Potential of the homeopathic remedy, Arnica Montana 30C, to reduce DNA damage in Escherichia coli exposed to ultraviolet irradiation through up-regulation of nucleotide excision repair genes

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OBJECTIVE: To examine to what degree an ultra-highly diluted homeopathic remedy, Arnica Montana 30C (AM-30C), used in the treatment of shock and injury, can modulate the expression of nucleotide excision repair genes in Escherichia coli exposed to ultraviolet (UV) irradiation.

METHODS: E. coli were cultured to their log phase in a standard Luria-Bertani medium and then exposed to sublethal doses of UV irradiation at 25 and 50 J/m² for 22.5 and 45 s, respectively. The UV-exposed bacteria were then supplemented with either AM-30C (drug) or placebo (P-30C). The drug-treated and placebo-treated bacteria were subjected to assay for DNA damage and oxidative stress 90 min after UV exposure. Several protocols like comet assay, gel electrophoresis for DNA ladder and intracellular reactive oxygen species (ROS) generation, and biomarker measurement like superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) were conducted. The mRNA expressions of the excision repair genes like ultraviolet repair uvrA, B and C genes (or also known as excision repair genes) were estimated by reverse transcription-polymerase chain reaction method.

RESULTS: The UV-exposed bacteria showed DNA damage and oxidative stress, as revealed by an increase in ROS generation, and a decrease in SOD, CAT and GSH activities. As compared to placebo, the AM-30C-treated bacteria showed less DNA damage and oxidative stress as manifested by a decrease in ROS generation, and an increase in SOD, CAT and GSH activities. AM-30C also up-regulated the expression of repair genes as compared to the control.

CONCLUSION: AM-30C helped repair the DNA damage through up-regulation of repair genes.
and also ameliorated the oxidative stress through the reduction of ROS generation and suitable modulation of anti-oxidative stress enzymes.

**KEYWORDS**: *Escherichia coli*; ultraviolet rays; homeopathy; *Arnica*; reactive oxygen species; DNA damage; DNA repair

The increase in ultraviolet (UV) radiation on the surface of the earth due to depletion of the stratospheric ozone layer has recently fuelled a renewed interest in studies related to ascertaining various deleterious effects of UV-radiation on living organisms\(^\text{(1)}\) and possible remedial measures. Radiation is one of the most effective and carcinogenic exogenous agents, that can interact directly with DNA or indirectly through generation of free radicals or reactive oxygen species (ROS), thus inducing DNA damage or altering the genomic integrity\(^\text{(2-5)}\). This can drastically affect normal life processes of all organisms ranging from lower prokaryotes to higher eukaryotes\(^\text{(6,7)}\). Therefore, most living organisms have developed an intrinsic mechanism to repair damaged DNA as early as possible.

DNA damage caused by UV exposure is repaired mainly by direct reversal of DNA damage, particularly in the case of formation of cyclobutane pyrimidine dimers, by processes such as photoreactivation, by excision repair when there is a mismatch in complementary bases, and by post-replication repair\(^\text{(4,8-10)}\). However, cells have developed a number of repair or tolerance mechanisms to counteract various forms of DNA damage. The biochemical and molecular repair pathways have been extensively investigated in some model organisms such as *Escherichia coli*, *Saccharomyces cerevisiae*, and humans\(^\text{(11)}\), where specialized repair proteins scan the genome continuously and encounter the DNA lesions by triggering several distinct intrinsic repair mechanisms, such as photo reactivation, nucleotide excision repair (NER)\(^\text{(12,13)}\) and some other specialized repair systems such as SOS response which is an error-prone repair mechanism that comes into action when the amount of UV-irradiated DNA damage exceeds the capacity of the faithful repair system (NER) to correct that damage. This process can allow cell survival but often at the cost of mutagenesis.

