Research Article

www.ijrap.net

NANOPARTICLES MEDIATED PHENOTYPIC MUTATION IN *INDIGOFERA TINCTORIA* L. (FAMILY: FABACEAE)

Bapi Ghosh, Animesh Kumar Datta *, Debadrito Das, Divya Vishambhar Kumbhakar, Ankita Pramanik
Department of Botany, Cytogenetics, Genetics and Plant Breeding Section, University of Kalyani, Kalyani,
West Bengal, India

Received on: 30/03/17 Accepted on: 27/04/17

*Corresponding author

E-mail: dattaanimesh@gmail.com

DOI: 10.7897/2277-4343.082129

ABSTRACT

Dry seeds (moisture content: 12.7%) of *Indigofera tinctoria* L. (family: Fabaceae; dye yielding plant of commerce with immense therapeutic uses) are treated with copper (Cu), cadmium sulphide (CdS), zinc oxide (ZnO) and copper oxide (CuO) nanoparticles (NPs) and also with ethyl methanesulphonate (EMS - 0.25, 0.50 and 1.00 %, 4 h duration) and gamma irradiations (50, 100, 150, 200 and 300 Gy) for inducing phenotypic mutation and causing variation in the gene pool which did not exist previously. Out of 11 macromutant types, 6 in EMS, 9 in gamma irradiations, 8 in Cu-NPs and CdS-NPs, 9 in ZnO-NPs and 11 in CuO-NPs are induced. NPs induce relatively higher mutation frequency than EMS and gamma irradiations. Potentiality of NPs inducing genetic variation in the form of macromutation is highlighted. The mutants (2n=16) show normal meiotic chromosome behaviour. Results obtained are discussed.

Keywords: *Indigofera tinctoria*, Nanoparticles, Conventional mutagens, Macromutation

INTRODUCTION

Nanoparticles ranging from 1 to 100 nm (at least one dimension) possess unique physico-chemical properties^{2,3} and ensure the potential impact in different aspects of life sciences¹⁻¹². Nanoparticles mediated research is opening up a new vista in agricultural science as they are reported to act as mutagen¹³ and can induce 'plant type' (phenotypic) mutation^{11,12,14}. Chemical synthesis of nanomaterials in the laboratory, operational simplicity and cost effectivity are significant aspects which triggers their application. Present article, as an additional endeavour, describes the potentiality of nanoparticles in inducing phenotypic mutation (copper-Cu, Cadmium sulphide-CdS, zinc oxide-ZnO and copper oxide-CuO) in *Indigofera tinctoria* L. (family: Fabaceae; common name: Nili; yield blue dye of commerce with immense therapeutic significance^{15,16}). The conventional mutagens gamma irradiations and ethyl methanesulphonate (EMS) are also considered in the study for comparison. It is always significant to widen the gene pool through mutagenesis, which can enhance bioactive compound(s) magnifying therapeutic uses of the species under study.

MATERIAL AND METHODS

Germplasm

Seed stocks (moisture content: 12.7%) of *Indigofera tinctoria* L. (family: Fabaceae) were procured from Medicinal Plant Garden, Narendrapur Ramkrishna Mission, Govt. of West Bengal.

Synthesis and Characterization of NPs

Cu-, CdS-, ZnO- and CuO- NPs were prepared following wet chemical co-precipitation methods and were opto-physically characterized using UV-Visible Spectrometer (UV-Vis, Shimadzu UVPC - 1601), Fourier Transform Infra-red Spectrometer (FTIR only for CdS-NPs, Jasco FT/IR - 6300), X

- ray Diffractometer (XRD, Shimadzu LabX, $\lambda=1.5406 \text{ \AA}$, 2 θ from 10°– 90°), Dynamic Light Scattering analyzer (DLS, DelsaTM NanoC, Beckman Coulter) and Field Emission Scanning Electron Microscope (FESEM, JEOL JSM – 7600F) for conformation of nano standard quality^{11-13,17} (unpublished data).

