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Anti-anemic and protective effects of *Grona triflora* ethanol leaf extract on phenylhydrazine-induced anemia in albino rats

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Abstract

Anemia is a global health condition characterized by reduced hemoglobin concentration and impaired oxygen transport. Medicinal plants rich in antioxidants and micronutrients are increasingly explored as alternative therapies. This study evaluated the anti-anemic effects of *Grona triflora* ethanol leaf extract in phenylhydrazine-induced anemia in albino rats. Extraction was done using cold maceration method. Acute toxicity was determine using Lorkes method. Thirty (30) Albino rats were used. Anemia was induced through intraperitoneal injection of phenylhydrazine at 60 mg/kg for 3 days. Rats with packed cell volume less than 29% were grouped into six groups of five rats each. Group I was the normal control while II was the negative control. Group III received 2 mg/kg Ferrous sulphate while groups IV, V and V received 250, 500 and 1000 mg/kg *Grona triflora* extract orally for three weeks. Automatic counter was used to investigate the effect of *Grona triflora* ethanol leaf extract on phenyl hydrazine induced anemia. The LD_{50} was 5000 mg/kg. Ferrous sulphate and different doses of ethanolic leaf extracts of *Grona triflora* significantly ($p<0.05$) increased the packed cell volume, haemoglobin, red blood cells, mean corpuscular volume, mean capsulated haemoglobin, and platelets in a dose dependent manner but decreased the white blood cells, lymphocytes and neutrophils within three weeks. These findings suggest that *Grona triflora* possesses hematinic and protective properties, supporting its traditional use in anemia management.

Keywords: Anemia; *Grona triflora*; Hematinic; Phenylhydrazine; Phytochemicals; Albino rats

1. Introduction

Anemia is defined as a condition in which hemoglobin concentration or red blood cell (RBC) count is insufficient to meet physiological needs [1]. It affects approximately 1.62 billion people globally, particularly in developing countries [2,3]. The condition arises from nutritional deficiencies, infections, genetic disorders, or toxin-induced hemolysis [4]. Phenylhydrazine-induced anemia is commonly used as an experimental model because it causes oxidative destruction of erythrocytes [5]. In many developing regions, plant-based therapies remain widely used due to their accessibility and affordability [6].

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Grona triflora, a member of the Fabaceae family, is traditionally used for treating various ailments including anemia. It is rich in phytochemicals and micronutrients essential for hematopoiesis [7]. However, its anti-anemic potential has not been fully validated scientifically.

This study aimed to evaluate the hematinic, biochemical, and protective effects of *Grona triflora* ethanol leaf extract in phenylhydrazine-induced anemia in rats.

2. Materials and methods

2.1. Plant collection and extraction

Leaves of *Grona triflora* were collected from a medicinal garden at Agulu. The plant leave was authenticated by a Taxonomist, in the department of Botany, Nnamdi Azikiwe University, Awka and a voucher number (NAUBT9908) was attached to it and deposited in the herbarium. The leaves were air dried and pulverized using electrical blender. Extraction was performed using 80% ethanol by cold maceration for 48 hours. The filtrate was concentrated using a water bath at 40°C.

2.2. Phytochemical screening

Standard methods were used to identify alkaloids, flavonoids, tannins, saponins, glycosides, and terpenoids [8,9].

2.3. Vitamin Analysis

Vitamin analysis was carried out for vitamin A, K, B6, C, E and Folate using standard method [10]

2.4. Elemental Analysis

Elemental analysis for iron, magnesium, calcium, zinc and copper was determined using standard method [11]

2.5. Experimental animals

Albino rats (120–150 g) were obtained and maintained under standard laboratory conditions with free access to feed and water. They were acclimatized to the environment for one week prior to experimental use. The study protocol was carried according to the rules and regulations of the Institutional Animal Ethical Committee (IAEC). Also, the ethical review committee of the Faculty of Pharmaceutical Sciences, Chukwuemeka Odumegwu Ojukwu University gave the animal study ethical clearance and issued an ethical approval number, PHACOOU/AREC/2023/030.

2.6. Induction of anemia

Anemia was induced by intraperitoneal injection of phenylhydrazine (60 mg/kg) for 2 days. Rats with PCV <29% were selected [12].