Since the NER pathway in *E. coli* has been extensively studied and is recognized as one of the major repair pathways to come into action in the case of DNA damage due to UV or ionizing radiations\(^\text{(11,14)}\), the authors chose to examine whether the potentized remedy duluted beyond Avivadro’s limit (10\(^{-6}\) in the present study) could have any role or influence on the activities or expression levels of these repair genes in *E. coli* that regulate synthesis of the UvrA, B, C endonuclease enzyme complex consisting of the UvrA, B, C protein, and DNA helicase II (sometimes also known as UvrD in this complex). In *E. coli*, NER is catalyzed by the products of mainly three genes (*uvrA*, *B* and *C*). The protein UvrA recognizes the damaged DNA and recruits UvrB and UvrC to the site of the lesion. UvrB and UvrC then cleave on the 3’ and 5’ sides of the damaged site, respectively, thus excising an oligonucleotide consisting of 12 or 13 bases. The action of helicase is then required to remove the damage-containing oligonucleotide from the double-stranded DNA molecule, and the resulting gap is filled by DNA polymerase I and sealed by ligase\(^\text{(8)}\).

The selection of the potentized remedy Arnica Montana 30C (AM-30C) was based on claims made in homeopathy literature asserting the use of this remedy in effectively combating internal shock and injury\(^\text{(15)}\). Earlier studies have demon-
strated that ultra-low doses of homeopathic remedies such as Ginseng 30C showed positive modulations of cytogenetical and haematological effects in whole body X ray-irradiated mice\(^{[10]}\). Similarly, potentized Arnica Montana, known for its wound-healing and anti-shock response properties, has been reported to have profound anticlastogenic actions in mice\(^{[17,18]}\). AM-30C has also been reported to ameliorate genotoxic effects of ultrasonic sound waves in mice\(^{[19]}\).

In recent years, there has been a renewed interest by large sectors of society in alternative forms of medicines due to the toxic nature and unwanted side effects of many orthodox medicines. Homoeopathy, that often uses micro-doses of very high dilutions of natural substances originating from plants, minerals or animal parts\(^{[20,21]}\), is one such alternative mode of treatment that is gaining popularity in many countries because of its none or negligible side effects although its efficacy is challenged by others. The initial drug substance is generally dissolved in aqueous ethanol (mostly 70%), known as the “mother tincture”. In the centesimal scale, when 1 mL of the mother tincture is diluted with 99 mL of aqueous ethanol (solvent vehicle of the drug) and given 10 jerks or successions, potency 1C is produced. Further, when 1 mL of the potency 1C is again diluted with 99 mL of aqueous ethanol and given 10 jerks, the potency 2C is produced, and so on. Therefore, when the drug attains potency 12C, it becomes diluted to \(10^{-24}\) (beyond Avogadro’s limit) and the existence of even a single molecule of the original drug substance in such high dilution becomes highly improbable. Although some researchers demonstrated the existence of nanoparticles of the original drug substance in such ultra-highly diluted homeopathic drugs\(^{[22]}\), the efficacy is often questioned by rationalists, as the precise mechanism of drug action is still scientifically unknown. Therefore, the authors of this study assert that in-depth research is warranted to validate the efficacy of ultra-high dilutions in simple living forms and attempt to understand the molecular mechanism of its action.

Thus, in the present investigation, the authors aimed to test the hypothesis that the potentized remedy, AM-30C, used against shock and injury, could show its efficacy in reducing DNA damage caused by UV irradiation in \(E. coli\), and if successful, to understand the possible molecular mechanism involved to accomplish it.

1 Materials and methods

1.1 Culture and growth conditions of \(E. coli\) \(E. coli\) (\(E. coli\) CA, \(F^+\) wild type molecular biology strain) were obtained from the Department of Biochemistry and Biophysics, University of Kalyani, India, and maintained as a primary culture in the standard Luria-Bertani (LB, 0.5% yeast extract, 1% tryptone, 1% sodium chloride and 2% bacto agar) plates and slants. All experiments were performed with starved synchronized cells by following the protocol used by Saha et al\(^{[23]}\). Briefly, cells were first grown at 37 °C up to the log phase about 10\(^6\) cells/mL (optical density at 600 nm for 10\(^8\) cells/mL was found to be 0.2 by Shimadzu PharmaSpec UV-1700 spectrophotometer) in LB medium. The grown cells were washed with and suspended in a starvation buffer (5.0 g of potassium chloride (KCl), 1.0 g of sodium chloride (NaCl), 1.2 g of Tris, 0.1 g of magnesium sulphate (MgSO\(_4\)), 1.0 mL of 1.0 mol/L calcium chloride (CaCl\(_2\)) in 1 000 mL distilled water; pH adjusted to 8.1) and then were allowed to starve with shaking at 37 °C for 1 h for synchronization of cells.

