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# Hepatic and Glucose Biotolerance Induced by the Aqueous Extract of Leaf of *Parkia biglobosa* in Rabbit

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## Abstract

The aqueous extract of leaves of *Parkia biglobosa* (Pb) is used in Africa to cure hypertension or for the treatment of immunocompromised individuals. To avoid intoxication of consumers, our study was carried out to evaluate the action of the extract on the level of glucose and liver enzymes [Akaline phosphatase (ALP), Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT)]. Two sets of five (5) batches of six (6) rabbits were constituted. The first set each received a single dose of Pb (25, 50, 75 and 100mg/kg body weight or BW) and 0.9% NaCl. The second set were given Methylprednisolone 15mg/kg BW (MP15) and mixtures MP15 and Pb (25, 50, 75 and 100 mg/kg of BW). Rabbit whole blood were collected in dry tubes from the marginal ear vein on the first day before injection (Do), then the 3rd, 9th, 15th and 21th days, was centrifuged. The serum obtained was used for assay of glucose, transaminases and ALP on *automate Cobas Integra 400 Plus*®. The doses of Pb 25, 50, 75mg/kg of BW did not induce significant changes in transaminases (ALT and AST), ALP and glucose compared to control group (0.9% NaCl) from D0 to D21. However, the dose of 100 mg/kg produced significant increase in transaminases and glucose ($p<0.05$) levels. The doses of aqueous leaf extract of *Parkia biglobosa*used were tolerated by the liver and did not involve an hyperglycaemia.

**Keywords:** Akaline Phosphatase, Glucose, *Parkia Biglobosa*, Rabbits, Transaminases

## Introduction

In developing countries, majority of the population used traditional herbs to cure their ailment. The practice of African medicine has lots of metaphysical and religious resonances which have unfortunately led to some misconceptions and misgivings (Etim, 2012). However, this medicine remains very effective. To this end, *Parkia biglobosa (Jacq) Benth. (Mimosaceae*) has several therapeutic properties depending on the part of plant used. According to Millogo-Kone *et al*., 2007, the leaves of *Parkia biglobosa* used in recipes in Africa for the treatment of shingles and the decoction of the bark of the trunk at a lower dose induced antimicrobial action . On this plant (*Parkia biglobosa*), Adjanohoun *et al*., 1987 reported several healing properties of the bark of *Parkia biglobosa* such as the treatment of amoebiasis, hookworm, the arscadiose, asthma, infertility and peptic ulcers. Studies have shown that hexane extracts, methanol and aqueous extract of bark have analgesic and anti inflammatory properties (Kouadio *et al*., 2000).

In addition, *P. biglobosa* seeds are used as local food condiment (Esenwah and Ikenebomeh, 2008) called "dawadawa" in Ghana, "soumbala" in Burkina or "iru" in Togo and Nigeria (Pelig-Ba, 2009). Moreover, there was also a relationship between the nutritional and medicinal properties of *Parkia biglobosa.* That corroborates the writings of Waziri *et al*., 2012 according to which, edible fruits have been used worldwide for decades as herbal medicines with the therapeutic and nutritional values. This is the case of seeds of *P. biglobosa*, used in soup preparation to cure hypertension (Bonnah *et al*., 1998; Tra Bi *et al*., 2008). Other studies have shown that treatment of hypertension could be done with leaves of *Parkia biglobosa* in decoction (Tra Bi *et al*., 2008) and also with the aqueous extract of the bark of concentrations between 1.18 and 18, 93mg/kg of BW (Kassi *et al*., 2008).

Moreover, Yapo *et al*. (2010) showed that doses of 50 and 100mg/kg of aqueous extract of leaves of *Parkia biglobosa* induce immunostimulation. Studies on the acute and subacute toxicity of *Parkia biglobosa* are rare despite its use by traditional healers in Africa. These studies were conducted to determine the effect of aqueous extract of leaves of *Pakia biglobosa* on some markers of liver and glucose serum metabolism in rabbits.

