EFFECT OF HYDROETHANOL LEAF EXTRACT OF *FLEURYA AESTUANS* ON HISTOLOGICAL AND RENAL FUNCTION TEST OF AN ALLOXAN INDUCED DIABETIC WISTAR RATS

Original Research Article

ABSTRACT

Aims: To examine the effect of *Fleurya aestuans* extract (FAE) on histological and renal function test (RFT) in an alloxan-induced type 2 diabetes rat model.

Study design: The experiment was conducted in the department of Human Physiology, Madonna University, Nigeria, between August and September 2020.

Methodology: Thirty-five (35) male Wistar rats were split into seven (7) groups at random. Group 1 was the control group (CG). Group 2 was the negative control group (NCG). Group 3 was the extract group with the lowest dose (LDEG). Group 4 was the medium dose extract group (MDEG). Group 5 was the extract group with the highest dose (HDEG). Positive control group 1 (PCG1) was made up of rats from group 6. Positive control group 2 (PCG2) was made up of rats from Group 7. Rats were given chloroform anesthesia and sacrificed by cutting the jugular vein and blood was collected into heparinized bottles for renal function test.

Results: *Fleurya aestuans* extract (FAE) at lowest, medium and highest doses given for 30 days resulted in significant reductions in renal electrolyte markers. In diabetic rats treated with FAE, urea and creatinine clearance were likewise declined. Histological experiments were also used to uncover the extract's ameliorative potential structural impacts on renal damage.

Conclusion: we have correctly anticipated that the bioactive elements of FAE may reduce the progression of renopathy using the ALX model. *F. aestuans* may operate as a possible adjuvant for antidiabetic therapy and should be further researched.

Keywords*:* *Fleurya aestuans*, Alloxan, Diabetes, Renal function test.

Introduction

Diabetic renal dysfunction (DRD) is one of the most common causes of death among diabetics (1). The thickening of the basement membrane, the proliferation of messengial cells, fibrosis, and death of podocytes all contribute to morphological and functional problems. In diabetic individuals, renal cell dysfunction causes increased excretion of urine albumin, urea, uric acid, and creatinine, fluid retention, glomerular lesions, and a reduction in glomerular filtration rate (GFR). Furthermore, chronic hyperglycemia and oxidative stress are two important contributors to the development of diabetic complications. Under normal circumstances, the hexokinase enzyme phosphorylates glucose for energy production, however persistent hyperglycemia causes hexokinase saturation. As a result of this saturation, extra glucose enters pathways like the polyol pathway. Chronic hyperglycemia causes oxidative stress by raising the number of reactive oxygen species in the body (ROS). As a result, changes in the metabolic pathways indicated above cause structural and functional abnormalities in nephrons, eventually leading to diabetic renopathy (2, 3).

Many diabetic patients choose complementary and alternative therapies in addition to the existing diabetes interventions such as oral hypoglycemic medications and insulin therapy, which has increased the demand for alternative therapies for diabetes control (4).

Fleurya aestuans is a plant that belongs to the Urticaceae family. It is known by a variety of Nigerian names, including fiyafiya and ofuefue (Yoruba), bulsum fage (Hausa), and ile-nkita (Igbo) as one of the unique herbal plants prized for its nutritional and therapeutic benefits (5). Traditional medicine systems employ the leaves of this plant to treat a variety of ailments. Tetrahydroxyflavone, flavonoids (quercetin and isoquercetin, rutin), terpenes (limonin), terpenoids, polyphenols, fatty acids, tannins, sterols, vitamin C, and tocopherols, among others, may be responsible for Fleurya aestuans' medicinal activity (6). Antidyslipidemic and anti-inflammatory properties are among the pharmacological properties of Fleurya aestuans (6, 7). Due to its widespread use in everyday food, Fleurya aestuans could be regarded a functional plant. Furthermore, its pharmacological benefits in treating a variety of ailments encourage its use as a functional plant (7).

The current study was designed to explore hydroethanolic extract of Fleurya aestuans leaf for attenuation of diabetic renal failure, following the trend of employing safe natural products along with functional plants, their bioactive ingredients, and therapeutic capabilities.

