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## Evaluation of antioxidative and antidiabetic potential of *piper betle leaf* in alloxan induced diabetic albino Rats

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### Original Research Article

### ABSTRACT

*Piper betle leaf* is deep green heart shaped belongs to family *Piperaceae*. This plant is used for the treatment of different diseases like diabetes, cancer, inflammatory responses, oxidant injuries etc. in the present study ethanolic extract of *Piper betle leaf* was used (300 mg/kg). Diabetes was induced in male albino rats by using Alloxan (150 mg/kg). Glutathione (GSH), Catalase (CAT), Malondialdehyde (MDA), Nitric oxide (NO), micronutrients (Vitamin A, Vitamin C and Vitamin E), serum glucose level and Advance glycation end products (AGE's) were estimated. The results showed that levels of the Malondialdehyde (0.087, 0.151 and 0.104 respectively), Advance glycation end products (0.026, 0.053, 0.025) and Nitric oxide (0.07, 0.438, 0.190) were found decreased while level of the GSH (0.184, 0.188, 0.207), CAT (0.087, 0.031, 0.091) and vitamin A (0.196, 0.136, 0.178), E (0.199, 0.140, 0.179) and C (0.163, 0.120, 0.164) were increased after administration of *Piper betle leaf* extract. Blood glucose level was also found to be decreased. It is concluded that *Piper betle leaf* can be used as antidiabetic drug because it is involved in lowering the blood glucose level and *Piper betle leaf* is a good source of anti-oxidants.

**Keywords:** Diabetes, Alloxan, Antioxidants, ROS, Albino Rats

### INTRODUCTION

*Piper betle leaf* usually known as pan and more than 14 million people use this daily Ekta *et al.*, (2016). These are climber, perennial and tropical shade loving plant. It is yellowish green to dark green and have a shiny epidermis. Due to the presence of some essential oils betle leaves are aromatic and varied taste from sweet to pungent (Ekta *et al.*, 2016). Many biologically active compounds are present in *Piper betle leaf* (Sripardha, 2014). It need well- drained and fertile soil for good crop. Main parts of *Piper betle leaf* plant like leaves, root, stems and fruit are usually used in various forms. It has sharp burning taste, good smell, improves taste and appetite, tonic to brain, heart and liver. *Piper betle leaf* shows various properties like anticancer, antiplatelet, anti-diabetic, antioxidant, antifungal, anti-inflammatory and antimicrobial, antifertility, antimalarial, anti-amoebic,

anti-hyperglycemic and radioactive properties. Chemical constituents are carbohydrates, steroidal components, alkaloids, tannins and amino acids. It also contains vitamins like riboflavin, nicotinic acid, vitamin A thiamine, vitamin C, and some minerals such as iodine, iron (0.004-0.006), phosphorus (0.04-0.7%) potassium (0.25-4.5%) calcium (0.1-0.4%) (Dakshina and Aditya, 2016). Ethanolic extract of *Piper betle leaf* is very effective to prevent the lipid peroxidation (Rekha *et al.*, 2014).

Hydroxychavicol is a 1-allyl-3,4-dihydroxybenzene and it is a major phenolic compound in *Piper betle leaf* which possess various properties like anti-platelet, antioxidant, anti-bacterial, anticancer, anti mutagen, antiinflammatory, and antifungal (Prabodh and William, 2012). The brief study of *Piper betle leaf* extract has reported that it contains large numbers of bioactive molecules. Concentration of biologically active compounds present in *Piper betle leaf*

depends on the variety of the plant, season and climate.

Some investigations reported that *Piper betle leaf* has high anti-diabetic effects. *Piper betle leaf* can be used in the treatment of diabetes (Grazia *et al.*, 2013). When extract of *Piper betle leaf* is given orally to the diabetic patients for a week than it results in lowering the blood glucose level. (Grazia *et al.*, 2013). All the living organisms including human beings uses antioxidative defense system which protect them against oxidant damage. Human body cells utilize the antioxidant compounds which are involved in the reaction with oxidizing agents and deactivate them. Vitamin C and Vitamin E are both considered as major membrane bounded and aqueous-phase anti-oxidants respectively. Catalase (CAT), superoxide dismutase (SOD) and Glutathione peroxidases (GSH) are main enzymatic anti-oxidants (Badrul *et al.*, 2012).

