**Phytochemical screening and Blood Glucose-Lowering effects of Ethanol Leaf Extract of Two *Corchorus* specieson Alloxan-Induced Diabetic Albino Rats**

**Abstract**

**Background:** Diabetes mellitus is a metabolic disorder with tremendous debilitating effects of high public health impact. Drugs employed in the treatment and management of the condition have remained unsuccessful in curbing the effects of the disease necessitating a turn to natural plant habitat for therapeutic remedies. The present study thus demonstrated the antihyperglycaemic effect of the ethanol extracts of *Corchorus tridens and Corcchorus olitorius* alloxan-induced diabetic rats as well as phytochemical investigations of the plants.

**Methods:** Two groups of thirty rats each were administered *C. tridens* ethanol extract (CTEE) and *C. olitorius* ethanol extract (COEE) respectively for three days after pretreatment with alloxan for induction of diabetes. Rats in both cohorts were randomized into six groups (A-F) of five rats and subsequently received treatments as follows: groups A-C received 50mg/kg, 100mg/kg and 200mg/kg body weights of the extracts respectively. Group D received neither alloxan nor the extract and served as the (Negative) control while Group E was administered with 0.2mg/kg of glibenclamide and Group F (Positive) was induced with alloxan but was not treated with either extract or antidiabetic drug. All animals received 200mg/kg body weight alloxan except rats in Group D. Phytochemical investigations were also carried out on plant extracts using methods according to Association of Analytical Chemists.

**Results:** A significant increase in blood sugar level up to 592mg/dl on administration with alloxan was observed in all groups except Group D in the CTEE cohort. On the sixth day of experiment, the mean blood glucose level of Groups A, B, C and E that received CTEE reduced by 47.3%, 53.3%, 35% and 58% respectively. Group F did not show any significant change in blood glucose level. On the other hand, groups A, B, and C that received COEE had a percentage blood glucose reduction of 19%, 53.6% and 87.5% respectively. Group D maintained normal range of 90.4-108.8 mg/dl blood glucose. Group E blood glucose level showed a glucose decline of 58.7% to 245.8+25.20 from 596+8.25 while Group F remained high at 488.5+25.82. These findings showed a significant difference between groups treated with CTEE and COEE (*p* < 0.05). Preliminary phytochemical analyses revealed the presence of alkaloids and flavonoids as the major secondary metabolites in the ethanol extract of the plants, which for the most part may allude to the plant’s ameliorating potentials in diabetes mellitus.

**Conclusion:** Based on these findings, it can be inferred that both *C. tridens* and *C. olitorius* may be useful as phytomedicines in lowering blood glucose levels in diabetes mellitus.

**Keywords:** *Corchorus* *tridens*, *Corchorus* *olitorius*, diabetes mellitus, phytomedicine, antihyperglycaemia, alloxan

# Introduction

By the turn of the 21st century, growing interest in the science of diabetology orchestrated the need for therapeutic remedies offering sustained treatments to individuals affected with diabetes mellitus. Diabetes mellitus, a metabolic disorder of public health concern, characterized by hyperglycemia, increased thirst and hunger with long-term complications of the kidney, retina and liver has progressively impacted negatively on quality of life and socio-economic status of affected individuals. According to the World Health Organization (WHO), an estimated 422 million adults were living with diabetes in 2014 representing 8.5% of the world population while in 2016, approximately 1.6 million deaths were directly caused by diabetes1. Similarly, in 2019, 9.3% global diabetes prevalence (463 million people) was recorded2,3. In Nigeria, the prevalence of diabetes mellitus is estimated to be between 0.9–15 %4. With such staggering statistics and a projected global increase of 10.2% (578 million) by 20302,3, alternative therapeutic remedies are required to tackle the diabetes burden. Despite the above, current healthy recommendations suggest dietary modifications, exercise and modern-day pharmaceuticals, which aim at ameliorating debilitating occurrences in affected individuals and include the oral antidiabetic drugs such as the sulfonylureas, biguanides, thiazolindinediones and injectable insulin5. Nonetheless, these drug agents have remained unsustainable in light of increased cost and access especially considering people living in rural low-income countries as in the Nigerian context, thus a turn to our immediate plant habitat for ethnobotanical options6 with particular interest in the *Corchorus* plant genus, a neglected and under-utilized plant of about 40-100 species including *Corchorus* *tridens* and *Corchorus* *olitorius,* which hold the thrust of this study.

