

Phytochemical Screening And Hypoglycemic Effect Of *Leptadenia Hastata*

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Abstract: The prevalence of diabetes mellitus is increasing at alarming rate globally and it is a major area of concern. The aqueous, ethanolic and n-hexane extracts of *L. hastata* leaf were screened for 20 phytochemicals while the aqueous leaf extract was further investigated for hypoglycemic activity. Eighteen albino rats were divided into six groups of three rats each; group I and II served as negative and positive controls while hyperglycemia was induced in groups III, IV, V and VI followed by oral administration of 100, 200, 300 and 400 mg/kg body weight of *L. hastata* aqueous leaf extracts for up to four weeks respectively. Animals were then tested for blood glucose level at days 14 and 28 post extract administration respectively. Phytochemical analysis showed the presence of Saponin, Tannin, Phenol, Cardiac glycoside, Anthranol glycoside, Flavonoid, Alkaloid, Terpenoid, Diterpenes, Triterpenes, Reducing sugar, Phytosterol, Coumarin, Steroids, Quinone, Essential oil, Phlobatanin, Ketone, Pentose and Anthraquinone in the ethanolic leaf extract of *L. hastata* while not all phytochemical were identified when the aqueous and n-Hexane extracts were analyzed. A significant reduction ($p \leq 0.05$) in blood glucose level at day 14 and day 28 of all the dosages administered were observed when compared with day 2 before the treatment. From the results, it can be concluded that the aqueous leaf extract of *L. hastata* demonstrated significant hypoglycemic and hypoglycemic effect in alloxan induced hyperglycemic rats possibly due to its bulk of secondary metabolites content. Our findings support its traditional use for managing diabetes mellitus cases.

Keywords: Diabetes mellitus; Hyperglycemia; Hypoglycemia; Phytochemicals; *Leptadenia hastata*;

Introduction

Diabetes mellitus (DM) is a group of metabolic disorder characterized by elevated blood glucose levels due to insufficient insulin production by the pancreas or the cells' resistance to insulin's role in facilitating glucose uptake (Jayaweera *et al.*, 2022). DM is now a widespread ailment, highly present in numerous countries across the globe, impacting individuals of all age groups in both developed and developing nations (Jayaweera *et al.*, 2022). The American Diabetes Association classified diabetes into two major categories; Type 1 diabetes (T1D), caused by autoimmune destruction of the β -cell of the pancreas by producing specific antibodies against its cells and type 2 diabetes (T2D) resulting from combination of resistance to insulin action and a secretory response by pancreatic β -cells that is inadequate to overcome insulin resistance, leading to relative insulin deficiency (Kumar *et al.*, 2021). According to World Health Organization (WHO) data, more than 250 million people are currently living with diabetes and this figure is anticipated to rise to more than 592 million by 2035, with T2D accounting for 80% to 90% of DM (Alqahtani *et al.*, 2020).

In Nigeria, one-third of all diabetes cases are said to occur in rural areas, with the balance occurring in metropolitan areas. In 2013, Nigeria has the largest burden in Africa (Ogbera and Ekpebegh, 2014), with 2.6 million cases, followed by South Africa with 1.9 million, and Tanzania with 1.7 million (Chiwanga *et al.*, 2016; IDF diabetes atlas, 2013). According to the survey by Adeloye *et al.*, (2017), 4.7 million Nigerians aged 20 to 79 were estimated to have had type 2 diabetes. One of the most reliable approaches to drug discovery has been found to be ethnobotanical surveys of medicinal plants (Ekpo *et al.*, 2008; Fabricant and Farnsworth, 2001).

Some medicinal plants have been reported to be useful in managing diabetes worldwide and have been used empirically in anti-diabetic remedies (Ibrahim *et al.*, 2010). Hypoglycemic activity of the plants is mainly due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes (Patel *et al.*, 2012). Thus, the consumption of herbal remedies has grown more than threefold in the past decade, giving rise to an entirely new sector known as the 'nutraceutical' industry (Osinubi, 2006).