**MATERIALS AND METHODS**

**IN-VIVO ANTIDIABETIC STUDY**

**Place of Work**

All experimental work was done in school of Biochemistry, Faculty of Allied Health Sciences, Minhaj University Lahore, after the approval of University Ethical and Research committee.

**Induction of Diabetes**

Diabetes was induced in Rats by using Alloxan (150 mg/kg)

**Study Design**

Comparative study. Present study was consisted of three Groups of rats. Each group contain 10 male albino rats.

<b>Group A (n=10)</b> (B.W = 200 gm)	<b>Group B (n=10)</b> (B.W = 200 gm)	<b>Group C (n=10)</b> (B.W = 200 gm)
Normal Control	Diabetic Control	Diabetic was treated with <i>Piper betle leaf</i> Extract for (21 days)

**Inclusion criteria**

Physically healthy rats are included in this study (200gm body weight, Male albino rats).

**Exclusion criteria**

Physically unhealthy, injured and low body weight (<200gm) rats are excluded from this study.

**Preparation of plant extract**

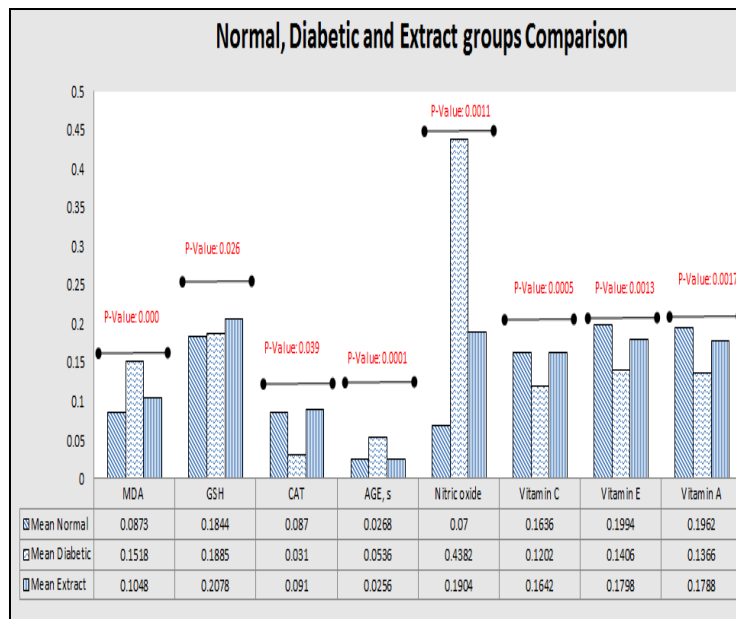
Fresh leaves of *Piper betle leaf* were collected and washed under distilled water. The leaves were powdered and soaked in 70% ethanol and shaken vigorously. The solution was filtered by using Whitman’s filter paper no1 and allowed the ethanol to evaporate completely. The resultant extract was stored in sterile glass vials at 4°C for subsequent studies.

**Blood/Sample Analysis**

Blood samples (5.0 ml) of each rat from all three groups of rats were taken in clotted gel vial. Blood was centrifuged at 4000 rpm for 10 minutes and serum was separated. It was further processed for the estimation of Reduced Glutathione (Moron *et al.*, 1979), Catalase (Aebi, 1974), Malondialdehyde (Ohkawa *et al.*, 1979), Nitric oxide (Moshage *et al.*, 1995), micronutrients i.e. Vitamin A (Rosenberg *et al.*, 1992), Vitamin C & Vitamin E (Roe and Keuther, 1943), Serum glucose level and Advance glycation end products (AGE’s) and their biomarkers.

**Statistical Analysis**

Statistical analysis was done by using SPSS (Ver.16).