*Corchorus* *tridens,* popularly called Wild Jute*,* Jew’s mallow or Horn-Fruited jute, is a tall, annual leafy herbaceous plant of the family, *Tiliaceae*, reaching a height of 2-4m, unbranched or few sides branched with alternate, simple lanceolate leaves 5-15cm long, an acuminate tip and a finely serrated or lobed margin. The flowers are small (2-2cm in diameter) and yellow in colour with five petals. The plant is locally known as *Ahahara* in Igbo, *Ewedu* in Yoruba and *Kukah* in Hausa. In the Southwestern part of Nigeria, it is popularly consumed in a soup known as ‘*Ewedu’* soup. The seeds are used as flavorings and herbal tea has also been prepared from the dried leaves. The leaves of *C. tridens* have been employed in the treatment of gastrointestinal conditions such as abdominal pain and diarrhea in herbal medicine7. Other uses as reported by previous studies were suggested in wound healing of various forms7. It has also been reported to be demulcent, deobstruent, diuretic, purgative, emollient, mucilaginous and tonic8. *C. tridens* has not been sufficiently investigated for phytotherapeutic potentials especially with regard to blood sugar lowering in the context of diabetes mellitus management.

*Corchorus olitorius*, commonly known as Bush Okro or Nate jute*,* is an annual, leafy vegetable belonging to the family of *Sparmaniaceae*9. It is 90-120cm tall with glabrous stems and leaves 6-10cm long and 3.5-5cm broad, with pale yellow flowers and black trigonous seeds10-12. It grows mostly in the tropical regions of the world including Nigeria13. In Nigeria, it is locally known as *Ahihara* in Igbo, *Malafiya* in Hausa and *Ewedu* in Yoruba9 where it is widely consumed as a vegetable in soup delicacy called *Ùkùèrè* in the Southwestern part of the country14. *C.* *olitorius* leaves are well used as emollient, diuretic, tonic and for purifying human body. While the leaves are very rich in proteins, β-carotene, iron, calcium, vitamins A, B, C, E, folic acid, zinc, amino acid and essential minerals15-18, they have been employed as herbal remedy against malaria or typhoid fever in traditional medicine19-22. The leaves of *C. olitorius* have also been reported to have hypoglycemic effect23 and antibacterial activity24,25. However, this hypoglycemic effect has not been substantiated well enough as scientific documentation are scanty on the subject.

In the light of the above background, this study was conducted with the objectives of investigating the ameliorating potentials of *C. tridens* and *C. olitorius* on the glycemic index of diabetic rats induced with 200mg/kg body weight of alloxan and determining the phytochemical composition of both plant species.

# Material and methods

## Chemicals

Alloxan and Glibenclamide were analytical grade products of Sigma-Aldrich Chemical Company, UK.

***Collection and Identification of Plant Materials***

The plant leaf samples of *C.* *tridens* and *C.* *olitorius*, used for the study were obtained fresh from Eke Awka market in Awka, Anambra State. The plants were authenticated by a taxonomist at the Department of Botany, Nnamdi Azikiwe University, Awka, Nigeria where voucher numbers were assigned to the samples before deposition at the Herbarium.

***Preparation of Plant Materials***

Both leaves of *C. tridens* and *C.* *olitorius* were dried at room temperature for one week after which each sample was ground into coarse powder using warring blender. Each plant leaf sample, 400 *g*, was soaked in the ratio of 1 g to 5 ml of 70% ethanol in air-tight containers for 24 hours with periodic stirring for proper extraction. Afterwards, the mixtures were sieved, filtered using a funnel and filter paper and concentrated under vacuum using a rotary evaporator at a temperature of 50oC in a water bath. This was evaporated to obtain the extracts. The concentrated volumes of the plant extracts were reconstituted in 20% Tween and stored until use for various phytochemical investigations and administration to animals.

***Phytochemical Analyses of C. tridens and C. olitorius Plant Extracts***

The gravimetric method of Harborne26 was used in the determination of the percentage alkaloid contents of the *C. tridens* and *C. olitorius* plant species while the AOAC methods27 were used in the determination of the presence of flavonoid, saponin and tannin.