Materials and methods

Preparation of extract

Fresh leaves of Fleurya aestuans (West Indian wood nettle) were collected from the University of Port-botanic Harcourt's farm and scientifically defined by the herbarium of the Department of Plant Sciences, Faculty of Sciences, University of Port-Harcourt, Choba, Nigeria, with the reference number UPH/P/263. The leaves were washed thoroughly and allowed to dry at room temperature. A plastic jar was used to store the dried leaves. The extract was prepared using Al-Attar and Abu Zeid's (8) method with certain changes. Using a manual granulating machine, the dried leaves were ground into fine powder. 350g of plant powder was weighed and dissolved in 400ml of Water-Ethanol combination (25:75) in an Extraction Jar for 72 hours. It was well macerated during this time in order for the solvent to absorb. The filtrate was then separated from the residue by sieving it through a Whatman No. 1 filter paper. After producing a clear filtrate, it was poured onto an evaporating dish and dried on a steam bath at 45 degrees Celsius. The drying process was closely watched until the mixture transformed into a paste. The crude ethanolic extract of *F. aestuans* leaves obtained yielded 75.3g, which was stored at 4^{oC} in a domestic refrigerator.

Lethal dose (LD_{50}) of the extract.

Lorke's (9) approach was used to determine the extract's lethality, with slight modifications.

There were two parts to this method:

Phase A

Nine (9) male rats were employed in this phase. They were divided into three (3) groups, each with three (3) rats. The hydro-ethanolic extract of the leaves of Fleurya aestuans was given to each group of rats in doses of 40, 400, and 600 mg/kg, respectively. The animals are kept under constant observation for 24 hours in order to track their behavior and mortality rates.

Phase B

Four (4) male rats were divided into four (4) groups of one (1) rat each in this phase. The rats were given 800, 1300, 3500, and 4600 mg/kg of the hydro-ethanolic extract of Fleurya aestuans leaves, respectively, and were subsequently monitored for a day for signs of acute intoxication and mortality.

The extract's LD50 was estimated using the following formula:

$LD50 = \sqrt{(D0 \times D100)}$

Where;

D0 = The highest dose that resulted in no death,

D100 = Lowest dose that resulted in death.

Induction of Experimental Diabetes

Diabetes was produced in all of the test groups by injecting 150mg/kg body weight of alloxan monohydrate intraperitoneally after an overnight fast (10). The accucheck glucometer and test strips were used to measure the blood glucose levels (BGL) on a daily basis. Diabetic rats were employed in the investigation because their blood sugar levels were higher than 200 mg/dl.

Experimental design

For the investigation, thirty-five (35) male Wistar rats were employed. The rats ranged in weight from 180.4 to 210.9 grams. They were purchased from the Madonna University Animal House in Elele, Nigeria. Prior to the start of the experiment, the animals were housed in plastic cages and fed commercial rat feed and water ad libitum for a week to acclimate. The animals were split into seven (7) groups, each with five (5) animals.

Group 1 functioned as the control group (CG), receiving unlimited rat food and water.

Group 2 was the negative control group (NCG), receiving ALX only.

Group 3 was the low dose extract group (LDEG), receiving 50 milligrams per kilogram of body weight of Fleurya aestuans leaf extract for 30 days.

Group 4 was the medium dosage extract group (MDEG), receiving 75 milligrams per kilogram of body weight of Fleurya aestuans leaf extract for 30 days.

Group 5 was the high dose extract group (HDEG), given 200mg/body weight of Fleurya aestuans leaf extract for 30 days.

Group 6 was the positive control group 1 (PCG1) rats, given 600 mg of glibenclamide per kilogram of body weight for 30 days.

Group 7 was the positive control group 2 (PCG2) rats, given 100 mg of tetrahydroxyflavone per kilogram of body weight for 30 days.

Collection of Blood Samples

Rats were given chloroform anesthesia and sacrificed by cutting the jugular vein. Blood was collected into heparinized bottles and centrifuged for 10 minutes at 3000rpm; the supernatant (serum) was collected and stored in the refrigerator for future use.

Determination of sodium (Maruna and Trider method) mmol/L.

The current method is based on the reaction of sodium with a selective chromogen, which results in a chromophore whose absorbance fluctuates in direct proportion to the sodium concentration in the test sample.

Determination of potassium (Tiets N.W method) unit mmol/L

The amount of potassium in a colloidal suspension is determined by using sodium tetraphenylboron in a specially prepared combination. The sample's turbidity is proportional to the potassium concentration.