1.2 UV irradiation For UV exposure, cell suspension (in a starvation buffer) at a concentration of about 10\(^8\) cells/mL was taken as a thin layer in a petri-dish (5 cm diameter), which was placed on ice in a tray and was shaken gently during irradiation. The UV lamp used to irradiate was of a strength 1.1 J/(m\(^2\)·s). The cells were irradiated with several UV doses of 10, 25, 50, 75 and 100 J/m\(^2\) for 9, 22.5, 45, 67.5 and 90 s, respectively, according to the UV strength. The repair experiments were performed under dark conditions to avoid the possibility of photo reactivation-mediated repair of the UV-damaged \(E. coli\) cells.

1.3 Selection of sublethal dose and final treatment groups From this UV-irradiated culture, 2% of cells each, were inoculated into a fresh LB media, which were subdivided into the following groups: UV-unirradiated LB control, LB plus UV dose-treated, LB plus UV dose plus drug AM-30C-treated, LB plus UV dose plus placebo-treated. From the dose response curve, two sublethal UV doses: dose 1 (25 J/m\(^2\)) and dose 2 (50 J/m\(^2\)) with cell survival percentages of approximately 60% and 26.5%, respectively, were finally selected and further experiments were proceeded with these two doses.

1.4 Replication Different parameters of study mentioned below have been conducted on three subsets of experiments each and for each subset of experiment, three replicates were studied (\(n = 9\)). For normalization of the data against a standard growth pattern of the bacteria, the mean values were produced.

1.5 Estimation of intracellular ROS generation The intracellular ROS generation of the experimental cells was also analyzed qualitatively by a flow cytometer (BD FACS-ARIA)\(^{[24]}\). Cells grew up to their log phase and the accumulated ROS was measured by flow-cytometry using the 2',7'-dichlorodihydrofluorescein diacetate (DCF-DA) dye after the UV doses 1 and 2 treatment and thereafter, addition of AM-30C and placebo-30C (P-30C), respectively. Cells were washed with
phosphate buffer saline (PBS) and then incubated for 30 min with 40 nmol/L DCF-DA in dark conditions. Fluorescence was collected in the green channel fluorescent filter 1 (525 nm) of the flow cytometer equipped with an argon laser (488 nm) and with standard filter set up.

1.6 Superoxide dismutase and catalase assay Specific activities of superoxide dismutase (SOD)\[^{[25]}\] and catalase\[^{[26]}\] in E. coli of different sets were quantified by spectrophotometric study.

1.7 Estimation of glutathione content For glutathione (GSH) assay\[^{[27]}\], cell extract was added to a reaction mixture of sulfosalicylic acid, sodium phosphate buffer (pH 8.0) and double distilled water. 5, 5'-dithiobis (2-nitrobenzoic acid) solution was added and the absorbance was read at 412 nm by the spectrophotometric method.

1.8 DNA gel electrophoresis Chromosomal DNA molecules were extracted from different sets of control and treated cells using the phenol-chloroform-isooamyl alcohol method and then were electrophoresed on 1.5% agarose gel in a Tris-acetate-EDTA (TAE) buffer with 0.5 µg/mL ethidium bromide (EtBr) and visualized under a UV trans-illuminator and photographed.

1.9 Comet assay For comet assay\[^{[28]}\], cells growing up to their log phase were suspended in 0.75% low melting agarose (containing 1 mg/mL lysozyme) and layered over a frosted microscopic slide. The slides immersed in a lysis buffer (pH 10.0) were followed by electrophoresis in an electrophoresis buffer (pH 13.0) for 20 min. After being washed with a neutralizing buffer (pH 7.5), those slides were stained with ethidium bromide (1 mg/mL) and examined under a fluorescence microscope (Leica DMLS, USA). The extent of DNA breakage was determined by measuring the comet tail length using the Motie Image Plus 2.0.0L software, China.