2.7. Experimental design

Thirty (30) Albino rats were assigned into six groups (n=5) animals per groups. The rats were administered different doses of the ethanol extract of *Grona triflora* orally by gastric intubation daily for 3 weeks following the method described by Demo [13]. The experimental animals were grouped as followed:

- Normal Control
- Experimental Control (Anemia + No treatment)
- Standard Control (Anemia + Ferrous sulphate 2 mg/kg body weight)
- Treatment group (Anemia + *Grona triflora* extract 250 mg/kg body weight)
- Treatment group (Anemia + *Grona triflora* extract 500 mg/kg body weight)
- Treatment group (Anemia + *Grona triflora* extract 1000 mg/kg body weight)

At the end of the three weeks' experimental period, the albino rats were sacrificed under chloroform. Blood sample was collected by cardiac puncture and 3 mL of blood was collected into an EDTA sample bottle for haematological assay

2.8. Acute toxicity study

LD_{50} was determined using Lorke's method [14].

2.9 Determination of Hematological Parameters

Blood samples were analyzed for Red Blood Cell count **(** RBC), White Blood Cell count **(**WBC), hemoglobin, Packed Cell Volume (PCV), Mean Corpuscular Volume **(**MCV), Mean Capsulated Haemoglobin (MCH) and platelets, using an automatic counter as described by Dacie et. al [15].

2.9. Determination of Liver and Kidney indices

Liver enzymes [alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST)], bilirubin and renal function parameters (urea, creatinine and electrolyte [sodium (Na^+), potassium (K^+), chloride (Cl^-) and bicarbonate HCO_3^-)] levels were estimated spectrophotometrically using standard laboratory kits (Randox Laboratory Ltd., Co. Antrim, UK)

2.10. Measurement of body weight

Rats body weights were taken before administration and every week to observe variation in body weight [16].

2.11. Statistical analysis

Data were expressed as mean ± SEM and analyzed using ANOVA followed by post hoc turkey's test. Values of $p<0.05$ were considered significant. Data obtained from the study was analyzed using Statistical Package for Social Sciences (SPSS-25).

3. Results

3.1. Phytochemical composition

The extract contained high levels of alkaloids, tannins, and saponins, with moderate levels of flavonoids and terpenoids.

Table 1 Phytochemical composition of *Grona triflora* ethanol leaf extract

Phytochemicals	Ethanol extract (CO)
Alkaloids	+++
Tannins	+++
Steroids	++
Flavonoids	++
Glycosides	++
Saponins	+++
Terpenoids	++
Proteins	+
Reducing sugar	+
Fats and Oils	+
Acidic Compounds	-

(-) => Not Present, (+) => Faintly Present, (++) => moderately present, (+++) => Highly present

3.2. Acute toxicity

No mortality was observed up to 5000 mg/kg, indicating that the extract is relatively safe.

Table 2 Acute toxicity (LD_{50}) of *Grona triflora* ethanol leaf extract

Phases	Dose (mg/kg)	Mortality
PHASE 1	10	0/3
	100	0/3
	1000	0/3
PHASE 2	2000	0/1
	3000	0/1
	4000	0/1
	5000	0/1

3.3. Vitamins Composition of *Grona triflora* leaf Extract

The Vitamins composition of *Grona triflora* leaf extract revealed the presence of high amounts of Vitamin C. The plant extract was also found to contain vitamin A, vitamin K, vitamin B6, vitamin E and Folic acid in substantial quantities

Table 3 Vitamins Composition of *Grona triflora* leaf Extract

Vitamins	Concentration
Vitamin A (µg/100g)	4553.21 ± 06.02
Vitamin K (µg/100g)	51.09 ± 0.10
Vitamin B6 (Mg/100g)	20.16 ± 0.01
Vitamin C (Mg/100g)	52.18 ± 0.02
Vitamin E (IU/100g)	16.76 ± 0.02
Folic acid (µg/100g)	1600.03 ± 10.01

Values are mean ± SD for 3 determinations

3.4. Mineral Composition of *Grona triflora* leaf Extract

Magnesium was found to be highest in the plant extract followed by calcium and iron while copper and zinc were found to be lowest.