Determination of Chloriide: (Levinson S.S Method) unit mmol/L

The calorimetric displacement of thiocyanate by chloride from mercuric thiocyanate, as well as the subsequent production of red ferric thiocyanate complexes, is measured.

Determination of bicarbonate (HC03) (Back Titration Method)

Excess standard HCL reacts with serum HCo3. Back titrates the leftover HCL with standard NaoH using phenol red as an indicator.

Determination of urea (Urease-Berthelot Method) mmol/L

In the presence of urease, urea in serum is hydrolyzed to ammonia. Berthelot's reaction is then used to measure ammonia photometrically.

Determination of creatinine (Direct End-Point Method) umol/L

In an alkaline solution, creatinine interacts with picric acid to generate a colored complex. The amount of complex produced is proportional to the concentration of creatinine.

Histological Examination

Following the procedure indicated by Bancroft and Gamble (11); the tissues were fixed using 10% formalin for 7 days.
The tissues were dehydrated using ascending grades of alcohol 30% 50% 70% (3-12hr) 90% (3-12hrs) Absolute (3-12hrs) Absolute 2 (3- 12hrs) Absolute 3 (3-12hrs).
The tissues were cleared (de-alcoholisation) using xylene (15- 30mins).
The tissue were impregnated using paraffin wax for 2-3hrs
The tissues were embattled with molten paraffin wax in a plastic ice- tray the tray was immediately transfer to cold water to harden the wax.
The block are attached a holder
The tissue were cut using base-sledge microtome
The section tissues are floated in warm-water and pick-up with a slide.
The slides were incubated to remove the wax end fix the tissues on the slide
The slides were stain with Haematoxylin and Eosin.
The slides were mounted using Canada balsam or DPX
The slides were examined using x 40 obj. lens

Statistical Analysis

All data was expressed as mean + S.E.M. and statistical analysis was performed. A one-way analysis of variance was used to compare the two groups (ANOVA). When P 0.05, values were considered significant.

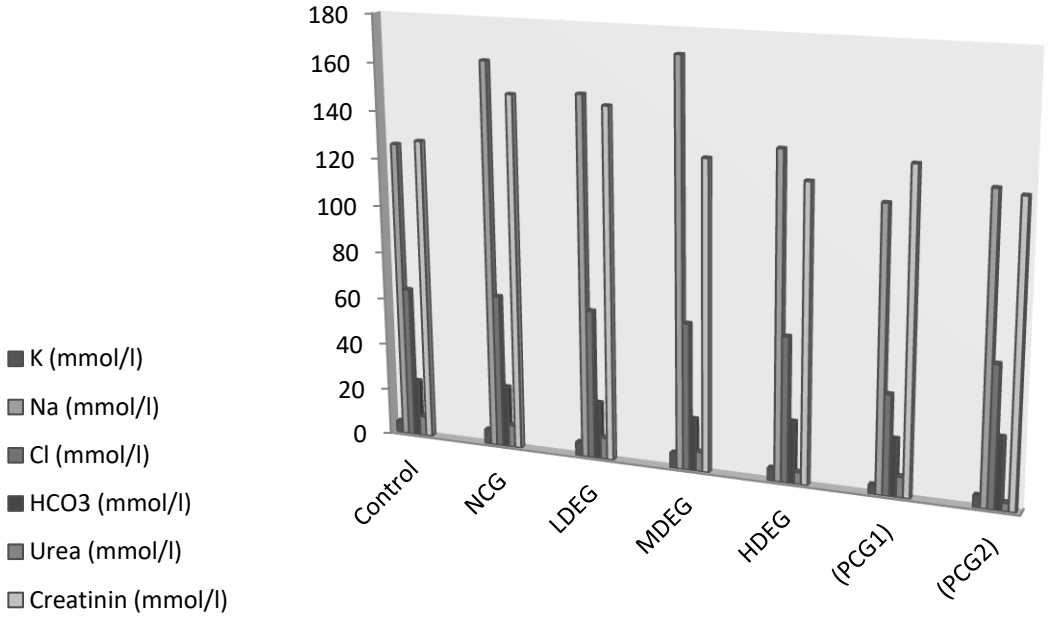
Results

The extract at lowest (40mg/kg) and highest lethal dose (4600mg/kg) did not produce any form of lethality in the study animals. Figure 1 demonstrated a significant increase in renal function parameters in the negative control group when compared to the control group. The extract at 200mg/kg caused a significant decrease ($p<0.05$) in plasma sodium ions and creatinin in contrast to the negative control group. When compared to the negative control group, glibenclamide at 600mg/kg induced a significant decrease ($p<0.05$) in plasma potassium, sodium, and chlorine ions. When compared to the negative control group, tetrahydroxyflavone at 100mg/kg induced a significant decrease ($p<0.05$) in soduim, chlorine ions, urea, and creatinin.