Treatments

Dry filled seeds of *I. tinctoria* were exposed to synthesized solutions of Cu-, CdS-, ZnO- and CuO- NPs (0.25, 0.50 and 1.00 $\mu\text{g/ml}$, 4 h durations) and EMS (0.25, 0.50 and 1.00 %, 4 h duration; solution prepared in 0.2 M phosphate buffer, pH adjusted to 6.8). The seeds were also treated with different gamma irradiation doses (50, 100, 150, 200 and 300 Gy, source ⁶⁰Co, absorbance dose rate 47.4 Gy/min, source to distance 12 cm). Dry control and bulk Cu-, CdS-, ZnO- and CuO- controls (0.25 $\mu\text{g/ml}$, 3 h) were also kept for study. Hundred seeds were treated in each lot.

Assessment of biological damages

Out of lethality, injury and seed sterility (M1 attributes relating to biological damages), only lethality could be assessed as per Konzak et al¹⁸. Injury (seedling length enhanced in all cases than controls) and seed sterility (seeds were found to mature non-synchronously) could not be estimated in the plant species.

Raising of M1 and M2 plant population

M1 generation was raised following sowing of 50 seeds from each treatment including controls in the experimental field plots of Department of Botany, Kalyani University (West Bengal plains, Nadia, latitude 22°50' to 24°11' N, longitude 88°09' E to 88°48' E, elevation 48 ft above sea level, sandy loamy soil, pH 6.85) in late February and were harvested at the month of December.

M2 population was raised in plant to row from selfed seeds (first formed flower was bagged in each case) of each surviving M1 plants. In both M1 and M2 generations plants were grown at distance 20 cm between plants and 25 cm between rows. Fertilizer application was not made at any stage of plant growth.

Screening of phenotypic mutants

Phenotypic (Macromutants) mutants were screened at M2 generation from seedling to maturity, and estimated as per 100 plants¹⁹. The mutant phenotypes were confirmed at M3 and different morphometric traits were ascertained.

Determination of Mutagenic efficiency and effectiveness

Viable mutation frequency²⁰ was used to calculate the efficiency and effectiveness of NPs, EMS and gamma irradiations using the formulae suggested by Konzak et al.¹⁸. The mutagenic efficiency was calculated as Mf/L ; while the effectiveness as $Mf/c \times t$ and Mf/Gy unit converted to kR (Mf = mutation frequency, L = lethality, c = concentration, t = duration, Gy = gray unit and kR = kiloroentgen).

Meiosis

Young inflorescences of 2 to 3 cm were collected from each plant type and controls at 8.30 am to 9.30 am and fixed in Carnoy's solution for 48 to 72 h (changes given at 24 h interval) and subsequently preserved in 70 % alcohol under refrigeration ($16^{\circ}C \pm 1^{\circ}C$). Male meiosis was performed from anther squash preparations. Pollen mother cells (PMCs) and pollen grains were stained in 2 % propionocarmine solution. Meiotic plates were studied at diplotene-diakinesis, metaphase I (MI) and anaphase I (AI) and suitably photomicrographed and magnified.

Fully stained pollen grains were designated as fertile in accordance with Marks²¹.

