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Effects of Camellia sinensis Leaves Extract on Some Biochemical Parameters in Alloxan-Induced Diabetic Rats

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Abstract
This study was designed to evaluate the effect of aqueous leaves extract *Camellia sinensis* on serum glucose levels and liver enzyme markers in alloxan-induced diabetic rats. Diabetes was induced by a single dose of intraperitoneal injection of 150mg/kg bwt of alloxan, and 100, 200 and 400 mg/kg bwt of the extract was administered orally to different groups of diabetic rats for 3 weeks. A significant decrease ($p<0.05$) in serum glucose, Total bilirubin (TB) and the liver marker enzymes (Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and alkaline phosphatase (ALP)) was observed in groups 4 and 5. The hypoglycemic effects as well as its effects on liver enzymes makes it an attractive recipe for the prophylactic treatment of diabetes.
Keywords: *Camellia sinensis,* diabetes, liver marker enzymes, rats.

1. Introduction

Diabetes mellitus is an increasingly common but serious metabolic disorder that has become a global issue. It is characterized by a state of insulin deficiency that leads to a rise in glycemia (Gupta *et al* 2005), initially involving changes in carbohydrate metabolism and secondarily of lipids and proteins (Fontes 2002).

Many hypoglycemic agents, such as the biguanides and sulfonylureas, are used alone or in combination with insulin to treat this disease. However, these medications can cause serious side effects (Hwang *et al*., 2005), this motivate a search for safer and more efficacious agents to control diabetes. In recent years, interest has increased in using natural products for pharmacological purposes, as a form of complementary or replacement therapy. and literature has show that there are more than 400 plants species showing antidiabetic activity (Arulrayan *et al*, 2007), that are categorized on the basis of plant's part.

Published reports have also shown that numerous extracts obtained from plants parts are effective hypoglycemic agents, with lesser side effects at lower cost than the usual antidiabetic agents (Pushparaj *et al*, 2000; Gupta *et al* 2005, Sohn *et al*., 2010).

Tea is one of the most widely consumed beverages in the world, second only to water, and its medicinal properties have been widely explored. The tea plant, *Camellia sinensis*, is a member of the Theaceae family, It is a native to mainland of China, South and Southeast Asia, but it is today cultivated across the world in tropical and subtropical regions (Wakawa and Ira, 2015).

Camillia sinensis is used traditionally in China and other Asian countries to cure puritis, urine retention, clears phlegm, thirst, internal heat, fatigue, sleeplessness, calms spirit, clears vision, quenches thirst, dispels summer heat, detoxifies poison, aids in digestion, sobers drunkenness (Brenton et al, 2004)**.** Several studies suggest that *Camellia sinensis* may contribute to a reduction in the risk of cardiovascular disease and some forms of cancer (Sakanaka 1997, Kno *et al* 2000, Dulloo *el al* 2000, Levites *et al* 2003, Cabrera et al 2006).

Thus the increasing use of medicinal plants in the treatment of many diseases is due to the extraction and development of several successful drugs and chemotherapeutic agents from plants as well as its availability, affordability and their minimal adverse effects and low costs (Mahesser *et al*, 2010).

This study is undertaken to appraised the effects of aqueous leaf extract of *Camellia sinensis* on glucose level and marker enzymes of liver injury, a highly specialized organ that regulates a wide variety of high-volume biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions in humans

2. Materials and Methods

2.1. Chemicals

Diagnostic kits for serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), alkaline phosphatase (ALP) and billirubin were purchased from Randox Laboratories Ltd. and other chemicals and solvents were of highest grade commercially available.

2.2. Camellia sinensis leaves

Freshly harvested leaves of the *Camellia sinensis* was used for the preparation of the crude extract. It was collected from highland tea farm land in Sarduana Local Government area of Taraba State-Nigeria (in the month of June). It was authenticated in the plant science department of Modibbo Adama University of Technology Yola and then it was dried under room temperature.

2.3. Drug preparation

The freshly dried leaves of *Camellia sinensis* was grounded into fine powdered form using laboratory mortar and pestle. 200g of the powder was weight and mixed with distilled water about three times volume and boil for 5-7min. The extract was filter through whatman filter paper (No 4) and the filtrate obtain was concentrated using rotary evaporator. It was then stored under frozen condition until use.

2.4. Breeding of Animals

A total number of twenty-five (25) male albino rats weighing between 90-150kg were purchased from the animal farm, National Veterinary Research Institute Vom, Jos Plateau State-Nigeria. They were housed in cages at room temperature under 12/12 night/dark and were fed with pelleted standard laboratory feed (Vital Feeds, Grand cereals and oil mills Jos) and water *ad libitum.* They were allowed to stay for 7days to acclimatize before the commencement of the work.

2.5. Experimental protocol

The rats were randomly divided into five (5) groups of four (4) rats per group and were given the extract as follows

Group 1: Normal Control (diet/water)
Group 2: Rats were given single dose of 150mg/kg bwt alloxan +diet/water
Group 3: (treated). Rats were given 100mg/kg b.wt. Leaf extract + 150mg/kg bwt alloxan +diet/water
Group 4: (treated). Rats were given 200mg/kg b.wt. Leaf extract + 150mg/kg bwt alloxan +diet/water
Group 5: (treated). Rats were given 400mg/kg b.wt. Leaf extract + 150mg/kg bwt alloxan +diet/water

The single dose of 150mg/kg bwt alloxan was administered intraperitoneally to induce diabetes (Akhtar *et al*., 2002)

2.6. Blood Collection

All the rats from the various groups were sacrificed using standard laboratory procedures and then blood samples were collected via ocular vein into clean containers and allow to stand for 10min. It was then centrifuged at 3000 rpm for 15min to obtain serum. The serum was then separated for the estimation of glucose and liver marker enzymes (transaminases and alkaline phosphatase) and total bilirubin.