Natural compounds (secondary metabolites) of medicinal importance, including terpenes, alkaloids, cardiac glycosides and others are primarily found in many higher plants (Deepak *et al.*, 2014). Avonoids, terpenoids, coumarins, phenolic compounds and other components found in herbal or plant products have been reported to lower blood glucose levels (Rao *et al.*, 2010). Ethnobotanic surveys have confirmed that plants like *Leptadenia hastata* and *Momordica balsamina* are used most notably for dietary purposes

have gained popularity as ingredients of polyherbal anti-diabetic formulations used by traditional herbal healers in Northern Nigeria (Aliero *et al.*, 2001; Aliero and Wara, 2009; Togola *et al.*, 2008).

L. hastata (Pers.) Decne, which belongs to the family Asclepiadaceae (Burkil, 1985), which are used for multiple medicinal purposes in Africa (Thomas, 2012). It is known as Yadiya (Hausa), Iranaji (Yoruba), Isanaje (Igbo), Sobotorooji (Fulani) and is a valuable herb with creeping latex stems, glabrescent leaves, glomerulus and racemes flowers as well as follicle fruits (Thomas, 2013). It is an edible non-domesticated vegetable found in wild throughout Africa (Bello *et al.*, 2011). *L. hastata* has been reported as an important source of dietary nutrients including fatty acids, beta carotene, protein and minerals, and pharmacologically active phytoconstituents such as phenolic compounds, triterpenoids, and glycosides (Hassan and Umar, 2006). The bark and leaves of *L. hastata* were found to contain mixtures of polyoxypregnane ester derivatives such as ester 12-O-aceylsarcostin, gagaminin, kidjolanin, metaplexigenin, and cyanforidin as well as triterpenes like lupeol, lupeol acetate, and lupeol palmitate (Aquino *et al.*, 1996).

The rationale behind treating/managing diabetes naturally, may lie in traditional plant remedies or herbal mixtures. Medicinal herbs are used to treat/manage certain medical conditions including diabetes in developing countries, specifically to lessen the cost of conventional medications for the populace (Bushnak *et al.*, 2021). It is in the wake of this background, that this study seeks to screen the phytochemical constituents, hypoglycemic, and hypoglycemic effects of the aqueous leaf extract of *Leptadenia hastata*.

Materials and Methods

Collection of Plant Samples

Fresh leaves of *L. hastata* were collected from Kano Zoological Garden, Kano State, Nigeria. The sample was immediately taken to the Laboratory of Life Sciences Department, Kano State Polytechnic, Nigeria for storage. The plant sample was identified and authenticated at the Herbarium Unit of Plant Biology Department, Bayero University Kano. The identification voucher number assigned is BUKHAN 0248 and sample specimen was deposited at the herbarium for future reference.

Extract preparation for Phytochemical Screening

30 g of fine powder of *L. hastata* was extracted with 300 mL of an appropriate solvents (distilled water, ethanol and N-hexane) (1:10 w/v) in a round bottom flask with magnetic stirrer for 24 h at room temperature. The extracts were then centrifuged at 5000 rpm for 15 min. An external magnetic field was applied to the magnetic stirrer to mix the solutions which facilitates the rotating of the small magnetic bar placed in the mixture of interest (Yashashri *et al.*, 2017).

Preparation of Aqueous Extracts of *L. hastata* for Hypoglycemic Assessment

The extraction was performed by soaking 30 g of the leaves powder of *L. hastata* in 250 mL of distilled water in a separate container, the volume of the extract, the difference between powdered mass and the mass of the residue were used for calculation the concentration of the extract. The concentration of extract was used for the calculation of dosages given to the treatment groups (group III, IV, V and VI).

Experimental Animals

Male and female albino rats were used for the experiment. They were procured from the Department of Biological Sciences, Bayero University Kano. The animals were housed in plastic cages in a well-ventilated room and had free access to water throughout the period of the experiment. They were fed with Vital Feed Growers (Palletized) Mash manufactured by Grand Cereals Ltd., a subsidiary of UAC of Nigeria.