1.10 RNA isolation, cDNA preparation and gene level expression study by reverse transcription-polymerase chain reaction Total RNA was isolated from E. coli cells using TRIzol reagent (Bangalore Genei, India)\[^{[29]}\]. To prepare cDNA, 2 µg of total RNA were reverse transcribed using oligo-DT primer in a final concentration of 100 mmol/L magnesium chloride (MgCl\(_2\)), 750 mmol/L KCl and 500 mmol/L Tris (hydroxymethyl) aminomethane (Tris-HCl) (pH 8.3), 10 mmol/L of each deoxy nucleotide triphosphate (dNTPs), 20 units of RNase inhibitor and 10 units of Moloney murine leukaemia virus (M-MuLV) reverse transcriptase (RT) (Chromous Biotech, India). The mixture was incubated at 37 °C for 1 h and terminated at 95 °C for 2 min. Polymerase chain reaction (PCR) was performed using Taq DNA polymerase (Chromous Biotech, India) in accordance with the manufacturer’s instruction. Briefly 5 µL of reverse transcribed product (cDNA) were used in a total volume of 25 µL\[^{[30]}\]. The primer sequences of the amplified genes are shown in Table 1. PCR was performed on an automated thermal cycler (Applied Biosystems, USA). For each PCR, pre-denaturation (95 °C for 3 min) was necessary before the amplification cycle (35 cycles) and after reaction there was a final extension phase at 72 °C for 10 min. Amplified cDNA products were separated on 1.0% agarose gel electrophoresis in a TAE buffer with 0.5 µg/mL ethidium bromide and visualized under the UV trans-illuminator and photographed. Densitometry was performed on a negative image using the ImageJ software. ImageJ software is a public domain software which was initially produced by Richard Stallman, at NIH, USA, and thereafter modified for various uses.

| Primer name | Primer sequence (5’-3’)
|-------------|------------------------|
| uvrA        | Forward: TAAGCTGCAAAACGTTGATGG
|             | Reverse: GTGCCAACATCAACCAATGG
| uvrB        | Forward: GTCTGGGCGATCCTGATTATA
|             | Reverse: CAATGTTTCACCACTCTCGT
| uvrC        | Forward: TCGGGGATCTTACGTGTCG
|             | Reverse: TGGAGTGGTTTGCACATCAG
| G-3-PDH     | Forward: CCCACTAAATCATCAATGGG
|             | Reverse: CCTCCCAACGACATCGG

G-3-PDH: glyceraldehyde-3-phosphate dehydrogenase.

1.11 Blinding The observers were blinded during observation and scoring of the data. The different coded vials (containing randomized populations of E. coli) of both the experimental (containing drug AM-30C) and control sets along with placebo (P-30C; 70% succussed alcohol) were not known to the observers as to which one belonged to the treated or control group; they were only deciphered later to remove any bias in observation.

1.12 Statistical analysis Data were presented as mean ± standard error of mean. Statistical analysis was performed by Student’s paired t-test, one-way analysis of variance and post hoc analysis using SPSS 10.0 software. P < 0.05 was considered significant.

2 Results

2.1 UV sublethal dose selection The UV dose-response curve is shown in Figure 1A. In this curve, initially the percentage of cell survival of E. coli decreased steadily which was followed by sharp decline in cell survival forming a gradual bending region (UV doses from 10 to 50 J/m²). That region of gradual bending is known as the shoulder region of the growth survival curve, whose corresponding dose is generally chosen as the sublethal dose where cells could repair most of the DNA lesion. Here the authors chose 25 and 50 J/m² as dose 1 and dose 2, respectively, where the cell survival percentages were found to be 61% and 26.5%, respectively (Figures 1B and 1C).
2.2 Intracellular ROS measurement by flow cytometric analysis

The intracellular ROS generation in the control and treatment groups are summarized in Figure 2. Generation of ROS showed the minimum in the control groups. Much ROS was generated when cells were treated with UV doses 1 and 2 which depicted a significant increase in the generation of intracellular ROS after UV irradiation. Treatment with AM-30C decreased ROS generation with respect to the UV dose-treated groups alone as well as with UV dose plus placebo-treated groups.