***Experimental Animals***

Sixty male albino rats of the Wistar strain weighing between 75-100 kg obtained from the animal house of the Department of Biochemistry, University of Nigeria, Nsukka, Enugu State, Nigeria, were used for the study. The rats were housed in animal cages in the Animal house of the Department of Applied Biochemistry, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria and allowed to acclimatize for seven days to the environment prior to the commencement of the experiment under a constant 12 hours light and 12 hours dark cycle at room temperature in accordance with National Institutes of Health guidelines of Animal Care and handling Laboratory animals. Food and water were administered *ad* *libitum* throughout the period of the study.

***Feed Composition***

The composition of the pelletized commercial rat chow (Vital Grower’s Feed) produced by Top Feeds Ltd., Delta State, Nigeria had a proximate composition of crude protein (16%), fat (5%), crude fiber (7%), ash (5%), calcium (0.9%), phosphorous (0.7%) and energy (11.1 kJ).

***Induction of Diabetes***

Blood samples were first collected from the tail veins of the rats using 1 ml syringe to measure the baseline blood glucose levels before induction of type II diabetes mellitus with alloxan. Freshly prepared solution of alloxan (0.1 g dissolved in 5 ml of freshly prepared sodium citrate buffer 0.1 M, pH 4.5) was injected intraperitoneally to the rats at a dosage of 200 mg/kg body weight at fasting state28. The rats were then fed after 30 minutes with 5% glucose solution (17.5g in 350ml of distilled water) after which they were allowed to fast for 12 hours. The rats were observed for signs of diabetes such as excessive thirst (polydypsia) and excessive hunger (polyphagia). Diabetes was confirmed after 48 hours of alloxan injection by collecting blood from tail vein and measuring blood sugar level using a blood glucose meter. Rats established to be diabetic with blood glucose level of more than 200mg/dl were used for further studies.

***Experimental Procedure***

Two groups of thirty rats each were administered *C. tridens* ethanol extract (CTEE) and *C. olitorius* ethanol extract (COEE) respectively. Rats in both cohorts were randomized into six groups (A-F) of five rats and subsequently received treatments accordingly. Alloxan (200 mg/kg body weight [*b.w.*]) was administered to all the groups except group D, which was the Negative control. The effect of the alloxan was observed for the first 48 hours for the induction of hyperglycemia indicated as the result on Day 2. The hyperglycemic rats after 48 hours received the extracts in groups A, B and C at 50, 100 and 200 mg/kg *b.w.* Group E received 0.2 mg/kg *b.w.* of the reference drug, glibenclamide and served as the reference group while group F received alloxan but was not treated and served as the Positive control. On the sixth day, blood was collected from the tail vein and estimated for blood glucose level. The body weights of the rats were also recorded on a daily basis, using an electronic weighing balance. Food and water were fed *ad libitum*.

***Estimation of Blood Glucose***

The glucometer (AccuCheck-Sensor) purchased from Roche Diagnostics was used to assay the blood glucose level after a fasting period of 12-16 hours. Before alloxan was administered, baseline blood glucose was measured, recorded and subsequently determined every 24 hours for seven days throughout the period of the study. Determination of glucose using the One-touch test strip glucometer is based on the principle of the reaction of glucose with oxygen in the presence of glucose oxidase yielding glucuronic acid and H2O229. Hydrogen peroxide subsequently oxidizes the dye on reaction mediated by peroxidase producing a blue colored form of the dye. The intensity of the coloration is proportional to the glucose concentration in the blood sample in mg/dl. To estimate the blood glucose, the tip of the tails of the rats were pricked with a blade and a drop of blood was squeezed under pressure into the test strip inserted in the glucometer.

***Data Analysis***

Data was subjected to analysis using the Statistical Package for Social Sciences (SPSS) Statistics version 25 (SPSS Inc., Illinois Chicago, USA). Results were expressed as the means ± standard error of mean of triplicate determinations. One-way Analysis of Variance (ANOVA) was used for comparison of the means between groups. Differences between means were considered significant at *p* < 0.05. Post-Hoc Tukey HSD Test was used for multiple comparisons of means.