## Materials and Methods

### Material

#### *Plant material and extract preparation*

The leaves of *Parkia biglobosa* (Jacq) Benth. (Mimosaceae) are collected from the Botanical Garden of the University of Cocody-Abidjan (Cote d'Ivore) in November 2009. The plant under the voucher specimen N$^{o}$01, was identified by Professor Laurent Aké-Assi of the Department of Botany, University of Cocody and deposited at the Botanical Garden herbatorium University of Cocody-Abidjan. Leaves were sorted, washed and dried under shade at room temperature 25 ± 2 °C. They are crushed, and 200g of grounded leaves were macerated in one liter of distilled water for 24 hours. The macerate was filtered two times through clean tissue, then three times on Whatman filter paper No.1. The filtrate is then allowed to dry for three days in an incubator at 50 °C to avoid deterioration of protein substances. The powder obtained after drying was dissolved in distilled water to give different amounts

of different doses of aqueous extract of *Parkia biglobosa* (Pb) leaf.

***Animals and experimental technique***

The rabbits of the species *Orictolagus cumiculus* (Laporideae) between 3 and 4 months weighing 1.5 ± 0.3 kg and caged in batches of six were used. They were acclimated to 25 ± 2°C in a 12-hour light/dark cycle in the pet room of the laboratory for 14 days prior to the experiment. The animals were fed on rabbit pellets and give tap water *ad libitum*.

The first experiment was to demonstrate the dose-response effect of Pb. Thus, five sets of six rabbits were formed. Each rabbit received intraperitoneally a single dose of *Parkia biglobosa* (25, 50, 75 and 100 mg/kg BW) and a control group that received 0.9% NaCl. The second experiment consisted of mixing a reference immunosuppressive agent (methylprednisolone, 15 mg/kg BW) and doses of Pb (50, 75 and 100 mg/kg BW). Animal blood sample were taken on the first day (D0) before the injection of various solutions and another on Day 3 (D3) after injection. Then the other samples are taken each week (7 days) for 21 days. All blood samples are taken from the ear marginal vein of rabbit. Whole blood was collected in dry tubes and centrifuged rapidly in a centrifuge JOUAN BR4i® brand (Buckinghamshire, England) at 3000 trs/min for 10 min to obtain serum. The equipment, including handling and sacrificing of the animals were in accordance with European Council Legislation 87/609/EEC for the protection of experimental animals (Mitjans *et al*., 2008)

**Determination of biochemical parameters**

The serum collected was used for liver enzymes assay which are ALP, ALT, AST and glucose with a *Cobas Integra 400 Plus* ROCHE DIAGNOSTIC® (Germany) at the Institute Pasteur in Abidjan (Côte d'Ivoire).

**Statistical analysis**

Statistic analysis was undertaken with *GraphPad Prism V5.01* software (Washington, USA). Groups of data were compared one-way analysis of variance (ANOVA). Due to the small population size, the non-parametric Dunnett test was performed to compare assess difference between the control group and the other groups. Differences were considered statistically significant at $p < 0.05$.

**Results**

**Effect of aqueous extract of leaf of *Parkia biglobosa* (Pb) on liver enzymes**

***Effect of aqueous extract of leaves of Parkia biglobosa (Pb) on alanine aminotransaminase (ALT)***

The doses 25, 50, 75 mg/kg BW of Pb did not induced significant variation in the level of ALT compared to control group (0.9% NaCl) from Day 0 to Day 21 shown in Figure 1. The concentration of ALT (14 ± 2.52 to 30 ± 7.77 UI/L) induced by these doses of Pb fall within the same range as those in the control group (15 ± 4.73 to 30 ± 3.51 UI/L) (Fig 1). However, Figure 1 showed a significant increase in the concentration of ALT on Day 0 ($p < 0.0001$), Day 15 ($p < 0.001$) and Day 21 ($p < 0.05$) with single dose of 100 mg/kg.