RESULT AND DISCUSSION

As compared to normal plant type (Figure 1:A), a total of 11 phenotypic (macromutants) mutant types are identified in M2 mutagenized population from a total of 4573 plants (Table 1). Out of 11 types, 6 in EMS, 9 in gamma irradiations, 8 in Cu-NPs and CdS-NPs, 9 in ZnO-NPs and 11 in CuO-NPs are spotted. Excepting *narrow leaf* mutant (Figure 1:C), all the mutants are viable and set seeds on harvest. The mutant phenotypes are confirmed at M3 as all the viable are bred true. Mutation affecting leaf trait(s) and branching nature are more prevalent in all the treating agents than stem characteristics and maturity. Over the M2 population, the mutants appear in the following order: EMS— *unbranched*>*branching from base*>*heterophyllous leaf* (Figure 1:B) = *dwarf*>*thick stem I* (Figure 1:D) = *lax branching*; gamma irradiations— *unbranched*>*heterophyllous leaf*>*early maturity*>*thick stem I* = *branching from base*>*lax branching*>*dwarf*>*thick stem II*>*funnel*; Cu-NPs— *unbranched*>*early maturity*>*heterophyllous leaf*>*narrow leaf* = *bushy* = *lax branching*>*branching from base* = *dwarf*; CdS-NPs— *lax branching*>*thick stem I*>*heterophyllous leaf* = *branching from base*>*thick stem II* = *early maturity*>*bushy*>*unbranched*; ZnO-NPs— *early maturity*>*lax branching* = *thick stem I*>*unbranched*>*branching from base* (Figure 1:F) = *dwarf*>*heterophyllous leaf* = *narrow leaf*>*bushy*; CuO-NPs— *unbranched*>*dwarf* = *lax branching*>*thick stem I*>*funnel*>*narrow leaf*>*thick stem II* = *branching from base* = *bushy* (Figure 1:G) = *early maturity*. Excepting CdS-NPs treatment, unbranched phenotype is most predominant among

the treating agents. Mutants namely, *branching from base*, *heterophyllous leaf*, *lax branching* (Figure 1:H) and *unbranched* (Figure 1:I) types are found to appear with all the treating agents (Table 1). No mutants are scored in controls.

Estimated mutation frequency (Figure 2:A-F) is 3.68% to 6.70% (viable: 5.12%, total: 5.12%) in EMS, 1.83% to 6.16% (viable: 4.96%, total: 4.96%) in gamma irradiations, 4.42% to 8.80% (viable: 5.86%, total: 6.48%) in Cu-NPs, 5.92% to 9.76% (viable: 7.39%, total: 7.39%) in CdS-NPs, 7.53% to 9.90% (viable: 7.86%, total: 8.30%) in ZnO-NPs, 6.54% to 8.85% (viable: 7.40%, total: 7.80%) in CuO-NPs. Results show that NPs induce mutation frequency is more than EMS and gamma irradiations. Considering total mutation frequency, the treating agents occur in the following order: ZnO-NPs> CuO-NPs> CdS-NPs> Cu-NPs> EMS> Gamma irradiations.

The morphometric traits are assessed from true breeding M3 mutant plants. The diameter ($2.47 \text{ cm} \pm 0.34$; range: 1.90 cm to 3.10 cm) of *thick stem mutant I* and *II* is significantly higher (data recorded 2 cm above base in all cases) than normal ($1.03 \text{ cm} \pm 0.16$; range: 0.70 cm to 1.54 cm) plants (t -value 4.86 at 18 DF; $p < 0.01$). Leaf yield at flowering stage of both mutants (*thick stem I* – $4.78 \text{ kg} \pm 0.39$; *thick stem II* – $3.24 \text{ kg} \pm 0.47$) is much higher than untreated normal plants ($2.42 \text{ kg} \pm 0.26$). The branching nature of *lax branching* mutant plants shows an average of 72.55° (65.8° to 79.3°) angle of divergence with the main axis in comparison to 41.8° (32° to 51.6°) in normal plants. Leaf yield in the mutant is $2.98 \text{ kg} \pm 0.42$. In normal plants branching initiate at a distance of $17.5 \text{ cm} \pm 1.42$ (13.0 to 22.0 cm) compared to 7.43 ± 0.15 (6.9 to 8.1 cm) in mutant namely, *branching from base* ($t = 6.84$, DF = 18, $p < 0.001$). Leaf yield in the mutant is maximum ($4.75 \text{ kg} \pm 0.58$) among the plant types (0.67 kg to 4.78 kg). Early maturity plant type flowered within 116 to 127 days from sowing compared to 152 to 169 days in normal plants. *Heterophyllous leaf* mutant possesses both small ($0.50 \text{ cm} \pm 0.06$; range- 0.38 to 1.26 cm) and large ($4.05 \text{ cm} \pm 0.72$; range: 3.40 to 4.70 cm) pinnae compared to $2.30 \text{ cm} \pm 0.21$ (1.40 to 3.20 cm) pinnae in normal plants. Compared to $1.56 \text{ cm} \pm 0.16$ height of normal plants, dwarf mutant plants are significantly lesser ($t = 9.62$, DF = 18, $P < 0.001$) in height ($0.38 \text{ cm} \pm 0.05$).