Induction of Hyperglycemia

Hyperglycemia was induced according to the protocol of Aruna *et al.*, (1999) as described by (Okoli *et al.*, 2010) with slight modification. A single intraperitoneal injection of Alloxan Monohydrate of 150 mg/kg of body weight were given to group II (positive control) and treatment groups (group III, IV, V and VI) respectively. After two days, the blood was drawn from the tail vein of the rats from all the groups by snipping to determine blood glucose level with an automated glucose analyzer device (ACCU-CHEK Active Blood Glucose Monitoring System, Roche Diabetes Care GmbH, Mannheim, Germany). Animals with blood glucose level ≥ 225 mg/dl considered hyperglycemic and used for the study.

Experimental Design

Eighteen albino rats were used and divided into six groups of three rats each; group I and II served as negative and positive control that was induced hyperglycemia respectively. Group III, IV, V and VI hyperglycemic were administered orally with 100, 200, 300 and 400 mg/kg body weight of aqueous leaf extracts of *L. hastata* respectively for up to four weeks. A glucometer was used to test the glucose level in the animals at two and four weeks, 24 h after the last administration respectively.

Phytochemical Screening of the extract sample

Phytochemicals screening was carried out for the aqueous, ethanol and N-hexane extracts by the standard method (Tiwari *et al.*, 2011).

Test for Saponins (Foam Test): 0.5 g of all the extracts of *L. hastata* were mixed and shaken with 2 mL of water. If foams produce persists for 10 min, it indicates the presence of saponins.

Test for Tannins (Gelatin Test): To all the extracts, 1 % gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

Test for Phenols (Ferric Chloride Test): *L. hastata* were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Test for cardiac glycosides (Legal's Test): Extracts of *L. hastata* were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

Test for Anthranol Glycosides (Modified Borntrager's): The extracts of *L. hastata* were treated with Ferric Chloride solution and immersed in boiling water for about 5 min. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammoniacal layer indicates the presence of anthranol glycosides.

Test for Anthraquinone: A few drops of magnesium acetate solution were added to the *L. hastata* extracts. Formation of the pink colouration is an indicative of anthraquinone.

Test for Flavonoids (Alkaline Reagent Test): *L. hastata* extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Test for Alkaloids (Dragendroff's Test): The *L. hastata* extract were dissolved individually in dilute Hydrochloric acid and filtered. The filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Test for Terpenoid (Noller's Test): *L. hastata* extracts was warmed with a piece of tin and a few drops of thionyl chloride. Appearance of violet or purple colouration indicates the presence of terpenoid.

Test for Diterpenes (Copper acetate Test): Extracts of *L. hastata* were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

Test for Triterpenes (Salkowski's Test): *L. hastata* were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

Test for Reducing Sugars (Benedict's test): Filtrates from the *L. hastata* extracts were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Test for Phytosterols (Libermann Burchard's test): *L. hastata* extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

Test for Coumarin: To 2 mL of each *L. hastata* extracts, a few drops of alcoholic sodium hydroxide was added. Appearance of yellow colour indicates the presence of coumarin.

Test for Steroid (Libermann- Burchard Test): To the 2 mL of *L. hastata* extract, a few drops of chloroform, 3 - 4 drops of acetic anhydride and one drop of concentrate sulphuric acid were added. Appearance of purple colour, which changes to blue or green colour, shows the presence of steroid.

Test for Quinone: Each *L. hastata* extracts were treated with a few drops of concentrated sulphuric acid or aqueous sodium hydroxide solution. Colour formation indicates the presence of quinoid compound.

Test for Essential oil (Spot test): A small quantity of dried powdered of *L. hastata* extract was pressed between two filter papers. Formation of grease spot indicates the presence of essential oil.

Test for Phlobatannins (hydrochloric acid test): To 2 mL of the crude solution of the each extract, dilute hydrochloric acid was added and the mixture was observed for red precipitate that indicates presence of Phlobatannins.

Test for Ketone: A few crystals of resorcinol and an equal volume of concentrated hydrochloric acid were added to 2 mL of crude solution of each *L. hastata* and then heated over a spirit lamp flame and observed for a rose colouration, that shows presence of ketone.