Histology of the Kidney

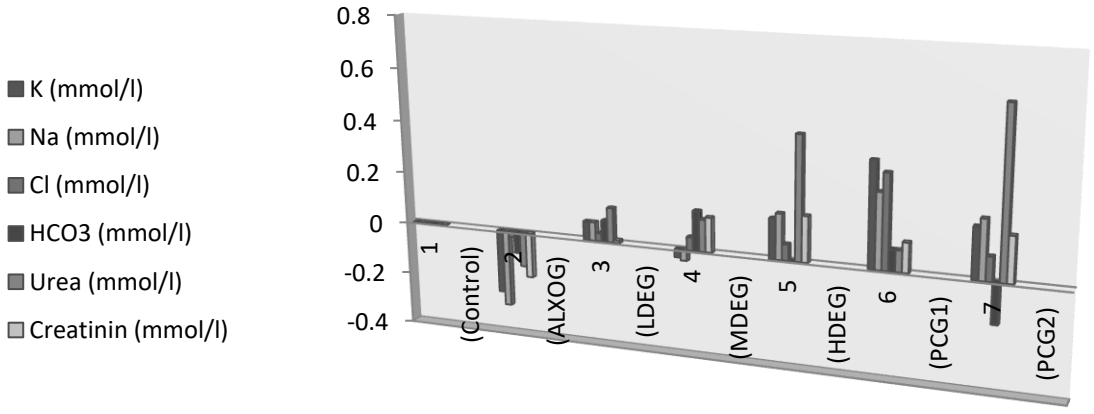
Plate 1-7 displays the photomicrographs of transverse sections of the rat's kidney in the control group and test groups.

Fig 1: Renal function markers



	Control	NCG	LDEG	MDEG	HDEG	(PCG1)	(PCG2)
K (mmol/l)	5	6.2	5.8	6.4	5.3	3.8	5
Na (mmol/l)	126	163	152	170	136	118	127
Cl (mmol/l)	64	65	63	62	61	42	59
HCO3 (mmol/l)	24	26	24	22	26	24	30
Urea (mmol/l)	8.4	9.5	9.2	8.3	5.1	8.7	3.6
Creatinin (mmol/l)	128	150	148	130	124	134	125