***Effect of aqueous extract of leaves of Parkia biglobosa (Pb) on aspartate aminotransaminase (AST)***

Figure 2 showed that from Day 0 to Day 9, doses of Pb (25, 50, 75 and 100mg/kg CP) did not induce an increase in the concentration of AST. Therefore, from Day

0 to Day 9, the average values of these enzymes in the presence of Pb doses are between 13 ± 1.15 and 23 ± 8.08 UI/L and were not significantly different from those of animals in the control group which is between 15 ± 1.5 and 23 ± 14.18 UI/L. However, on Day 15 doses of 75 and 100mg/kg of BW increased significantly ($p < 0.05$) and temporarily concentrations of AST compared to the control value before dropping rapidly to their initial values on Day 21 (Fig. 2).

***Action of P. biglobosa on the serum concentration of alkaline phosphatase (ALP)***

Figure 3 represents the effect of the Pb amounts on alkaline phosphatase. It shows that all the Pb amounts used induced a significant reduction in the concentration of this enzyme compared to the median values with Day 0. The significant minimal median values ($p<$ 0,001) of this reduction compared to Day 0 are reached in Day 3 for Pb25 (99.16 ± 20.98 UI/l); for Pb75 (55.35 ± 12.90 UI/l) and for Pb100 (60.67 ± 22.50 UI/l). As for Pb50, the minimal significant value (59.00 ± 21.66 UI/l) is reached in Day 9. Consequently, the interval of the median values of the concentrations induced by the Pb amounts lies between 55.35 ± 12.90 and 148.00 ± 22.91UI/l. This reduction in the concentration of ALP induced by the Pb amounts is independent of the amounts and tends towards the reversibility to Day 21 (Fig 3).

**Effect of aqueous extract of leaf of *Parkia biglobosa* (Pb) on glucidic behavior**

***Effect of aqueous extract of leaves of Parkia biglobosa (Pb) on serum glucose***

Figure 3 showed that from Day 0 to Day 21, the doses of Pb (25, 50, 75 and 100 mg/kg BW) did not induce significant changes in serum glucose compared to control. However, doses of 50 and 100mg/kg BW induced a significant increase ($p <0.05$) in serum glucose of 1.35 ± 0.18 and 1.33 ± 0.10 g/L respectively compared to control group (1.08 ± 0.01 g/L) on Day 15 (Fig. 4).

***Effect of aqueous extract of leaves of Parkia biglobosa (Pb) on hyperglycemia induced by methylprednisolone***

In Figure 5, we observed that the methylprednisolone 15 mg/kg BW induced a significant increase in blood glucose on day 3 ($p <0.05$), Day 9 ($p <0.001$), Day 15 ($p <0.05$) and Day 21 ($p <0.001$) compared to the control group (0.9% NaCl). However, mixtures of MP15 and Pb of different doses (50, 75 and 100mg/kg BW) have not resulted in significant change of blood glucose compared to control group from Day 0 to Day 21 (Fig. 5).

**Discussion**

Most parts (stems and bark of the trunk, leaves and fruits) of *Parkia biglobosa* used in traditional medicinal recipes for the treatment of several diseases. Different studies of several authors have found that the concentrations used are lower than 100 mg/ml. In this study it was shown that there have been no significant changes in the levels of ALP, ALT and AST in the presence of Pb. This indicates no effect of aqueous extract of leaves of *Parkia biglobosa* on the liver. In fact, any increase in the concentration of markers of liver enzymes like ALP, AST and ALT testify to liver failure (Ogunlade *et al*, 2012; Akachi *et al*., 2010; Shi *et al*., 2010). However, ALT is mainly found in the liver and an increase in ALT values in serum is a sign of liver disease (Javad *et al*, 2011).