2.3 Measurement of the specific activity of SOD

Results of the specific activities of SOD in the control and treatment groups are summarized in Figure 3. The specific activity of SOD in E. coli cells decreased after UV doses 1 and 2 treatments, in comparison with the control cells. Again, AM-30C-treated cells showed a significant increase in SOD specific activity. The addition of placebo did not make any significant difference in specific activity of SOD in E. coli in UV dose 2-treated cells but was found to have marginally increased in UV dose 1-treated cells.

2.4 Measurement of the specific activity of catalase

The specific activities of catalase in the control and treatment groups are summarized in Figure 4. In the UV-irradiated dose 1 group, the specific activity of catalase in E. coli cells significantly decreased compared with the control group. Addition of AM-30C to both the UV doses 1- and 2-treated cells showed a significant increase in catalase activity as compared to only UV-exposed ones.
2.5 GSH content assay The amounts of intracellular GSH (free GSH to react with salicylic acid and 5, 5'-dithiobis (2-nitrobenzoic acid) and respond to absorbance at 412 nm) are presented in Figure 5. The concentration of the free GSH in E. coli cells significantly decreased after UV doses 1 and 2 treatments as compared to the control ones. Addition of AM-30C to the treatment groups showed a considerable increase in the intracellular concentration of free GSH as compared to the only UV-treated groups. The addition of placebo did not make any significant difference in intracellular GSH concentration from that of only UV-treated groups.

2.6 DNA damage studied through gel electrophoresis Figure 6 represents the data of genomic DNA determined through agarose gel electrophoresis of different control and treatment groups of E. coli. UV-treated groups showed a smeared damaged DNA (smear indicates extensive DNA fragmentation) with respect to UV-unirradiated control cells showing a clear single band, representing an intact DNA. AM-30C-treated groups also showed a clear single band, while the placebo-treated cells also showed DNA smearing like that of the only UV-treated E. coli.

Figure 3 Data of superoxide dismutase assay
A bar graph showing specific activity of superoxide dismutase (μmol/(L·mg·min)) was plotted. Values were measured in three independent experiments carried out in triplicate and were expressed as mean ± standard error of mean. * * P<0.01, vs control; ** P<0.01, vs dose 1 (UV at 25 J/m²); ▲ ▲ P<0.01, vs dose 2 (UV at 50 J/m²). AM30C: Arnica Montana 30C; P30C: placebo-30C.

Figure 4 Data of catalase assay
A bar graph showing specific activity of catalase (μmol/(L·mg·min)) was plotted. Values were measured in three independent experiments carried out in triplicate and were expressed as mean ± standard error of mean. * * P<0.01, vs control; ▲ ▲ P<0.01, vs dose 1 (UV at 25 J/m²); ▲ ▲ P<0.01, vs dose 2 (UV at 50 J/m²). AM30C: Arnica Montana 30C; P30C: placebo-30C.

Figure 5 Data of glutathione assay
Intracellular glutathione concentration in E. coli cells (μmol/L per 10⁸ cells) was plotted. Values were measured in three independent experiments carried out in triplicate and were expressed as mean ± standard error of mean. * * P<0.01, vs control; ▲ ▲ P<0.01, vs dose 1 (25 J/m²); ▲ ▲ ▲ P<0.01, vs dose 2 (50 J/m²). AM30C: Arnica Montana 30C; P30C: placebo-30C.

Figure 6 DNA damage assay through gel electrophoresis Images of DNA gel after electrophoresis containing DNA samples of different treatments. A: Dose 1-treated; B: Dose 2-treated. Ln1: Control; Ln2: Ultraviolet-treated; Ln3: Ultraviolet plus Arnica Montana-30C-treated; Ln4: Ultraviolet plus placebo-30C-treated.

2.7 Comet assay The DNA damage by comet assay in different control and treatment groups is summarized in Figure 7. Comet tail length was found to be the minimum in control cells. This indicates that there was almost no DNA damage in UV-unirradiated cells (a greater comet tail length depicts greater DNA damage). The comet tail lengths significantly increased in doses 1- and 2-treated groups when compared to the control. Addition of AM-30C to UV-irradiated cells resulted in a considerable decrease in comet tail lengths at both of the doses. The placebo-treated groups also showed a considerable increase in comet tail
length like that of the UV-treated groups. Thus the values indicate that the drug reduced the quantum of DNA damage induced by UV radiation.