Fig 2: % diff of renal function markers



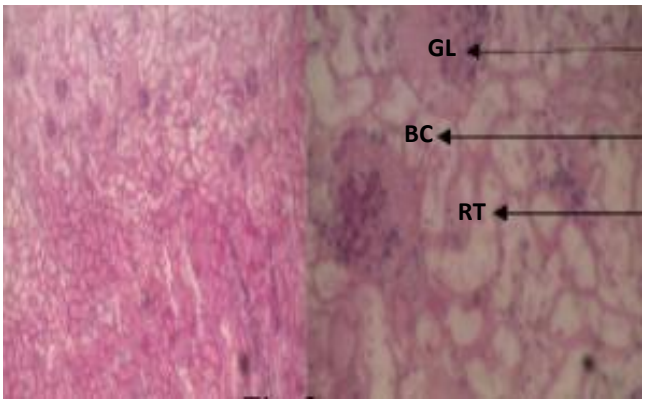


Plate 1 - CG (H&E) x 600

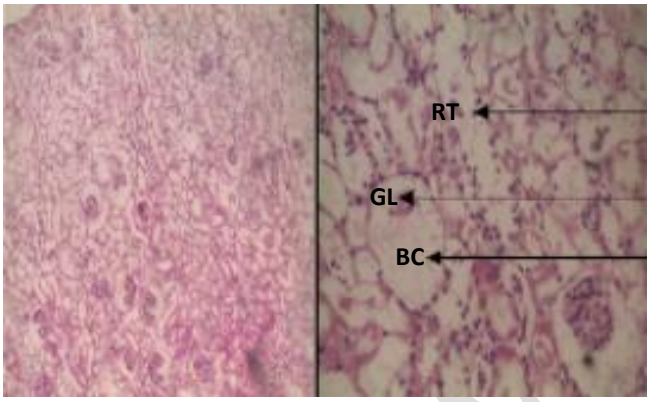


Plate 2 - NCG (H&E) x 600

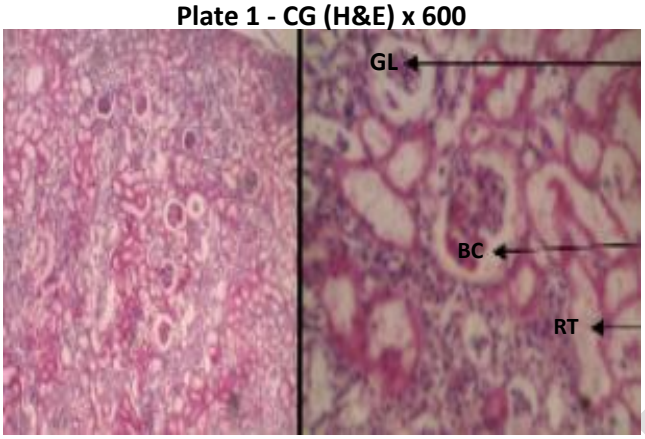


Plate 3 - LDEG (H&E) x 600

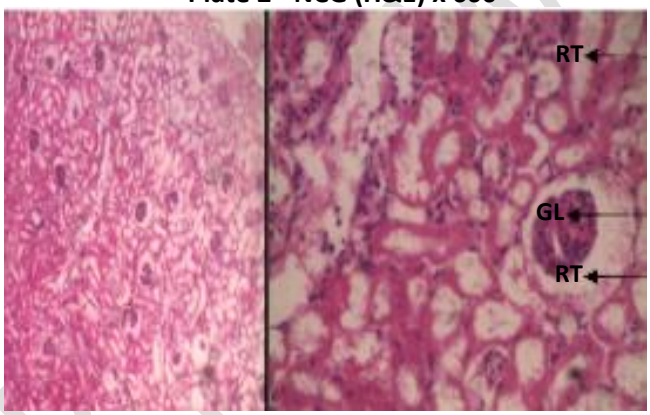


Plate 4 - MDEG (H&E) x 600

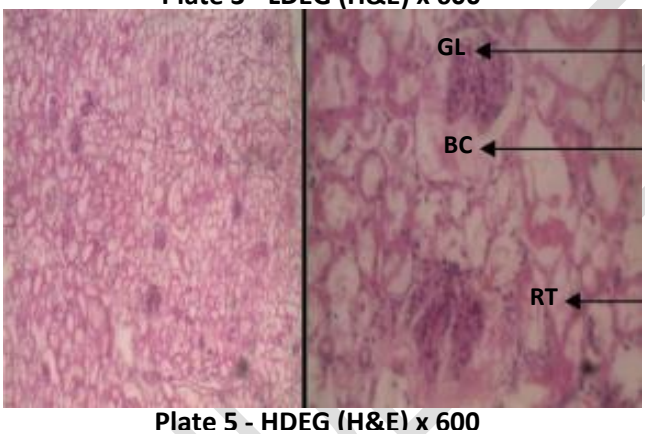


Plate 5 - HDEG (H&E) x 600

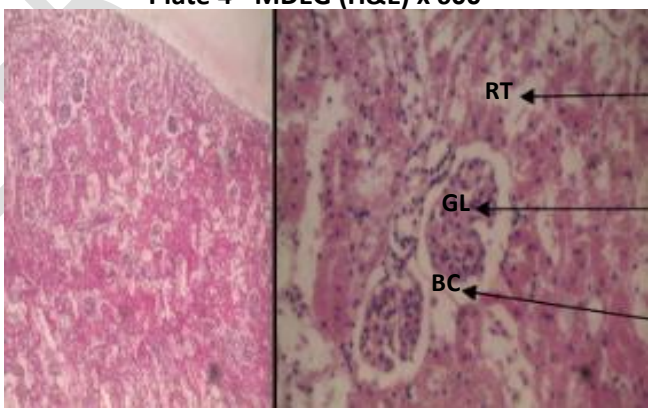


Plate 6 - PCG1 (H&E) x 600

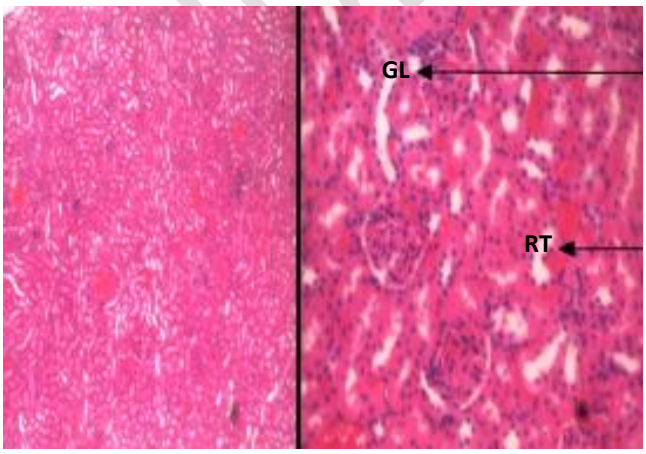


Plate 7 – PCG2 (H&E) x 600

Discussion

Diabetic kidney disease is the decline of kidney function over time in people who have diabetes. Protein loss in the urine owing to glomeruli destruction can become large, resulting in a low serum albumin level, which can lead to generalized body swelling (edema) and the nephrotic syndrome. Similarly, the estimated glomerular filtration rate (eGFR) may gradually decrease from a normal of over 90 ml/min/1.73m2 to less than 15, indicating that the patient has end-stage renal disease [6]. It normally progresses slowly over time. Pathophysiologic problems begin with blood glucose levels that have been poorly regulated for a long time (12).

Hippocrates, the father of medicine as a logical discipline, researched and summarized many therapeutic characteristics of plants. Throughout history, many traditional systems, including Nigerian traditional medicine systems, have used plants as medicine. A plant must include biologically active components that contribute to improved health or reduced illness risk in addition to being nourishing in order to be medicinally active or useful. These are well-thought-out plants that would be found in a typical diet.

Biomarkers such serum urea and creatinine were observed to be higher in the negative control group in the current investigation, indicating the development of diabetic renopathy. The elevated renal parameters were reversed when Fleurya aestuans was given orally, and the mitigating effects were dose dependant. In comparison to normal control rats, diabetic rats NCG) have higher electrolyte concentrations. Treatment with Fleurya aestuans extract considerably lowered these levels.