Some authors have shown that the hepatoprotective effect of a substance depend on a decrease in concentrations of

AST and ALT level whose concentration are high in the presence of a hepatotoxic substances like carbon tetrachloride (CCl4) (Yang *et al*., 2010), or carbonated drinks according to Jeroh *et al*, 2012. The aqueous extract of leaves of *Parkia biglobosa* does not act as CCl4 on the liver at doses below 100 mg/kg BW. However, the doses of 75 and 100 mg/kg BW have resulted in a significant increase in the concentration of AST on Day 15. The concentration of ALT was significantly increased on Day 15 by the dose of 100 mg/kg BW compared to the control. Despite this increase, the dose of Pb100 mg/kg BW can not cause liver toxicity, because the mean concentrations of AST (47 ± 3.51 UI/L) and ALT (46 ± 2.00 UI/L) remained within the normal range in rabbits that are 21.24 ± 9.89 UI/L for AST and 45.52 ± 20.54 UI/L for ALT (van Praag, 2010; Coulibaly *et al*., 2007). Concerning the ALP, all the experimental Pb doses involved a significant reduction their median values of Day 3 with Day 15. That shows it not action of the aqueous extract of the leaf of *Parkia biglobosa* on this hepatic enzyme. In conclusion, the aqueous extract of leaves of *Parkia biglobosa* does not induce hepatotoxicity with immunostimulating and antihypertensive doses of 50 to 100 mg/kg BW. This would imply that these doses are well tolerated by the liver, contrary to *Fumaria parviflora* (Javad *et al*, 2011) what may cause a hepatic damage in long-term administration by low doses.

Experiments have shown that serum glucose was significantly increased only on the Day 15 for the concentration of 100 mg/kg BW (1.33 ± 0.10 g/L) compared with the control group (1.08 ± 0.01 g/L). This increase would suggest that Pb100 is hyperglycemic compared with lower concentrations that are Pb25, Pb50 and Pb75. However, in rabbits, the normal range of blood glucose is 1.15 ± 0.45 g/L (van Praag, 2010; Sakande *et al*., 2003). Comparing this range, it is noteworthy that the serum glucose levels induced by Pb100 are in the range of normal values of serum glucose in rabbits. This leads us to reject the hypothesis mentioned above that the dose Pb100 would be hyperglycemic. We conclude thus, despite the rise in glucose concentration compared to the control group, Pb does not cause any significant increase in serum glucose in healthy rabbits.

The stability of the concentration of glucose by *Parkia biglobosa* is corroborated by the significant decrease in glucose concentration induced by the mixture Pb50, 75, 100 and MP15 compared to the significant increase in serum glucose concentration induced by a dose of MP15 alone (Fig. 4). In fact, according to the work of Jin and Jusko (2009), the dose of 15 mg/kg of methylprednisolone (MP15) stimulated more gluconeogenesis (insulin insensitivity) and lipogenesis than other doses. This suggests that aqueous extract of leaves of *Parkia biglobosa* inhibited the hyperglycemic effect of methylprednisolone.

## Conclusion

The aqueous extract of leaves of *Parkia biglobosa* has immunostimulatory effects via the immune cells and a hypotensive effect, did not induce toxic effects on the liver. So the experimental doses used did not result to higher glycemia.

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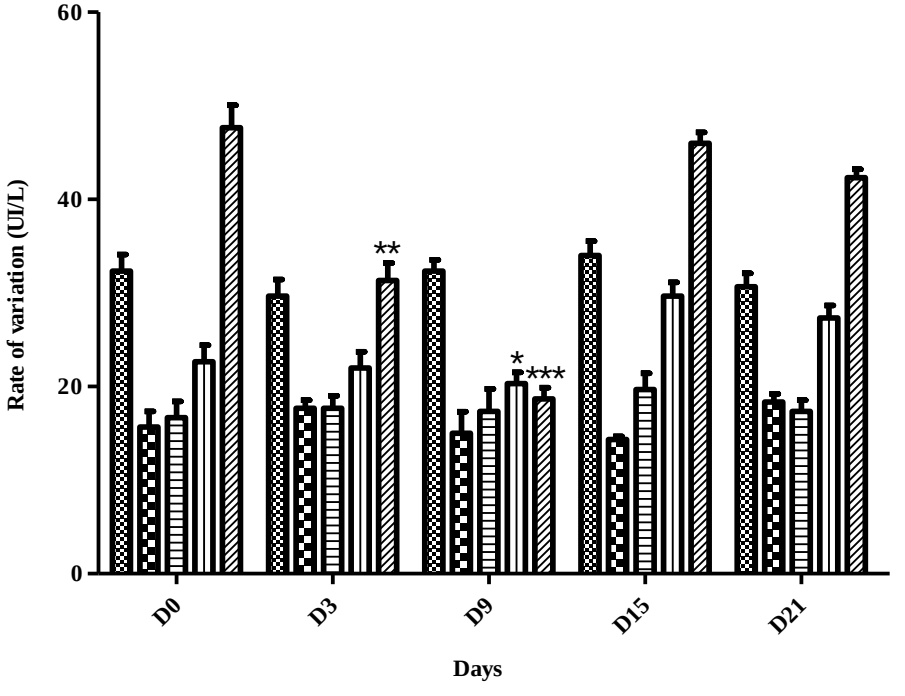