Mutagenic effectiveness and efficiency

The mutagenic effectiveness (Table 2,3) relates doses to mutation is found to be significant in 0.25 and 0.50 %, 4h EMS; 50 Gy to 200 Gy in gamma irradiations; 1.00 $\mu\text{g/ml}$, 4h in Cu- and CdS-NPs; 0.25 $\mu\text{g/ml}$ and 0.50 $\mu\text{g/ml}$, 4h in ZnO-NPs and 0.50 $\mu\text{g/ml}$, 4h in CuO-NPs. Among treating agents it seems that effectivity is maximum in CuO-NPs followed by ZnO-NPs, CdS-NPs, Cu-NPs, EMS and gamma irradiations. The mutagenic efficiency defined as the relation of number of mutational events to undesirable effects like lethality and it is mostly efficient in threshold doses of treatments with the treating agents.

Meiosis

Meiotic configurations in normal and mutants are present in Table 4. Meiocytes show $2n=16$ in all the plant types always (Figure 3:A-I). Bivalents are mostly in rod configuration (Figure 3:A-C). Mean chromosome association per cell at MI is $7.79\text{II} + 0.44\text{I}$ in normal and it varies from $6.97\text{II} + 1.17\text{I}$ (*narrow leaf*) to $7.89\text{II} + 0.22\text{I}$ (*funnel*) among the mutants. Bivalents per cell are random ($p > 0.05$) in distribution compared to non- randomness of univalent ($p < 0.001$) among the plant types. Equal (8/8)

anaphase I segregation of chromosomes (Figure 3:A) ranges from 92.30% to 100.00% in the plant types. Rarely few cells show laggard(s) (1 to 2) and unequal separation (9/7 – Figure

3:I) of chromosomes. Pollen fertility in control is 97.94% and it varies from 58.16% to 94.37% among mutants.

Table 1: Phenotypic mutant types in *L. tinctorial*

Mutant types	Frequency (%)					
	EMS	Gamma irradiation	Cu-NPs	CdS-NPs	ZnO-NPs	CuO-NPs
<i>Heterophyllous leaf</i>	0.96	0.79	0.77	0.96	0.44	0.92
<i>Narrow leaf</i>	0.00	0.00	0.62	0.00	0.44	0.39
<i>Thick stem I</i>	0.64	0.61	0.00	1.50	1.18	0.79
<i>Thick stem II</i>	0.00	0.26	0.00	0.68	0.00	0.26
<i>Branching from the base</i>	0.96	0.53	0.46	0.96	0.74	0.26
<i>Bushy</i>	0.00	0.00	0.62	0.27	0.29	0.26
<i>Funnel</i>	0.00	0.08	0.00	0.00	0.00	0.52
<i>Unbranched</i>	1.28	1.05	1.54	0.14	1.03	1.98
<i>Lax branching</i>	0.64	0.44	0.62	2.19	1.18	0.79
<i>Early maturity</i>	0.00	0.61	1.39	0.68	2.22	0.26
<i>Dwarf</i>	0.80	0.08	0.46	0.00	0.74	1.19
Total plants scored	625	1140	648	730	674	756

Table 2: Mutagenic effectiveness and efficiency in EMS and gamma irradiated samples

	Treatments		Percent of control		Viable mutation frequency (%)	Mutation effectiveness	Mutation efficiency
	Concentration/ Gy	Duration (h)	Lethality	Injury		Mf/c×t Mf/kR	Mf/L
EMS	0.25	4	40.0	–	3.68	3.68	0.09
	0.50	4	16.7	–	6.70	3.35	0.40
	1.00	4	46.7	–	5.59	1.40	0.12
Gamma Irradiation	50	–	6.7	–	1.83	0.36	0.27
	100	–	23.3	–	3.44	0.34	0.15
	150	–	26.7	–	5.46	0.36	0.20
	200	–	53.3	–	6.16	0.31	0.12
	300	–	26.7	–	5.40	0.18	0.20