2.7. Statistical analysis

All the data generated from the study was subjected to statistical analysis and the result was expressed as Mean + SEM. Student t-test was used to determine the statistical difference between 2 mean values at 95% level of confidence ($p<0.05$).

3. Results

The result is the table below show the various effects of aqueous leaves extract *Camellia sinensis* on serum glucose levels and liver enzyme markers in alloxan-induced diabetic rats. The results of the liver marker enzymes are an indicative of the functioning of the liver in diabetic state.

The effects of the plant extract on these liver function indices are in varying doses administered to different groups of diabetic and normal rats to see also if the plant has a dose dependent effect. It also shows the effects of the plant extract on blood glucose level and total bilirubin under diabetic state and if it is as well dose dependent.

Table 1

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
AST (µ/L)	13.33 ± 4.37	29.00 ± 9.86[a]	24.00 ± 2.65	21.50± 1.50**	19.39 ± 7.53[c,d]
ALT (µ/L)	11.67 ± 1.76	20.33 ± 4.67*	20.03 ± 0.83	16.50 ± 0.96[c]	15.63 ± 1.38[c]
ALP (µ/L)	210.13± 3.50	285.30±3.38*	277.24±17.19	241.36±14.81[c]	244.72±11.96[c]
Glc mmol/L)	10.67 ± 0.20	19.10 ± 0.08*	15.26 ± 1.06	14.97 ± 0.34[c]	14.73 ± 0.38[c]
TB (mg/dL)	14.80 ± 0.69	22.30 ± 0.12*	19.65 ± 0.65	16.82± 0.87**	17.72± 0.87**

Results are Mean ± SEM (n= 4), *Significantly different from group 1, ** Significantly different from group 2, c: Significantly different from group 2, d**:** Not significantly different from group 3

4. Discussion

Hyperglycemia is strongly associated with diabetic complications and its effective control is an important goal in the management, treatment and prevention of diabetes. *Camellia sinensis* has been on the focus with great interest due to its beneficial properties in the prevention and treatment of human disease (Wakawa and Ira 2015). Several epidemiological and experimental studies have provided evidence that green tea has antioxidant activity, anticarcinogenic potential, antidiabetic activity and preventive effects towards cardiovascular disease (Shokrzadeh *et al*., 2009) and liver damage (Wakawa and Ira 2015)

This study was carried out to determine the effects of *Camellia Sinensis* leaf extract on blood glucose level and some indices of liver function in diabetic rats. The table of results showed an observed relative increase in the levels of the parameters in group 2, which may be attributed to the diabetic state or the effect of alloxan used to induce diabetes in the rats.

There is an observed decrease in the mean values of AST and ALT in groups 4 and 5 relative to the observed increase in diabetic induced rats. The administration of *Camellia sinensis* leaf extract also significantly reduced the level of glucose and total bilirubin in Group 4 and 5

It was observed that pre-treatment of liver with the extract of *Camellia sinensis* caused a relative difference in the concentration of the marker enzymes and bilirubin concentration which is an index of jaundice (a condition indicating liver injury), possibly due to either increase production or decrease uptake by the liver, decrease conjugation and secretion from the liver or blockage of bile duct which might be as a result of toxicant administration (Wakawa and Ira 2015)

The findings in this study clearly showed that the oral administration of green tea extract improved the levels glycemic control as observed in other studies (Wolfram *et al* 2006, Igarashi *et al*.2007). It was suggested that EGCG (epigallocatechin gallate) one of the catechins in *Camellia sinensis*, enhances oral glucose tolerance in severely diabetic mice (Wolfram *et al* 2006)

The increase in insulin-stimulated glucose uptake, inhibition of the intestinal GLUT system and decrease in expression of genes that control gluconeogenesis are the possible mechanisms observed for the hypoglycemic effect of *Camellia sinensis.* Numerous studies have demonstrated that green tea and its constituent phytochemicals (catechins) possess antioxidative properties as they improve total antioxidant capacity, suppress destructive oxygen free radicals and prevent oxidative stress damage (Cabrera *et al* 2003, Mustata *et al* 2005, Nagao *et al* 2005, Juskiewicz *et al* 2008). It was demonstrated that green tea consumption increases the plasma total antioxidant activity and improves the actions of liver enzymes (Coimbra *et al* 2006)

Thus in this study, it is observed that blood glucose level reduced significantly and the actions of liver enzymes improved.

5. Conclusion

From the above study and results, it shows that the oral administration of *Camellia Sinensis* leaf extract has a hypoglycemic effect and also maximizes the activities or functions of important liver enzymes. It also shows that the prolonged use and high consumption of this leaf extract will invariably cause hypoglycemia when not checked. Therefore, from the results, it might be safe to conclude that the *Camellia Sinensis* reduces blood sugar level.

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