# Results

***Phytochemical Screening***

The results of preliminary qualitative phytochemical screening of the ethanol extract of the leaves of *C. tridens* and *C. olitorius* are shown in the Table I below. The findings reveal the presence of flavonoids, alkaloids, cardiac glycosides, saponins, phenols, sterols and anthracine glycosides in both plant species. Tannins and cyanogenic glycosides were totally absent in both samples.

# Table I Qualitative Phytochemical Screening of the leaves of *C. tridens* and *C.* *olitorius*

|  |  |  |
| --- | --- | --- |
| **Phytochemical** | ***C. tridens*** | ***C. olitorius*** |
| Alkaloids | + | + |
| Anthracine glycosides | + | + |
| Cardiac glycosides | + | + |
| Cyanogenic glycosides | - | - |
| Flavonoids | + | + |
| Phenols | + | + |
| Saponins | + | + |
| Sterols | + | + |
| Tannins | - | - |

* = Absent; + = Present

***Mean Blood Glucose Levels***

The alloxan had the highest hyperglycemic effect for the *C.* *tridens* experimental groups on group E (reference group) followed by group F and then A, C, B and D in decreasing order. The effects of *C. tridens* extracts administered after 48 hours (Day 2) were shown from day 3 to 6. Group A treated with the 50 mg/kg *b.w.* extract reduced from 446.8±103 on day 3 to 225.8±42 on day 6. Group B (100 mg/kg *b.w.*) and C (200 mg/kg *b.w.*) dropped from 300±55 and 265±27 to 140.2±17 and 201.2±41 respectively. The reference (glibenclamide) group E decreased from 589.4±8 to 245.8±41 while the positive control (F) which received no treatment after alloxan administration increased from 315.8±45 to 390.3±30. The negative control (D) group which was not treated with alloxan but consumed the experimental diet/feed only started with a value of 105.5±6 on the third day and ended with a value of 100±9 on the 6th day with fluctuations in glucose concentrations between day 3 to 6 not exceeding the value of 10 mg/dl.

# Table II Mean Blood Glucose Levels of Rats fed diet supplemented with *C. tridens* ethanol extract

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Group** | **Mean Blood Glucose Level (mg/dl)** | | | | | | |
| Baseline | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 |
| A | 104+4.00 | 104.6+19 | 404.8+107 | 446.8+103 | 391.8+107 | 391.8+86 | 225.8+42 |
| B | 101.8+6.00 | 93.2+17 | 202+129 | 300+55 | 225+83 | 167+29 | 140.2+17 |
| C | 100.4+5.00 | 219.2+12 | 310.2+15 | 265+27 | 215.4+14 | 275+18 | 201.2+41 |
| D | 85+2.3 | 89.3+4 | 105.5+6 | 101.5+4 | 91.5+8 | 94.5+5 | 100+9 |
| E | 88.2+5.00 | 348.8+22 | 592+11 | 589.4+8 | 528.8+7 | 301.6+33 | 245.8+41 |
| F | 84.3+5.00 | 117.8+26 | 450.5+18 | 315.8+45 | 478.5+40 | 376+35 | 390.3+30 |

*Values are expressed as Mean ± SEM of triplicate determinations.*

Similarly, the mean blood glucose level (mg/dl) of rats fed with *C. olitorius* supplemented diet after six days are reported in the Table 3 above. The alloxan had the highest hyperglycemic effect for the *C.* *olitorius* experimental groups on group E (reference group) followed by group F, C, B, D and A in decreasing order. The effects of *C. olitorius* plant extract administered after 48 hours were shown from day 3 to 6. Group A treated with the 50 mg/kg *b.w.* extract reduced from 446.8±103 on day 3 to 225.8±42 on day 6. Group B (100 mg/kg *b.w.*) and C (200 mg/kg *b.w.*) dropped from 300±55 and 265±27 to 140.2±17 and 201.2±41 respectively. The reference (glibenclamide) group E decreased from 589.4±8 to 245.8±41 while the positive control (F) which received no treatment after alloxan administration increased from 315.8±45 to 390.3±30. The negative control (D) group which was not treated with alloxan but consumed the experimental diet/feed only started with a value of 105.5±6 on the third day and ended with a value of 100±9 on the 6th day with fluctuations in glucose concentrations between day 3 to 6 not exceeding the value of 10 mg/dl.