Table 4 Mineral Composition of *Grona triflora* leaf Extract in mg/100g

Mineral Element	Concentrations
Iron	18.01±0.01
Magnesium	304.32±4.12
Calcium	19.55±0.03
Zinc	0.09±0.01
Copper	1.10±0.12

Values are mean ± SD for 3 determinations

3.5. Hematological effects

The result of the study showed that rats treated with 250 mg/kg, 500mg/kg and 1000mg/kg had significantly ($p<0.05$) normal levels of PCV, HGB, MCV, MCH, PLT, RBC, WBC count, lymphocytes and neutrophils compared to Negative control. However, an increase in PCV, HGB, MCV, MCH, PLT, RBC, WBC count, lymphocytes and neutrophils values was observed in a dose dependent manner compared to negative control. In summary the effect of PHZ after induction was normalized in the three treatment doses (250 mg/kg, 500 mg/kg and 1000mg/kg) as well as the standard drug

Treatment significantly increased PCV, hemoglobin, RBC, and platelet levels in a dose-dependent manner. These findings suggest enhanced erythropoiesis and recovery from hemolysis induced by phenylhydrazine [4]. The improvement may be attributed to the presence of iron, folate, and vitamin C, which are essential for hemoglobin synthesis and iron absorption [11].

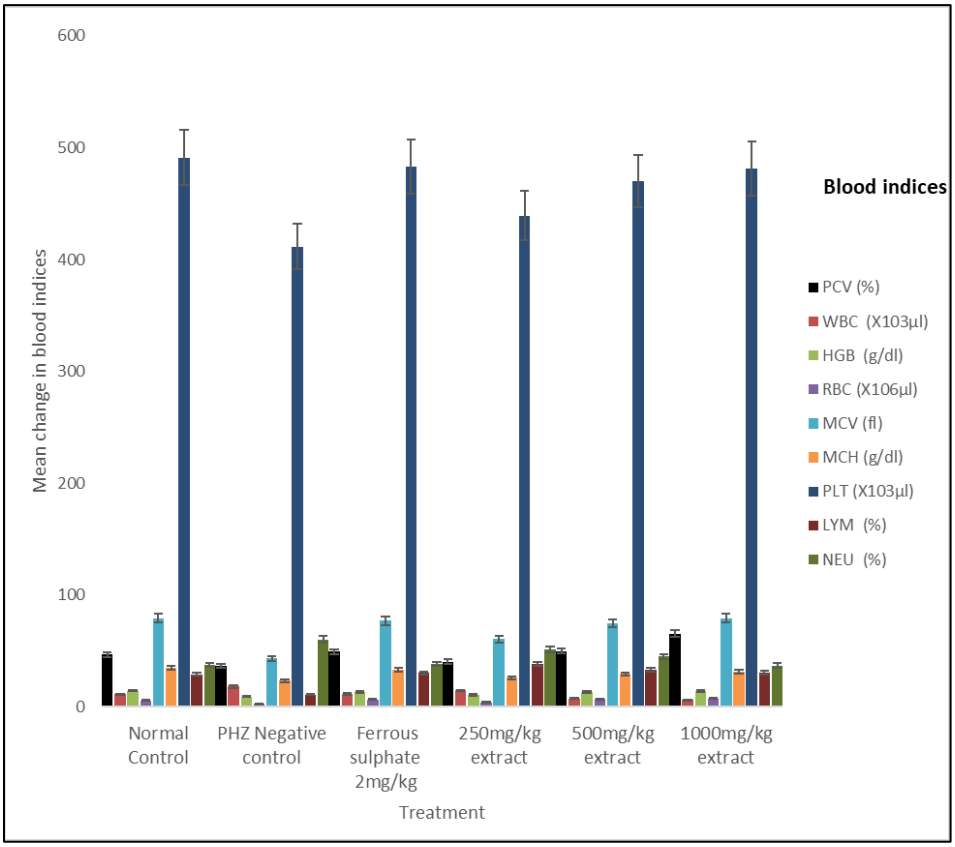


Figure 1 Effect of *Grona triflora* leaf Extract on different hematological indices.