**Fig-1**Variation of the rate of ALT according to the dose of *Parkia biglobosa* taken with time.

NaCl 0,9%; Pb25: *Parkia biglobosa* 25mg/kg BW; Pb50: *Parkia biglobosa* 50mg/kg BW; Pb75: *Parkia biglobosa* 75mg/kg BW; Pb100: *Parkia biglobosa* 100mg/kg BW .

Number of experimental rabbit n=30. Level of significance: *$p < 0.05$; **$p < 0.001$ ; ***$p < 0.0001$. Experiment doses (*e.g.* Pb25 = aqueous extract of leaves of *Parkia biglobosa* 25 mg/kg BW)

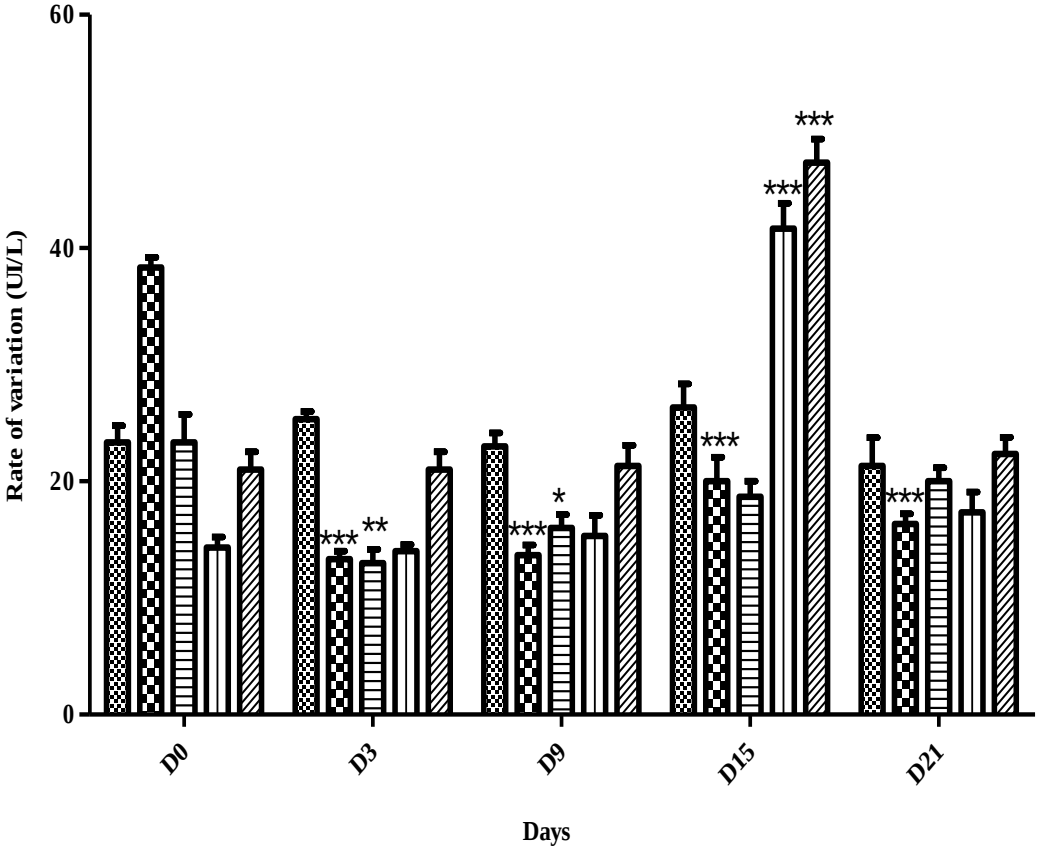


**Fig-2.** Variation of the rate of AST according to the dose of *Parkia biglobosa* taken with time

NaCl 0,9%; *: Parkia