Test for Pentoses: To 2 mL of the solution of the extracts of *L. hastata*, were added an equal volume of concentrated hydrochloric acid containing little phloroglucinol. This was then heated over a spirit lamp flame and observed for red colouration, indicative of presence of pentoses.

Statistical Analyses

The data were collected from various parameters of *L. hastata* and were presented as mean \pm standard deviation. Sample means were subjected to repeated measures analysis of variance (ANOVA) statistical analysis using Statistical Package for Social Sciences (SPSS Version 20.0 IBM Inc.) Duncan's multiple range test (DMRT), post-hoc statistical test was used after an ANOVA to identify which specific pairs or groups of means are significantly different at ($p \leq 0.05$).

Results

The result of the qualitative phytochemical screening is presented in Table 1. The results, which revealed the presence of Saponin, Tannin, Phenol, Cardiac glycoside, Anthranol glycoside, Flavonoid, Alkaloid, Terpenoid, Diterpenes, Triterpenes, Reducing sugar, Phytosterol, Coumarin, Steroids, Quinone, Essential oil, Phlobatanin, Ketone, Pentose and Anthraquinone in all the solvents used that is aqueous, ethanol and n-hexane extracts of the *L. hastata* leaf. It was found that all the twenty phytochemicals screened are present in ethanol extract. However, most of the phytochemicals are present in the aqueous and n-hexane extracts with the exception of Terpenoid, Diterpenes, Triterpenes, Phytosterol, Steroids, essential oil and Saponin, Tannin, Phenol, Alkaloid, Reducing sugar, Coumarin, Quinone, Phlobatanin, and Anthraquinone which were absent in aqueous and n-hexane extracts respectively.

Table 1: The qualitative result of phytochemical screening of aqueous, ethanol and n-hexane extracts of *L. hastata* leaf.

Parameters	Extract		
	Aqueous	Ethanolic	n-Hexane
Saponin	+	+	-
Tannin	+	+	-
Phenol	+	+	-
Cardiac glycoside	+	+	+
Anthranol glycoside	+	+	+
Flavonoid	+	+	+
Alkaloid	+	+	-
Terpenoid	-	+	+
Diterpenes	-	+	+
Triterpenoids	-	+	+
Reducing sugar	+	+	-
Phytosterol	-	+	+
Coumarin	+	+	-
Steroids	-	+	+
Quinone	+	+	-
Essential oil	-	+	+
Phlobatanin	+	+	-
Ketone	+	+	+

Pentose	+	+	+
Anthraquinone	+	+	-

key: + = present, - = absent

Table 2 presents the results of hypoglycemic effect of the aqueous *L. hastata* leaf extract on blood glucose level in the Alloxan induced hyperglycemic rats. After day 2 post alloxan inducement of hyperglycemia, animals in groups II, III, IV, V and VI developed hyperglycemia. From our results, the negative control group maintained a steady glucose level while hyperglycemia was observed to worsen over time in the positive control group. The extract treated groups exhibited dose- and time-dependent reduction in blood glucose levels with higher doses producing stronger and sustained glycemic control by Day 28.

Repeated-measures ANOVA revealed a significant effect of time on blood glucose levels ($F(2, 24) = 1997.56, p \leq 0.05, \eta^2 = 0.994$). Furthermore, a significant time * treatment group interaction was observed ($F(10, 24) = 294.69, p \leq 0.05, \eta^2 = 0.992$), indicating that the pattern of glucose change over time differed significantly among experimental groups. The positive control shows progressive hyperglycemia over time while the extract-treated groups show a dose- and time-dependent reduction in glucose, most pronounced by Day 28. The 400 mg/kg bw group approaches normoglycemia, comparable to the negative control by Day 28, suggesting strong antihyperglycemic potential of our extract. A significant between-subjects effect of treatment group was observed on blood glucose levels ($F(5, 12) = 583.66, p \leq 0.05, \eta^2 = 0.996$) when averaged across Day 2, Day 14, and Day 28, indicating time-dependent treatment responses.