# Table III Mean Blood Glucose Levels of Rats fed diet supplemented with *C. olitorius* ethanol extract

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Group** | **Mean Blood Glucose Level (mg/dl)** | | | | | | |
| Baseline | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 |
| A | 103.8+45.12 | 90.4+24.68 | 101.0+43.70 | 111.4+39.60 | 94.4+32.66 | 108.0+18.72 | 100.8+5.59 |
| B | 102.2+4.73 | 97.8+16.68 | 210.6+97.45 | 206.4+39.40 | 169.6+60.61 | 167.8+64.69 | 194.8+97.83 |
| C | 87.4+6.34 | 55.6+16.05 | 382.0+65.95 | 446.0+77.74 | 262.5+40.61 | 249.7+56.56 | 255.5+41.71 |
| D | 85.0+3.39 | 89.3+4.12 | 105.5+2.64 | 76.5+11.68 | 91.5+2.97 | 95.0+4.42 | 100.0+1.24 |
| E | 88.2+1.72 | 346.8+71.45 | 596.0+8.25 | 589.4+7.03 | 528.8+36.08 | 301.8+21.72 | 245.8+25.20 |
| F | 84.3+2.38 | 122.8+16.81 | 450.5+30.12 | 328.3+22.02 | 488.5+25.82 | 375.3+40.32 | 390.3+32.00 |

*Values are expressed as Mean ± SEM of triplicate determinations*

# Discussion

# Diabetes mellitus is a chronic disease characterized by high blood glucose levels as a result of absolute or relative deficiency of circulating insulin levels. Phytotherapy as a growing alternative in the treatment of diabetes mellitus is receiving much attention with virtually every plant being investigated for their ethnobotanical efficacy in disease management. This has even become more so in resource limited countries where cost and access to modern medications are out of reach for those living with the condition, thus the need for urgent options in medicinal plant remedies. Findings of the present study showed the presence of phytochemicals in appreciable quantities. Phytochemicals have been implicated in pharmacologic activities of medicinal plants as these plants have been used in folklore medicine for ameliorative effects in conditions such as rheumatism, arthritis, hypercholesterolemia among others30,31. The presence of flavonoids, phenols, sterols and cyanogenic glycosides in the present study agree with work of Ndukwe *et al*32 while disagreeing with the finding of the same study on the presence of tannin. Phenolic compounds are a class of white, volatile crystalline solid compounds, to which flavonoids, tannins, flavones, lignans etc. belong to, that are easily absorbed through intestinal tract walls offering their antioxidant potential32,33. In several studies of the potencies of phytochemicals, flavonoids have been shown to be water-soluble antioxidant capable of scavenging free radicals thus preventing cell damage and conferring anticancer activity33-35. Flavonoids have also been shown to lower blood glucose levels thus its use in natural remedy for most common conditions6. The presence of flavonoids together with other phytochemicals such as alkaloids, tannins, saponins and phenolic compounds in the extract suggests the antioxidant and free radical scavenging potentials of the leaf extract as similarly reported by Lukaciniva *et al*36. Thus, according to Usunobum and Okolie37, flavonoids, tannins, terpenoids, alkaloids and phenolic group found in plants are free radical scavengers that prevent oxidative cell damage and induce mechanisms that affect cancer cells, and inhibit tumor invasion. This as reported by Usunobun and Okolie37 is due to the presence of conjugated ring structures and carboxylic groups which inhibit lipid peroxidation.