biglobosa* 25mg/kg BW; Pb50: *Parkia biglobosa* 50mg/kg BW; Pb75: *Parkia biglobosa* 75mg/kg BW; Pb100: *Parkia biglobosa* 100mg/kg BW .

Number of experimental rabbit n=30. Level of significance: *$p < 0.05$; **$p < 0.001$ ; ***$p < 0.0001$. Experiment doses (*e.g.* Pb25 = aqueous extract of leaves of *Parkia biglobosa* 25 mg/kg BW)

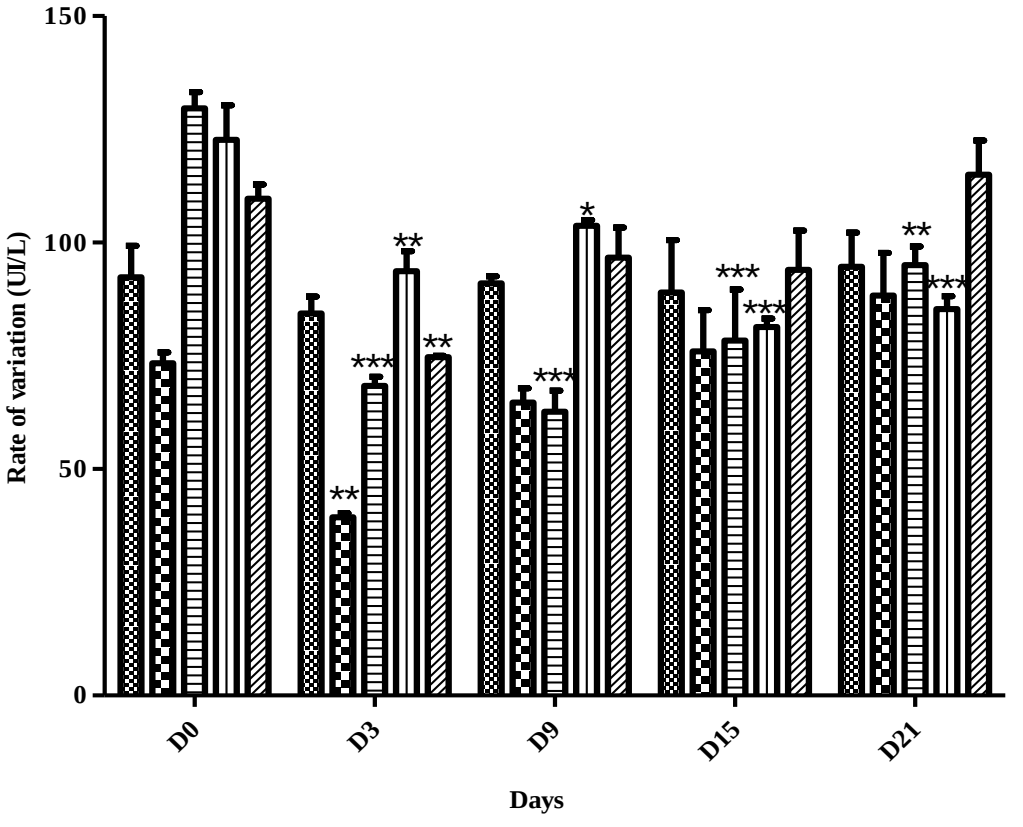


**Fig-3** Variation of the rate of ALP according to the dose of *Parkia biglobosa* taken with time

NaCl 0,9%; *Parkia biglobosa* 25mg/kg BW; Pb50: *Parkia biglobosa* 50mg/kg BW; Pb75: *Parkia biglobosa* 75mg/kg BW; Pb100: *Parkia biglobosa* 100mg/kg BW .

