>
<td>Lactate dehydrogenase (LDH)</td>
<td>37.15±6.00 (10)</td>
<td>23.23±2.18 (10)*</td>
</tr>
<tr>
<td>High density lipoprotein (HDL)</td>
<td>83.90±10.9 (10)</td>
<td>113.8±6.80 (10)*</td>
</tr>
<tr>
<td>Glucose</td>
<td>61.08±4.46 (10)</td>
<td>39.67±7.23 (10)*</td>
</tr>
<tr>
<td>Alanine transaminase (ALT)</td>
<td>2.290±0.22 (10)</td>
<td>1.850±0.05 (10)</td>
</tr>
<tr>
<td>Aspartate aminotransferase (AST)</td>
<td>2.870±0.70 (10)</td>
<td>2.877±0.56 (10)</td>
</tr>
<tr>
<td>Urea</td>
<td>34.90±11.0 (10)</td>
<td>32.90±7.86 (10)</td>
</tr>
</tbody>
</table>

All the value have been presented as mean ± Standard error (n)

* Indicate level of significance P<0.05 when compared to control.
Effect of Acacia on different biochemical parameters:
Oral administration of 800 mg/kg dose of Acacia for 7 consecutive days was not found to alter the liver, heart and kidney enzyme significantly. It produced slight but non-significant decrease in the serum ALT/GPT as shown in Table 1, but this decrease was also statistically non significant (p>0.05) as shown in Table 1. Heart profile showed that it increase the serum AST/GOT in comparison to control rats, although this increase was statistically non significant (p>0.05), as shown in Table 1. A non-significant decrease in the serum urea was also observed in the AAq (800 mg/kg) treated rats in comparison to control rats. These reductions were statistically non significant (p>0.05) showing the normal profile of kidney.

Autopsy:
Autopsy revealed that no gross changes were observed in organs like liver, spleen, heart and kidney. Drug did not cause any internal body hemorrhage.

DISCUSSION
In the present study rats were feed AAq (800mg/kg) for 7 consecutive days. The observations showed that the oral administration of AAq tortilis at the dose of 800mg/kg showed significant (p<0.05) hypoglycemic effect (Fig 1). The results indicate the presence of some powerful hypoglycemic activity which suppressed the elevation of sugar level. Previously Singh et al., 1976 showed that Acacia catechu extract decreased blood sugar of normal rats. It is believed that the extract causes the pancreas to secrete more insulin in normal animals (Singh et al., 1976). Another study showed the effect of gum Arabica on carbohydrate metabolism and they found it be beneficial to the diabetic patient; it suppressed the diabetes during feeding period only (Singhal et al., 1982). Acacia coriacea also significantly reduced the initial rise in plasma glucose levels (p<0.05) and the area under the plasma glucose curve (p<0.005) in six non-diabetic subjects (Thorburn et al., 1987). Some other studies also showed that flavonoids, alkaloids and saponins which are present in Acacia tortilis leaves have been documented to have blood glucose reduction effect (Bolkent et al., 2000; Diatewa et al., 2004). These findings were in accordance with the present results confirming the hypoglycemic effect of Acacia tortilis (Fig 1). In the present study AAq (800mg/kg) induces hypocholesterolemia in normal rats. It also prevented the elevation of lipid profile significantly because administration of AAq decrease the level of LDL and increase the level of HDL significantly (Table 1).

The mechanism(s) of hypolipidemic effect of the Acacia tortilis leaves may be similar to some of those proposed previously by Stacey and Michael Chillemi (2007), they suggest that fruit is rich in essential fatty acids and is said to be very beneficial for good coronary health. β-sitosterol (beta sitosterol) and phytosterol that competes with dietary cholesterol for absorption, it is thought to help to reduce blood cholesterol levels. Moreover, during the present study AAq is believed to improve coronary healthy by elevating high-density lipoprotein levels (HDLs or "good" cholesterol) in the blood, while decreasing the low-density lipoproteins (LDLs, or "bad" cholesterol). It may reduce the risk of arteriosclerosis, stroke and heart attacks (Gordon et al., 1989).

Phytochemical analysis has established that leaves of Acacia tortilis contained a variety of phenolic compounds (flavones, flavonols, flavonones, anthocyanins, leucoanthocyanins, coumarins and tannins) and alkaloids (tertiary and quaternary alkaloids) in varying concentrations (Nakafuero et al., 2007). In agreement with our results, that ingestion of polyphenols of red wine (Pal et al., 2003) and green tea (Bursill et al., 2001) increase LDL-receptor expression in a human hepatocyte cell line in culture. In addition, they interfere with solubilization of cholesterol in the digestive tract of rats, thereby reducing cholesterol absorption (Raederstorff et al., 2003) thus
Hypoglycemic and hypocholesterolemic effects of Acacia tortilis

In animals treated with AAq (Fig 2) a decrease in body weight was also observed which might be related to a decrease in serum cholesterol or glucose level. It is also reported that Acacia tortilis contain flavanol (flavan-3-ol) monomers (Nakafeero et al., 2007), have a tendency to decreased body weight and waist circumference (Chantre and Lairon, 2002).

Therefore the present study suggests that administration of AAq (containing flavanol catechins) inhibits the formation of oxidized lipids such as LDL, and thus decreasing the risk for developing arteriosclerosis. Moreover, catechin intake decreases body fat. These results suggest that catechins by decreasing the body weight (Fig 2) contribute to the prevention of diseases, particularly obesity.

The present study suggests that extracts of Acacia tortilis leaves (AAq) possess significant hypocholesterolemic and hypoglycemic activity. Keeping this view in mind the autopsy and toxicological studies were carried out.

Oral administration of AAq (800mg/kg) was found to be safe because no apparent toxicity was observed under the experimental conditions it did not cause lethality or organ toxicity (liver, heart and kidney). Although to confirm the toxicity of Acacia tortilis leaves extract biochemical studies were carried out showed that Acacia did not affect other blood chemistry parameters. The non-significant data collected after 7days were interpreted as biological variability normally observed in rats.

CONCLUSION

In conclusion, this study is unique; showed that oral administered aqueous extract (AAq at the dose of 800mg/kg) for 7 days only causes significant hypoglycemia and hypolipidemia in normal rats. In the light of present pharmacological and toxicological studies, Acacia tortilis appears to be a valuable plant, which can be useful, at least as an adjunct, in the therapy of diabetes and hypercholesterolemia or in a condition in which hyperglycemia and dyslipidemia coexist quite often. Further the toxicological, hematological and autopsy results further confirm the wide marginal safety of Acacia tortilis leaves extract.

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