3.6. Effect of *Grona triflora* leaf Extract on liver indices

The effects of *Grona triflora* leaf Extract on liver biomarkers of Albino rats induced anaemia with phenylhydrazine are shown in (Table 5). PHZ caused significant suppression of liver enzyme levels, indicating toxicity or metabolic disturbance. Treatment with both ferrous sulphate and different doses of the plant extract restored enzyme levels to near-normal ($P<0.05$). The extract demonstrates strong hepatoprotective and restorative effects.

Table 5 Effect of *Grona triflora* leaf Extract on liver indices

GROUP	ALT (U/L)	AST (U/L)	ALP (U/L)
Normal Control	19.43 ±0.20*	58.59 ± 1.64*	76.76 ± 2.42*
PHZ Negative control	7.33 ±0.10	24.47 ± 0.33	29.69 ± 0.22
(Ferrous sulphate 2mg/kg)	18.73 ±0.56*	58.77 ±0.44*	77.43 ±0.37*
250mg/kg extract	18.65 ±0.39*	59.41 ±0.32*	79.30 ±0.27*
500mg/kg extract	19.12 ± 0.29*	58.71 ±0.28*	78.47 ±1.36*
1000mg/kg extract	18.69 ± 0.47*	59.41 ± 1.54*	77.77 ±0.68*

Values are Mean ± SEM, (n = 5) *Significantly ($p< 0.05$) compared to negative control

3.7. Effect of *Grona triflora* leaf Extract on Kidney indices

The PHZ group shows a marked reduction in all kidney indices. This suggests severe disruption of kidney function and electrolyte balance, likely due to PHZ-induced toxicity or hemolytic damage affecting renal physiology. Values for

creatinine, urea, and electrolytes are restored close to normal control levels in Rats treated with ferrous sulphate and different doses of the leaf extract after induction of anemia. All parameters show significant improvement ($p < 0.05$) when compared to the negative control (Table 6)

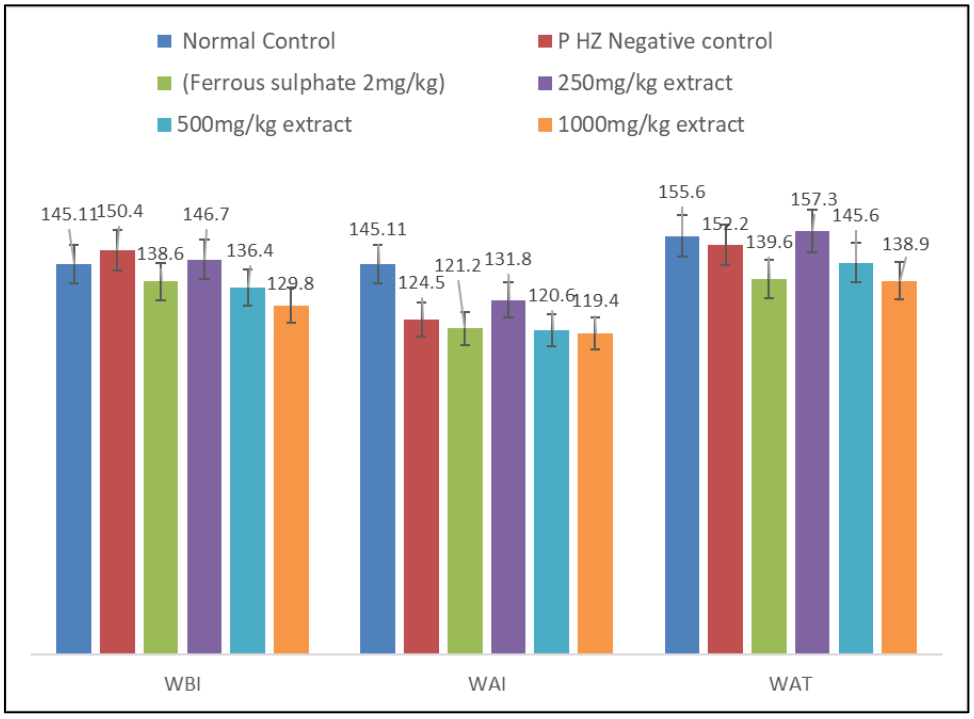
Table 6 Effect of *Grona triflora* leaf Extract on Kidney indices