Number of experimental rabbit n=30. Level of significance: *$p < 0.05$; **$p < 0.001$ ; ***$p < 0.0001$. Experiment doses (*e.g.* Pb25 = aqueous extract of leaves of *Parkia biglobosa* 25 mg/kg BW)

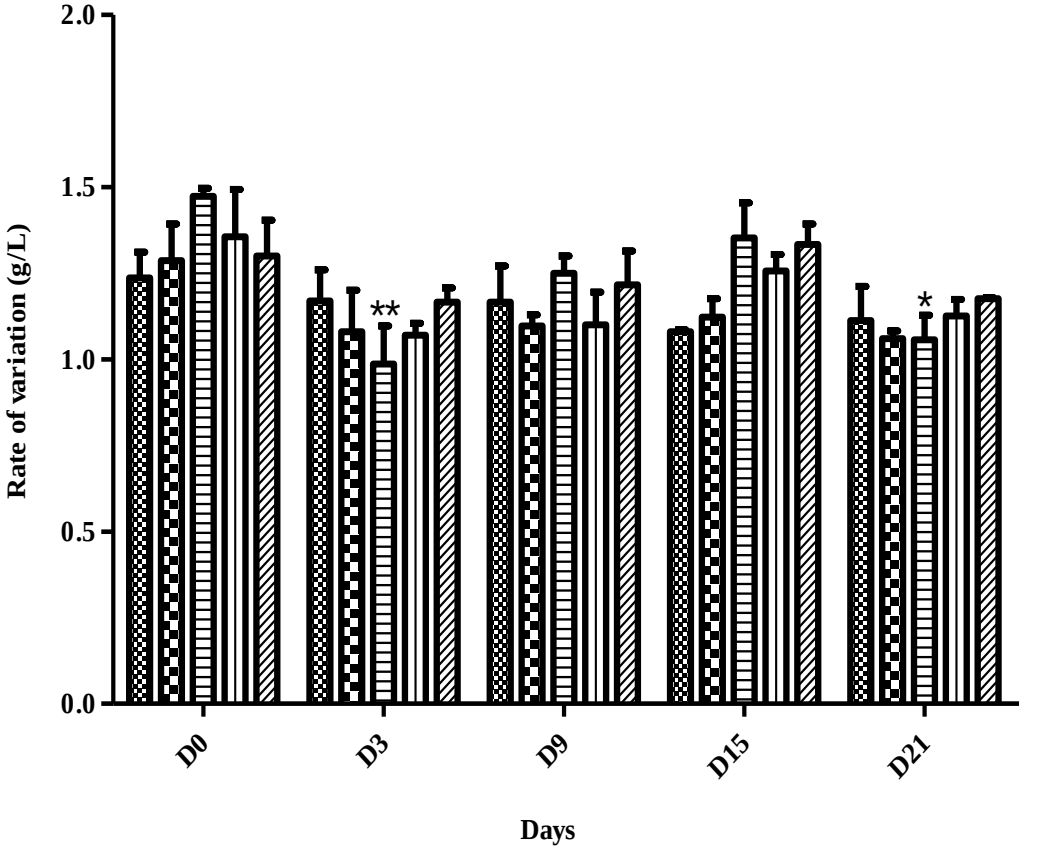


**Fig-4** Dose-response effect of *Parkia biglobosa* on glucose in time

NaCl 0,9%; Pb25: *Parkia biglobosa* 25mg/kg BW; Pb50: *Parkia biglobosa* 50mg/kg BW; Pb75: *Parkia biglobosa* 75mg/kg BW; Pb100: *Parkia biglobosa* 100mg/kg BW .