Table 3: Mutagenic effectiveness and efficiency in NPs treatments

	Treatments		Percent of control		Viable mutation frequency (%)	Mutation effectiveness	Mutation efficiency
	Concentration/ Gy	Duration (h)	Lethality	Injury		Mf/c×t Mf/kR	Mf/L
Cu-NPs	0.25	4	36.7	–	3.98	3.98	0.11
	0.50	4	30.0	–	5.97	2.99	0.20
	1.00	4	50.0	–	7.82	1.96	0.16
CdS-NPs	0.25	4	43.3	–	5.92	5.92	0.14
	0.50	4	33.3	–	6.30	3.15	0.19
	1.00	4	56.7	–	9.76	2.44	0.17
ZnO-NPs	0.25	4	50.0	–	7.17	7.17	0.14
	0.50	4	43.3	–	8.96	4.48	0.21
	1.00	4	60.0	–	7.53	1.88	0.12
CuO-NPs	0.25	4	46.7	–	8.16	8.16	0.17
	0.50	4	36.7	–	8.85	4.43	0.24
	1.00	4	66.7	–	6.54	1.64	0.10

Table 4: Meiotic configurations and pollen grains fertility in normal and mutant plant types

Plant Types	Diplotene			No. of PMCs scored at Metaphase I	Mean/cell at MI		No. of meiocytes scored at Anaphase I	Frequency of equal (8/8) segregation of chromosomes (%)	Pollen fertility	
	No. of cells scored	Rod bivalent	Ring bivalent		II	I			No. of pollen grains scored	Frequency (%)
Control	86	5.88	1.87	420	7.79	0.44	170	94.12	437	97.94
Heterophyllous Leaf	51	6.29	1.52	75	7.33	1.33	63	96.82	508	69.68
Narrow leaf	63	6.45	1.40	68	6.97	1.17	52	92.30	533	58.16
Thick stem I	48	6.37	1.38	152	7.41	1.17	52	100.00	530	83.20
Thick stem II	56	5.94	1.89	155	7.25	1.14	73	100.00	519	77.84
Branching From base	72	5.76	2.14	52	7.58	0.85	48	97.90	553	79.20
Bushy	69	6.21	1.65	92	7.63	0.74	69	100.00	594	64.14
Funnel	58	5.67	1.93	81	7.89	0.22	47	100.00	452	91.81
Unbranched	47	5.92	1.96	76	7.80	0.39	46	100.00	459	91.50
Lax branching	44	5.68	2.09	154	7.78	0.43	55	100.00	487	93.22
Early maturity	61	5.83	2.02	138	7.76	0.64	54	100.00	383	92.95
Dwarf	53	5.79	1.88	84	7.85	0.29	73	100.00	427	94.37

Figure 1:A-I. Normal (A) and mutant (B-I) plant types in *Indigofera tinctoria*. B. Heterophyllous leaf; C. Narrow leaf mutant with plant having normal pinnae (→); D. Thick stem I; E. Thick stem II; F. Branching from base; G. Bushy; H. Lax branching; I. Unbranched.

Figure 2:A-F. Graphical representation of viable mutation frequency

Figure 3:A-I. Meiotic configurations at diplotene (A-C); metaphase I (D-G) and anaphase I (H-I) cells in *Indigofera tinctoria* (2n=16). A. 8II; B-C. 7II+2I (→); D. 8II; E. 7II+2I (→); F. 6II+4I (→); G. 3II+10I (→); H. 8:8 separation; I. 9:7 unequal separation. Scale bar = 10 μm.