# The present study investigated the antidiabetic activity of orally administered *C.* *olitorius* and *C. tridens* species on alloxan-induced experimental diabetes mellitus in albino rats. Findings showed that alloxan increased the blood sugar level in the albino rats across treatment groups for both CTEE and COEE. This effect was largely seen 24 hours after alloxan administration with a peak blood glucose level of 348.8±22 for CTEE reference group E. Alloxan induces experimental diabetes by the destruction of β-cells of the islets of Langerhans and causes massive reduction in insulin release, thereby inducing hyperglycaemia38. Insulin deficiency results to changes in metabolic mechanisms in the experimental animals including increased blood glucose and increased enzyme levels in the liver such as alkaline phosphatase, transaminases39. However, a hyperglycemic effect was seen 24 hours after administration of alloxan followed by successive increase in blood sugar level as a result of inhibition of insulin release by the ability to destroy β cells of the pancreas. Further, it was observed at the end of the trial on Day 6, that there was a decrease in blood sugar level by 47.3% in group A which received 50 mg/kg of CTEE. There was also a decrease by 53.3% in blood sugar level of rats in group B that were administered 100mg/kg of CTEE while a 35% reduction in blood glucose was noted in group C rats that received 200mg/kg of CTEE. Similarly, rats in group E treated with 200mg/kg of glibenclamide showed a mean reduction by 58% in blood glucose level whereas group F had an 18% reduction in blood sugar level. These findings were also found to be significantly different (*p* < 0.05). This study also showed the ability of the plant extract to ameliorate the toxic effects of alloxan thereby reversing the hyperglycemic index which is the central theme associated with diabetes mellitus. Likewise, in COEE treated groups, a 19% decrease in blood sugar level of rats that received 50mg/kg in group A was observed, 53.6% was obtained for rats in group B that received 100mg/kg and 87.5% reduction was noted for rats in group C that received 200mg/kg. The control group remained normal at the range of 90.4-108.8mg/dl while the reference drug group showed a blood glucose reduction of 58.7% to 245.8mg/dl after an increase to 596mg/dl with alloxan induction. The alloxan group retained a high glucose level. These effects may be due to the presence of insulin-like substances, in the extracts, as explained by Collier *et* *al*40 stimulating β-cells to generate more insulin41 and abnormal amounts of fibers which meddle with carbohydrate absorption42 or its regenerative impact on pancreatic tissue43,44. It may then be adduced that the hypoglycemic activity of both *Corchorus* leaf extracts may be by increasing the sensitivity of *β*-cells to glucose thus elevating insulin levels as with the case of *Scoparia dulcis*45. Since diabetes mellitus is a condition of oxidative stress, the activity of CTEE and COEE could also have de-potentiating effect on the deleterious indices of oxidative stress. This was explained by a study that the complications of diabetes mellitus are as a result of the build-up of free radicals with concomitant compromise of antioxidant enzymes, which in the case of alloxan-induced diabetes reduces the action of liver antioxidant enzymes46,47.

# Furthermore, the phytochemical investigation of ethanol leaf extracts of *C. olitorius* and *C. tridens* revealed the presence of flavonoids, alkaloids, cardiac glycosides, saponins, phenols, sterols and anthracine glycosides in both plant species. Comparatively, *C. olitorius* had considerable presence of the phytochemicals than *C. tridens*. This may explain its potency and use in tradomedicine over *C. tridens*. However, both plants have been shown to be useful in herbal medicine largely attributed to the presence of these secondary principles, which have also been shown to be bioactive for the management of diabetes. Previous studies have shown that certain flavonoids exert hypoglycemic potentials and are able to recreate damaged pancreatic β-cells48. Thus, as opined by Arise *et al*25, the glucose-lowering effect of the ethanol extract of *C.* *olitorius* may be partly due to the presence of more than one of the antihyperglycemic principles known to impact blood glucose levels directly as well as synergistically. Saponins and phenols express hypoglycemic action by inhibiting intestinal absorption of glucose and glycogenolysis, hence restoring integrity of β-cells and consequently insulin release43.

# Conclusion

The present study showed that ethanol leaf extract of *C. olitorius* and *C. tridens* possess hypoglycemic properties in rats induced with diabetes through alloxan administration. It indicates that the ethanol leaf extracts of these plants contain biologically active components. This suggests their efficacy in the maintenance of glucose homeostasis and may be used as therapeutic alternatives in the management of diabetes mellitus.

**Statements and Declarations**

**Ethical statements**

All animals used for this study were kept and handled in accordance with National Institutes of Health guidelines of Animal Care and handling Laboratory animals.

**List of Abbreviations**

*b.w.* body weight

COEE *C. olitorius* ethanol extract

CTEE *C. tridens* ethanol extract

*C. olitorius* *Corchorus olitorius*

*C. tridens* *Corchorus tridens*

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1.

2.

3.

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