Number of experimental rabbit n=30. Level of significance: **p* < 0.05; ***p* < 0.001 ; ****p* < 0.0001. Experiment doses (*e.g.* Pb25 = aqueous extract of leaves of *Parkia biglobosa* 25 mg/kg BW)

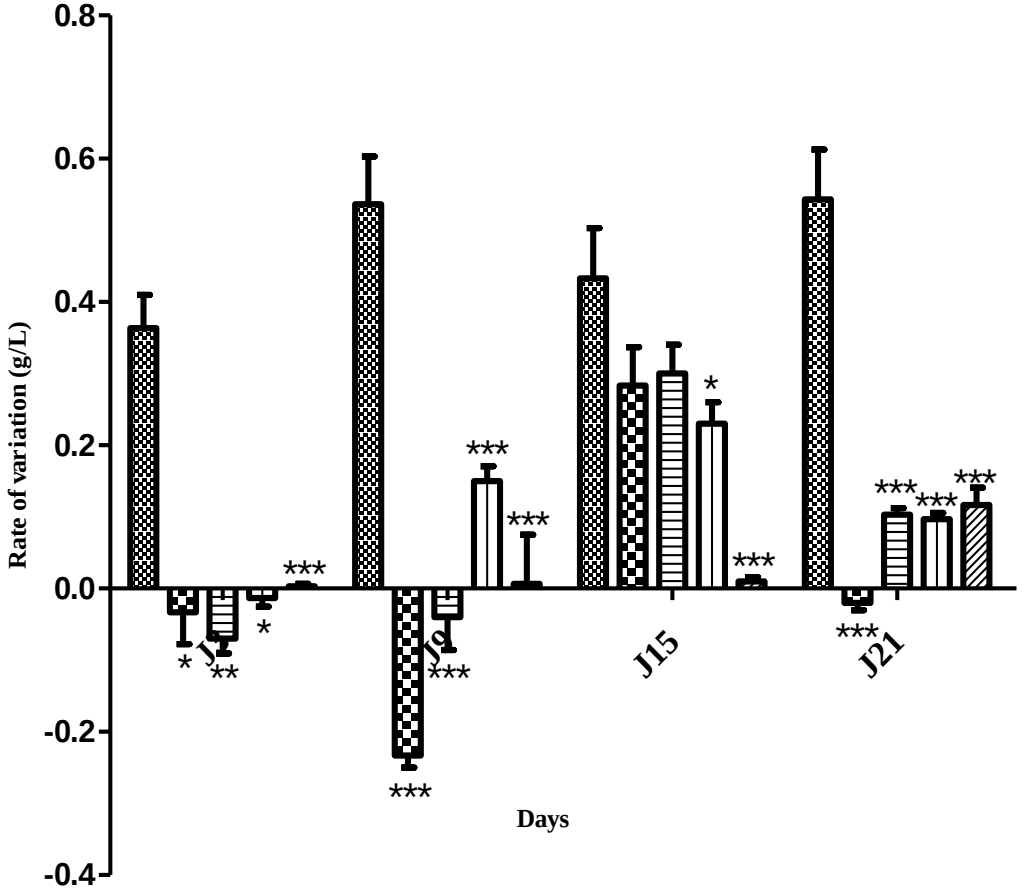


**Fig-5**. Variation in level of blood glucose according to doses of *Parkia biglobosa* taken in presence of methylprednisolone with time

MP 15; Pb50+MP15; Pb75+MP15; Pb100+MP15; Ip50+MP15

Number of experimental rabbit n=30. Significance level: *$p < 0.05$; **$p < 0.001$. Mixtures (*e.g.* Pb50+MP15= aqueous extract of leaves of *Parkia biglobosa* 50 mg/kg BW + methyprednisolone 15 mg/kg BW)