GROUP	Creatinine (mg/dl)	Urea (mg/dl)	Na+ (mEq/L)	K+ (mEq/L)	C- (mEq/L)	HCO3 – (mMol L^{-1})
Normal Control	0.83 ± 0.17*	28.29±0.66*	145.31 ±0.34*	4.09 ± 0.13*	99.00 ±0.87*	23.8±1.72*
PHZ Negative control	0.12 ±1.23	14.11 ± 0.41	97.34 ±1.25	1.11± 0.60	23.11 ±0.16	16.17±1.18
(Ferrous sulphate 2mg/kg)	0.81±0.53*	28.32±0.90*	144.64 ±0.21*	4.12 ± 0.54*	92.50 ±0.32*	23.08±0.09*
250mg/kg extract	0.82±0.34*	28.11±0.66*	146.43± 0.77*	3.77 ±0.31*	91.86 ± 0.43*	24.76±1.00*
500mg/kg extract	0.84±0.56*	28.33±0.52*	144.52 ±0.63*	4.20 ± 0.49*	94.16 ± 0.55*	23.98±0.54*
1000mg/kg extract	0.83±0.78*	28.37±0.37*	144.90 ±0.31*	4.59 ±0.13*	99.00 ± 0.67*	23.54±0.65*

Values are Mean ± SEM, (n = 5) *Significantly ($p< 0.05$) compared to negative control

3.8. Effect of *Grona triflora* leaf Extract on body weight

There was reduction in the body weight of the animals after induction with PHZ. Generally, an increase in body weight was observed in all the rats during the treatment period. Although significant increase ($P<0.05$) was observed in the body weight of rats that received 2 mg/kg ferrous sulphate, 250, 500 and 1000 mg/kg of *Grona triflora* leaf Extract compared to the group treated only with phenylhydrazine.



WBI= Weight before induction; WAI= Weight after induction; WAT= Weight after treatment

Figure 2 Effect of *Grona triflora* leaf Extract on body weight on Body weight of studied animals

4. Discussion

The phytochemical analysis of the ethanol leaf extract of *Grona triflora* revealed the presence of terpenoids, flavonoids, alkaloids, saponins, and tannins. It has been reported that phenylhydrazine causes oxidative damage to red blood cells by increasing the formation of reactive oxygen species [17]. These phytochemicals act as powerful antioxidants, protecting cells by preventing or repairing damage caused by free radicals or highly reactive oxygen species [18]. The

presence of these phytochemicals might have contributed to the antihaematinic activity of the ethanol leaf extract of *Grona triflora* observed in the present study.

The vitamin content of the plant revealed appreciable amounts of some haematinic vitamins, such as folic acid, vitamin A, vitamin C, vitamin K, vitamin B6, and vitamin E. Deficiency of folic acid and other vitamins involved in erythropoiesis has been reported to cause macrocytic, megaloblastic, and pernicious anemia [19]. These haematinic agents have been found to be effective in relieving the symptoms of anemia in pregnancy and infancy. Vitamins A and C contribute to iron uptake, while vitamin C enhances the intestinal absorption of non-haem iron by reducing ferric ions to the ferrous form or by forming a soluble complex in the alkaline pH of the small intestine, thereby improving iron absorption [20]. This might explain the observed increase in haemoglobin levels in the present study.

The ethanol leaf extract of *Grona triflora* contributed to the faster reversal of phenylhydrazine-induced anemia in rats treated with the extract for three weeks. A similar outcome was observed when anemic rats were treated with *Tectona grandis* and *Piper guineense* [21,22].

The mineral composition of *Grona triflora*, as revealed in this study, is highly relevant in the context of anemia. Calcium and magnesium are useful in the formation of blood and intracellular and extracellular fluids of body cells. They also function as constituents of bones and teeth and are involved in the regulation of nerve and muscle function [23]. The iron value obtained in this study is higher than those reported for some selected leafy vegetables in Nigeria. Iron is a component of haemoglobin, myoglobin, and cytochromes. Previous studies have reported that zinc plays a major role in the synthesis of haemoglobin [24], and zinc deficiency has been associated with anemia. Copper-containing enzymes catalyze the oxidation of ferrous iron to ferric iron, which is necessary for the absorption and utilization of iron in haemoglobin formation [25]. Potassium is important in the management of sickle cell anemia; it plays a major role in heartbeat regulation and assists in nerve impulse transmission. Abnormal activation of the potassium chloride cotransport system has been implicated in cell potassium loss and dehydration observed in sickle cell anemia [26]. Magnesium, as part of the protein synthesis machinery, is also involved—along with calcium—in muscle contraction and blood clotting [13].