A significant difference ($p \leq 0.05$) was observed when the mean glucose level of group I which is normoglycemic was compared with the means of groups II (positive control) and, groups III, IV, V and VI which received 100, 200, 300 and 400 mg/kg of aqueous leaf extract of *L. hastata* respectively for up to four weeks after which the blood glucose levels were measured after post extract administration on day 14 and day 28 respectively. There was significant ($p \leq 0.05$) reduction in blood glucose level on day 14 and day 28 of all the dosages administered when compared with day 2 before treatment. However, there was no significant difference observed at ($p > 0.05$) when blood glucose level of group VI of day 14 (140.67 ± 6.35 mg/dl) was compared with blood glucose level of day 14 of group I (140.33 ± 2.08 mg/dl).

Table 2: Effect of aqueous leaf extract of *L. hastata* (AELH) on blood glucose level in hyperglycemic rats.

Group	Dose	Glucose Concentration (mg/dl)		
		Day 2	Day 14	Day 28
Group I	Negative Control	139.33 ± 1.53^{Ae}	140.33 ± 2.08^{Ae}	138.00 ± 1.00^{Ab}
Group II	Positive Control	230.33 ± 2.52^{Bd}	239.33 ± 4.04^{Ba}	251.33 ± 7.09^{Aa}
Group III	100 mg/kg bw	275.33 ± 4.51^{Ab}	198.33 ± 2.52^{Bb}	100.67 ± 7.09^{Ce}
Group IV	200 mg/kg bw	247.00 ± 5.57^{Ac}	184.67 ± 5.03^{Bc}	111.00 ± 3.61^{Cd}
Group V	300 mg/kg bw	241.33 ± 6.11^{Ac}	175.67 ± 4.73^{Bd}	121.67 ± 4.16^{Cc}
Group VI	400 mg/kg bw	301.67 ± 4.73^{Aa}	140.67 ± 6.35^{Be}	103.00 ± 3.46^{Cde}

Values are mean \pm SD (n = 3). Different lowercase letters a, b, c, d, e within the same column indicates significant differences among groups at the same time point. Different uppercase letters A, B, C within the same row indicate significant differences across time within the same group (Bonferroni-adjusted, $p \leq 0.05$).

Discussion

Phytochemical screening and hypoglycemic effect of *L. hastata* were investigated. Table 1 divulged the qualitative phytochemical analysis of aqueous, ethanol and N-hexane extracts *L. hastata*. All parameters of phytochemicals screened were presence in ethanol extract. Our findings are in line with that Bello *et al.*, (2011) who reported that the *L. hastata* leaf contains phenolic glycosides, tannins, flavonoids, proanthocyanidins, alkaloids and saponins. Equally also, the dichloromethane extract of *L. hastata* showed the presence of alkaloids, steroids, terpenoids, saponins, flavonoids, Carbohydrate and Glycoside are present while tannins, and saponin were absent in their studies (Umaru and Umaru, 2018).

In another study on, the root of *L. hastata* phytochemicals including alkaloids, flavonoids, saponins, phenols, tannins, glycosides, carbohydrates and triterpenes were detected in both aqueous and methanolic extracts while steroid were absent in both extracts (Namadina *et al.*, 2019). The results from the current study and the reports from literature, polar solvents are excellent for extracting vital compounds from *L. hastata*.

Like results from leaf extract of *L. hastata*, Saponins, flavonoids, alkaloids, pentose, tannins and ketones were also detected in the root extract of *L. hastata* suggesting the pharmacologically active components, which supports its ethnobotanical claims of traditional

herbalist in Northern Nigeria in the treatment/management of various ailments (Sanda *et al.*, 2013). *L. hastata* leaves possesses a rich phytochemical and nutrients, which can be utilized as pharmacological agents in the treatment of some diseases as well as meet the nutritional requirement of the body (Ahmed *et al.*, 2024).