**Fig.1.** Graphical Representation of Normal, Diabetic and Extract Groups of Albino Rats

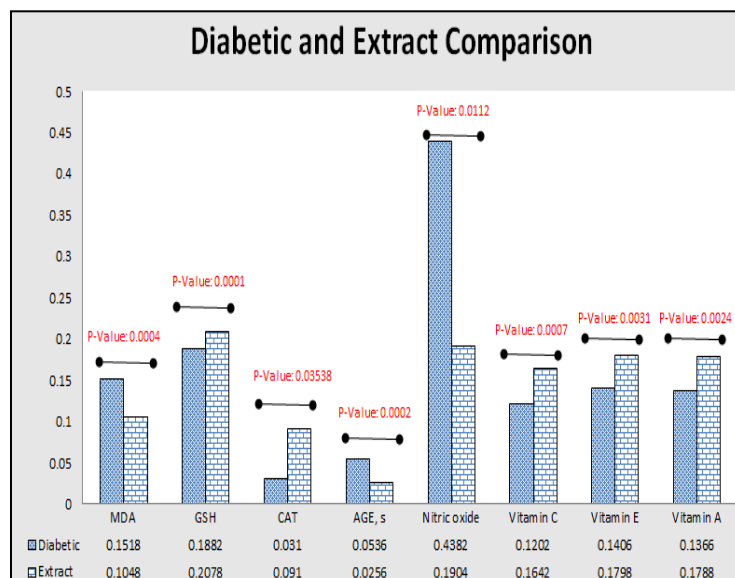
It was observed that average values of MDA for normal group was 0.087, diabetic group was 0.151 and extract group was 0.104 (Figure 1). The F value for testing of ANOVA was 24.865 and

its P value was found to be 0.000054 which was less than 0.05. This shows that *Piper betle leaf* extract decreased the lipid peroxidation by lowering the MDA level in rats. For GSH it was observed that average values for normal group was 0.184, diabetic group was 0.188 and extract group was 0.207. The F value was 5.904 and its P value was found to be 0.026686. It was observed that *Piper betle leaf* extract increased the serum GSH level in extract induced group as compared to diabetic group.

Average values for normal group was observed to be 0.087, diabetic group was 0.031 and for extract group was 0.091 for CAT as shown in Fig-1. The F value was 4.197 and its P value was calculated to be 0.039337 which was less than 0.05. Serum level of advanced glycation end products (AGE,s) in normal group was observed to be 0.026 while in diabetic group was 0.053 and in extract group was 0.025. The F value was 20.027 and its P value was found to be 0.000150. This shows that *Piper betle leaf* extract is involved to decrease the serum AGE, s level. Serum Nitric Oxide level in normal group was 0.07 whereas in diabetic group was 0.438 and in extract group was 0.190. The F value P value were in normal range as shown in Fig-1. Serum NO level was decreased in the plant extract group as compared to diabetic rats.

For vitamin C average values for normal group was 0.163, diabetic group was 0.120 and extract group was 0.164. The F value was 15.058 and its P value was found to be 0.000535 which was less than 0.05. This shows that *Piper betle leaf* extract had increased the serum vitamin C level as compared to diabetes induced group. It was measured that average values for normal group was 0.199, diabetic group was 0.140 extract group was 0.179. The F and P value were found to be in normal range. This shows that plant extract had elevated the serum vitamin E level than the diabetic group as shown in figure-1. Results demonstrated that average values of Vitamin A in normal group was 0.196, diabetic group was 0.136 extract group was 0.178. Increased level of serum vitamin A reveals that *Piper betle leaf* might be rich source of vitamins. Results shows that blood glucose for normal group was observed to be 115.8, diabetic group was 128 and extract group was 108 as shown in Figure-1. Data reveals that *Piper betle leaf*

extract involved to decline the blood glucose level in extract group as compared to the diabetic group.



**Fig. 2.** Graphical representation of different biochemical markers in Diabetic and Extract groups of Albino Rats

T test was applied for comparing the different variables among two groups that was Diabetic and Extract group. The data for all the marker such as MDA, GSH, CAT, AGEs, NO, and various vitamins i.e. E, A and C has been shown in figure-2. T value and P values of all the parameters have also been shown in figure-2. All the values were in normal range for these two tests as shown in the Fig-2.

## DISCUSSION

*Piper betle leaf* have unique properties that made it valuable part of plant and is also known as medicinal plant and have potential to fight against pathogens. A lot of studies on the leaf of betle plant demonstrate that it contained many bioactive compounds. Extracts of *Piper betle leaf* are used for the treatment of different diseases due to its essential properties like, antidiabetic and antioxidative. Studies demonstrated that *Piper betle leaf* extract was used for the treatment of diabetes and oxidative stress. *Piper betle leaf* was found to be beneficial in lowering the blood glucose and lipid peroxidation levels (Mannan *et al.*, 2014).