2.8 Gene expression analysis by RT-PCR The mRNA expression levels of uvrA (GenBank accession number ECK4030), uvrB (GenBank accession number ECK0788) and uvrC (GenBank accession number ECK1912) genes and housekeeping gene (glycerolaldehyde-3-phosphate dehydrogenase, GenBank accession number US8205) of E. coli cells in different control and treatment groups are summarized in Figure 8.

The arbitrary band intensity level of uvrA, B and C genes showed increased expression levels both in UV doses 1- and 2-treated cells when compared to UV-unirradiated control cells, whereas, in the case of AM-30C-treated groups, the arbitrary band intensity resulted in a significant increase in both of the dose levels. Addition of placebo did not make that much significant difference in band intensity from that of only UV dose-treated groups except for dose 2 plus placebo-treated group which revealed significant over-expression of uvrA only. The intensity of the band was known to be expression-dependent, namely, a more intense band indicates more genetic level expression.

![Figure 7 DNA damage analysis by comet assay](image)

Figure 7 DNA damage analysis by comet assay

A: Photographs showing the DNA damage by comet assay.
B: Graphical representation of mean values of comet tail lengths (μm). Values were measured in three independent experiments carried out in triplicate and were expressed as mean ± standard error of mean. ** P<0.01, vs control; ΔΔ P<0.01, vs dose 1 (25 J/m²); ΔΔΔ P<0.01, vs dose 2 (50 J/m²).

(a) represents the ultraviolet-irradiated control cells, (b) and (c) represent ultraviolet doses 1- and 2-treated cells, respectively; (d) and (e) represent dose 1 plus Aronia Montana-30C-treated cells and dose 2 plus Aronia Montana-30C-treated cells, respectively; (d) and (g) represent dose 1 plus placebo-30C-treated cells and dose 2 plus placebo-30C-treated cells, respectively.

Figure 8 Gene expression analysis by RT-PCR

Arbitrary band intensities of uvrA, B and C were analysed against the housekeeping gene G-3-PDH under different conditions. L1: Control; L2: Dose 1 treated; L3: Dose 1 plus Aronia Montana-30C treated; L4: Dose 1 plus placebo-30C treated; L5: Dose 2 treated; L6: Dose 2 plus Aronia Montana-30C treated; L7: Dose 2 plus placebo-30C treated. G-3-PDH: glyceraldehyde-3-phosphate dehydrogenase; RT-PCR: reverse transcription-polymerase chain reaction.

3 Discussion

Results of the present study revealed DNA damage and generation of oxidative stress in E. coli as a result of exposure to UV radiation, more in quantity at the longer and higher exposures. UV irradiation-
included generation of oxidative stress was revealed by different assay protocols. Furthermore, after exposure to UV radiation, the expressions of uvrA, B and C genes responsible for repair of DNA damage showed an up-regulation, as compared to the control.

UV radiation induces damage through the generation of free radicals or ROS\textsuperscript{4,5}. The highly toxic superoxide anion (O$_2^-$) seriously disrupts normal metabolism through oxidative damage to cellular components. Oxygen-free radicals can also be converted to reactive hydroxyl radicals, which again can cause DNA damage. Intracellular ROS attack both the bases and the sugar moieties, producing single and double-strand breaks in the backbone, adducts of base, sugar groups and cross-links to other molecules that block replication\textsuperscript{26,28}. Therefore, elimination of superoxide anion is definitely necessary for the survival of cells\textsuperscript{29}. Results of the present study showed that AM-30C significantly reduced intracellular ROS generation. This was supported by an increase in activities of some biomarkers like SOD, catalase and free intracellular GSH content which decreased after UV exposure. SOD as part of the defense system against oxidative damage in aerobic organisms\textsuperscript{33-35} catalyzes superoxide anion (O$_2^-$) to oxygen (O$_2$) and hydrogen peroxide (H$_2$O$_2$), which then gets reduced to water (H$_2$O) by H$_2$O$_2$-scavenging enzyme catalase\textsuperscript{36}. Therefore, SOD and catalase are the weapons to restrict the accumulation of ROS. In these findings, both SOD and catalase showed a marked increase in their specific activities on AM-30C treatment. Some molecules such as ascorbic acid and GSH are constitutively present and help maintain an intracellular reducing environment or scavenge reactive oxygen and protect themselves from the attack of ROS.