Administration of the ethanol leaf extract of *Grona triflora* significantly ($p < 0.05$) increased haematological parameters in the experimental groups in a dose-dependent manner. Significant increases were observed in the levels of PCV, Hb, platelets, MCV, MCHC, neutrophils, and RBC. A previous study reported similar results when ethanol leaf extract of yellow mombin was administered to rats [27]. However, a significant decrease ($p < 0.05$) was observed in the levels of WBC, lymphocytes, and neutrophils. Since white blood cells, lymphocytes, and neutrophils are indices of the body's immune response to infection, the observed decrease suggests an effect of the ethanol leaf extract of *Grona triflora* on these immune parameters. Treatment with 1000 mg/kg body weight was found to be more effective in ameliorating the effects of phenylhydrazine than other doses.

Liver function parameters used to detect liver disease or potential damage include serum levels of the enzymes AST, ALT, and alkaline phosphatase, as well as bilirubin levels. Typically, liver injury causes an increase in ALT, while the release of ALT and AST from the cytosol occurs due to hepatocyte damage, especially membrane injury [13]. The results of this study showed a significant decrease ($p < 0.05$) in the levels of ALP, AST, and ALT in the anemic untreated group compared to the normal control group. This shows that phenylhydrazine caused a significant suppression or alteration of liver enzyme activit**y**, indicating liver dysfunction or damage. However, groups treated with *Grona triflora* showed a significant increase ($p < 0.05$) in these enzyme levels, bringing them closer to those of the normal control group. Administration of 1000 mg/kg of *Grona triflora* restored enzyme levels to near-normal values, comparable to those observed in the group treated with the standard drug (ferrous sulphate). This suggests not only the safety of the leaf extract at appropriate doses but also its protective and restorative potential in cases of liver damage.

Elevated blood urea and creatinine levels are commonly associated with impaired renal function. In this study, however, the PHZ-induced anemic (negative control) group showed markedly reduced levels of these parameters, indicating disruption of normal kidney function and metabolic imbalance. Treatment with the ethanol leaf extract of *Grona triflora* significantly ($p < 0.05$) restored urea and creatinine levels toward those of the normal control group. The values observed in the extract-treated groups fell within the normal physiological range, comparable to the standard treatment group, suggesting that the extract did not exert nephrotoxic effects but rather demonstrated a protective and restorative influence on renal function.

In this study, the levels of sodium, potassium, chloride, and bicarbonate were markedly reduced in the PHZ-induced negative control group, indicating significant electrolyte imbalance. However, treatment with the ethanol leaf extract of *Grona triflora* and ferrous sulphate significantly ($p < 0.05$) restored these electrolyte levels toward those of the normal

control group. Although slight variations were observed, the values in all treated groups remained within normal physiological ranges, suggesting effective correction of electrolyte disturbances and absence of adverse effects on renal electrolyte regulation.

Additionally, injection of phenylhydrazine reduced body weight gain in rats compared to the normal control group. This may be due to reduced appetite, decreased disaccharidase activity, and structural and functional changes in the small intestine leading to nutrient malabsorption in anemic rats [28]. These findings are consistent with previous reports of significant weight loss in phenylhydrazine-exposed animals [29].

5. Conclusions

The ethanol leaf extract of *Grona triflora* demonstrated significant anti-anemic, hepatoprotective, and nephroprotective effects in phenylhydrazine-induced anemic rats. These effects are likely due to its rich phytochemical, vitamin, and mineral composition. The findings support the traditional use of *Grona triflora* in anemia management and highlight its potential as a natural therapeutic agent.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

All animal experiments were conducted in compliance with the 2010/63/EU Directive on the protection of animals used for scientific purposes and approved by the institution's animal ethical committee (PHACOOU/AREC/2023/030).

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