The start of diabetic renopathy is marked by functional abnormalities such as elevated electrolytes, urea, and creatinine levels. Uremia (excess urea in the urine) is associated with diabetic renopathy (13). In diabetic rats (NCG), a significant increase in urine urea and creatinine levels indicates poor kidney function and a change in electrolyte metabolism. As a result, treatment with Fleurya aestuans stabilized these levels, demonstrating its therapeutic effect in the treatment of renal dysfunctions.

The histoarchitecture of the kidneys is normal in Plate 1 (CG). Plate 2 (NCG) depicts aberrant renal corpuscles with glomerular degeneration and an enlarged Bowman's capsule capacity. Plate 3 (LDEG) revealed that the epithelial lining of the renal tubules has been lost. Hypertrophied renal corpuscles (RC) with normal glomeruli and increased Bowman's capsule capacity. Plates 4 (MDEG), 5 (HDEG), 6 (PCG1), and 7 (PCG2) reveal that the renal corpuscles (RC) and renal tubules (RT) of the rat kidney are normal, indicating that the diabetic renopathy produced by ALX medication has been reversed.

Chronic hyperglycemia and oxidative stress cause structural and functional changes in the body. In ALX-diabetic rats (NCG), structural anomalies in the kidney (basement membrane thickening, mesangial expansion, and hypertrophy) result in an elevated kidney parameters (14). Oral delivery of FAE to diabetic groups greatly reduced diabetic kidney dysfunctions, which is consistent with Donate research (2).

Conclusion

Overall, this study suggests that Fleurya aestuans leaves could be beneficial in the treatment of diabetic renal impairment caused by hyperglycemia in individuals.

Ethical approval

Our institutional ethical committee reviewed and approved the study, which was given the reference number UPH/CEREMAD/REC/MM78/052.

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