In the UV-exposed E. coli administered AM-30C, there was less DNA damage as determined from the analysis of data through comet assay and DNA gel electrophoresis. Therefore, it is evident that AM-30C has an ameliorative effect on UV-exposed E. coli, which is not an “ethanol effect” as the placebo-treated E. coli did not show such ameliorative responses. As DNA repair in E. coli is known to be accomplished through the activities of the specific repair genes, this study focuses on the expression levels of these genes. Interestingly, there was clear evidence of the over-expression of these genes, depicting an up-regulation and enhanced activities of these repair genes. Since the over-expression of these genes being absent in the E. coli treated with the placebo (except for uvrA expression in dose 2 plus placebo-treated group), it could be logically concluded that AM-30C must have played a role in the alteration of the expression level of these repair genes. To ascertain if the up-regulation of these genes by AM-30C could be further confirmed, the authors performed RT-PCR on mRNA of uvrA, B and C genes, and again, the results were confirmatory of the over-expression of the mRNA of these specific proteins. Therefore, the results of the present investigation actually vindicated the hypothesis advocated by Khuda-Bukhsh and his collaborators since long that one major way by which the potentized homeopathic drug would act is by the regulation of expression of the relevant genes. Khuda-Bukhsh et al\textsuperscript{17,20,21,27-29} also found much evidence of alteration in gene expression in a mammalian model (Mus musculus) after administration of potentized homeopathic drugs and demonstrated change in many signal proteins in cancerous mice\textsuperscript{40}. Other researchers are encouraged to verify these results and design experiments of a similar kind to reveal further steps of gene regulatory mechanisms involved.

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5 Competing interests

The authors declare that they have no competing interests.

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顺势疗法药物山金车 30C 通过上调核苷酸切除修复基因的表达减少紫外线照射后大肠杆菌的 DNA 损伤

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目的：检测高度稀释的顺势疗法药物山金车 30C 是否能够调节暴露于紫外线照射下大肠杆菌的核苷酸切除修复基因的表达。

方法：大肠杆菌在标准培养基中培养至对数阶段，然后接受亚致死剂量的紫外线照射（25 和 50 J/m² 分别照射 22.5 和 45 s）。接受不同剂量紫外线照射的大肠杆菌分别与山金车 30C 及安慰剂 30C 共同培养，90 min 后检测其 DNA 损伤情况及氧化应激状态。采用多种方法及指标如彗星实验、梯度凝胶电泳、细胞内活性氧生成及测量其他生物活性指标如过氧化物歧化酶、过氧化氢酶及谷胱甘肽活性 DNA 损伤情况及细胞氧化应激状态。逆转录聚合酶链反应检测大肠杆菌细胞紫外线损伤修复基因 uvrA、b、c（核苷酸切除修复基因）mRNA 的表达情况。

结果：照射后的大肠杆菌出现 DNA 损伤及氧化应激反应，表现为细胞内活性氧生成增加及过氧化物歧化酶、过氧化氢酶和谷胱甘肽活性降低。与安慰剂组相比，山金车 30C 降低了大肠杆菌的 DNA 损伤及氧化应激反应，表现为细胞内活性氧生成减少及过氧化物歧化酶、过氧化氢酶和谷胱甘肽活性增强。与对照组相比，山金车 30C 上调了大肠杆菌细胞紫外线损伤修复基因的表达。

结论：山金车 30C 能够通过上调紫外线损伤修复基因的表达修复紫外线引起的 DNA 损伤，并通过减少细胞内活性氧的生成及调节抗氧化酶活性降低细胞的氧化应激反应。

关键词：大肠杆菌；紫外线；顺势疗法；山金车属；活性氧；DNA 损伤；DNA 修复