Present study shows that by giving the

*Piper betle leaf* extract (300 mg/kg) for three weeks to alloxan induced diabetic rats, blood glucose level was significantly decreased which showed the antidiabetic activity of *Piper betle leaf*. Many studies reported that diabetic mellitus showed higher levels of oxidative stress due to high blood glucose level in human beings and in diabetic animal models. Due to the formation of free radicals anti-oxidative system of body disturbs which results in high oxidative stress. High glucose level inhibited the anti-oxidative enzymes to perform their proper functioning (Mannan *et al.*, 2014). *Piper betle leaf* contained phenolic compounds which control the production of free radicals and reactive oxygen species (ROS) which showed anti-diabetic properties. *Piper betle*, *Piper longum* and *Piper nigrum* belong to the family *Piperaceae* and it was reported that they showed antidiabetic activity. In present study ethanolic extract of *Piper betle leaf* were used whereas some other scientist used aqueous extract of *Piper betle leaf* and explored that *Piper betle leaf* was involved in lowering the blood glucose level (Nalina and Rahim, 2007).

According to the present study extract of *Piper betle leaf* in ethanol had more potential to overcome the oxidative stress and act as good source of antioxidants as compared to other solvents such as acetone, aqueous and methanol extract. These results showed that *Piper betle leaf* plant contained polar compounds which are more soluble in ethanol solvent as compared to others. The results of present study is complimentary with others in such a way that in present study, freshly prepared alloxan monohydrate was injected in male albino rats to induce the diabetes. *Piper betle leaf* has a high antioxidant capacity and contains several bioactive compounds.

In the present studies malonaldehyde (MDA) level was observe to decrease by giving *Piper betle leaf* extract to alloxan induced diabetic albino rats and results were significant. Malondialdehyde (MDA) is considered as an important biomarker for lipid peroxidation. By giving the oral doses of *Piper betle leaf* extract it was observed that it significantly reduced the level of malondialdehyde (MDA) by comparing the control group. Increased level of malonaldehyde (MDA) increases the lipid peroxidation process which results in tissue damage and anti-oxidant defense system of body disturbs. Present study shows that *Piper betle leaf* extract involved in the

mechanism of hepato-protection due to its anti-oxidant activity. Results of present study are also significant and show increased level of Glutathione (GSH) and Catalase (CAT) after the orally administration of *Piper betle leaf* extract to albino rats.

Present work shows that *Piper betle leaf* is good source of vitamins. Extract of *Piper betle leaf* was given to the diabetic albino rats for three weeks and increase in the values of vitamins A, C and E was observed. The level of advance glycation end products (AGE,s) in blood serum is significantly decreased. Values for advance glycation end products (AGE,s) is significantly low in those albino rats which were treated with extract of *Piper betle leaf*. Reports of previous researches showed that *Piper betle* is famous plant having many important constituents. Advanced glycation end products (AGEs) and Protein glycation production are linked with several pathophysiological conditions such as diabetes mellitus. *Piper betle leaf* extract reduced the glycation process which is induced due to high glucose level. *Piper betle leaf* contain many phenolic compounds. It was investigated that these phenolic constituents can play important role as anti-glycation property. The extract of *Piper betle leaf* is supposed to have medicinal property to inhibit the protein glycation process. It was also observed that all extracts of *Piper betle* leaves showed direct removing ability of Nitric oxide (NO). *Piper betle leaf* contain constituents such as, hydroxychavicol, alpha-tocopherol and eugenol which can reduce NO level on the albino rats. So it is confirmed that *Piper betle leaf* have ability to increases the levels anti-oxidants (Narong, 2015).

## CONCLUSION

Diabetes is the most burning issue now a day's all over the world. In present study male albino rats were injected with alloxan drug to induce diabetes. Serum tests of diabetic rats showed increased level of glucose, MDA, AGE, s and NO while decreased level of GSH, CAT and vitamins. It is concluded that *Piper betle leaf* can be used as antidiabetic drug because it is involv in lowering the blood glucose level. It is also revealed that *Piper betle leaf* plant is a good source of antioxidants and also involve in decreasing the MDA, AGE, s and NO level while increases the GSH, CAT and vitamins A, E and C level.

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