The findings of the current research are in agreement with the results of Muhammad *et al.*, (2024), who reported the qualitative phytochemical analysis of the aqueous, ethanol and n-hexane extracts of *Hyphaene thebaica* which was found to have the presence of Saponin, Tannin, Phenol, Cardiac glycoside, Anthranol glycoside, Flavonoid, Alkaloid, Terpenoid, Diterpenes, Triterpenes, Reducing sugar, Phytosterol, Coumarin, Steroids, Quinone, Essential oil, Phlobatanin, Ketone, Pentose and Anthraquinone compounds which have a vital function in fighting against diseases producing pathogens.

As observed in the result of the hypoglycemic effect of aqueous *L. hastata* leaf extract on blood level in induced hyperglycemic rats. There was significant difference between day 14 and day 28 when compared with post alloxan induced hyperglycemia (day 2) at $p < 0.05$. This implies that the dosages (100, 200, 300 and 400 mg/kg) used in this research were effective in lowering the blood glucose level. The effects are dose and time dependent, that is as dose and duration increases, the blood glucose level decreases. This hypoglycemic effect of the leaf extract of *L. hastata* could be due to the presence of phytochemicals analyzed in this present research or it could be linked to one or more mechanisms. The possible mechanism includes the activation of β -cells and subsequent secretion of insulin and stimulation of the insulin receptors. It might also be due to the enhancement of transport of blood glucose to the peripheral tissue. It can also be linked to the inhibition of the alpha-glucosidase activity (Babu *et al.*, 2003). The leaf extract of *L. hastata* administration for up to two and four weeks reduced blood glucose level significantly when compared with the groups of day two post alloxan induced hypoglycemia.

This implies that, the significant drop in glucose level of day two of group VI post alloxan induced hyperglycemia from 301.67 ± 4.73 mg/dl to 140.67 ± 6.35 mg/dl of group VI day 14 might be due to the presence of phytochemicals and the higher dose (400 mg/kg bw) of aqueous leaf extract of *L. hastata* they received. Sand *et al.*, (2024) reported that, there was significant reduction in blood glucose level of *L. hastata* root from 318.50 ± 55.2 to 163.00 ± 37.10 and 377.50 ± 45.10 to 273.00 ± 29.70 at 200 and 400 mg/dl of 18 h post of the extract respectively. These findings support the present study.

Moreover, day 28 of the post treatment groups (group III, IV, V and VI) showed reduced blood glucose level significantly at all the dosages of *L. hastata* leaf extract administered (100, 200, 300 and 400 mg/kg) when compared with day 2 post hyperglycemic groups (group II, III, IV, V and VI). Therefore, hypoglycemic and anti-diabetes of aqueous leaf extract of *L. hastata* was achieved at dosages of day 14 and 28 respectively.

The dosages used in this research are considered safe according to the report of Tamboura *et al.*, (2005) who conducted their experiments by on male albino mice using concentrations of 1000-2000 mg/kg body weight of *L. hastata* aqueous extract (leaves and stems). The mice were injected with the extract intraperitoneally and were observed during 48 to 72 h. According to their findings *L. hastata* is considered safe to use due to its high LD quotient value.

Conclusion

In this present work, the aqueous leaf extract of *Leptadenia hastata* demonstrated significant hypoglycemic and anti-diabetic effect in hyperglycemic rat's model. This is attributed due to the bulk of secondary metabolites present in it. These findings support its traditional use for treating/managing diabetes mellitus cases.

Declarations

Author contribution statement

Saratu Ahmad Othman, Mustapha Garba Muhammad, Musbahu Muhammad Sani: Conceived, designed and performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote and edit the manuscript.

Abubakar Shuaibu Ismail, Haruna Tsoho Bala and Mustapha Ishaq: Performed the experiments; Contributed reagents, materials, take care and supply food and water to the experimental animals throughout the period of the experiment.

Funding statement

This research project was supported by the Tertiary Education Trust Fund (TETFund) with TETFund Reference No: TETF/DR&D/CE/POLY/KANO/RG/2025/VOL.I.

Competing interest statement

The authors declare that there is no conflict of interest.

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