CONCLUSION

Present study highlights the potential significance of Cu-, CdS-, ZnO- and CuO- NPs in inducing phenotypic mutants as that alike to the conventional mutagens namely, EMS and gamma irradiations. The mutants are outcome of genetical consequences rather than cytological. Furthermore, mutation frequency is relatively higher in NPs treatments. The mutant types are nearly similar in all the treating agents. It reflects that NPs can act in identical loci of genomes as that of the conventional mutagens. Therefore, it opens up the possibility of using nanoparticles as mutagens for inducing genetic variation. High leaf yield in most of the induced mutants is significant for the plant ideotype.

ACKNOWLEDGEMENT

Authors are thankful to UGC consortium Center, Center for Research in Nanoscience and Nanotechnology (CRNN), Department of Zoology and Chemistry, Kalyani University for instrumentation facilities and to Dr. Sudha Gupta for necessary help.

REFERENCES

1. Roco MC. Broader societal issues of nanotechnology. J Nanopart Res 2003; 5:181–189.
2. Remédios C, Rosario F, Bastos V. Environmental nanoparticles interactions with plants: morphological, physiological, and genotoxic aspects. J Bot 2012; 2012:1–8.
3. Masarovičová E, Kráľová K. Metal nanoparticles and plants. Ecol Chem Eng S 2013; 20:9–22.
4. Lam CW, James JT, McCluskey R, Hunter RL. Pulmonary toxicity of single-wall carbon nanotubes in mice 7 and 90 days after intratracheal instillation. Toxicol Sci 2004; 77:126–134.
5. Caruthers SD, Wickline SA, Lanza GM. Nanotechnological applications in medicine. Curr Opin Biotechnol 2007; 18:26–30.
6. Nowack B, Bucheli TD. Occurrence, behavior and effects of nanoparticles in the environment. Environ Pollut 2007; 150:5–22.
7. Scrinis G, Lyons K. The emerging nano-corporate paradigm: nanotechnology and the transformation of nature, food and agri-food systems. Int J Sociol Agric Food 2007; 15:22–44.
8. Singh M, Singh S, Prasada S, Gambhir IS. Nanotechnology in medicine and antibacterial effect of silver nanoparticles. Digest J Nanomater Biostruct 2008; 3:115–122.
9. Nair R, Varghese SH, Nair BG, Mackawa T, Yoshida Y, Kumar DS. Nanoparticulate material delivery to plants. Plant Sci 2010; 179:154–163.
10. Castiglione MR, Giorgetti L, Geri C, Cremonini R. The effects of nano-TiO₂ on seed germination, development and mitosis of root tip cells of *Vicia narbonensis* L. and *Zea mays* L. J Nanoparticle Res 2011; 13:2443–2449.
11. Halder S, Mandal A, Das D, Gupta S, Chattopadhyay AP, Datta AK. Copper nanoparticle induced macromutation in *Macrotyloma uniflorum* (Lam.) Verdc. (Family: Leguminosae): a pioneer report. Genet Resour Crop Evol 2015a; 62:165–175.
12. Halder S, Mandal A, Das D, Datta AK, Chattopadhyay AP, Gupta S, Kumbhakar DV. Effective potentiality of synthesised CdS nanoparticles in inducing genetic variation on *Macrotyloma uniflorum* (Lam.) Verdc. BioNanoSci 2015b; 5:171–180.
13. Kumbhakar DV, Datta AK, Mandal A, Das D, Gupta S, Ghosh B, Halder S, Dey S. Effectivity of copper and cadmium sulphide nanoparticles in mitotic and meiotic cells of *Nigella sativa* L. (black cumin) – can nanoparticles act as mutagenic agents? J Exp Nano Sci 2016a; 11:1–17.
14. Kumbhakar DV, Datta AK, Das D, Ghosh B, Pramanik A, Saha A. Copper and cadmium sulphide nanoparticles can induce macromutation in *Nigella sativa* L. (black cumin). JPDS 2016b; 8:371–377.
15. Ghosh B, Datta AK, Mandal A, Das D, Kumbhakar DV. Meiotic Configurations and Secondary Chromosome Associations in *Indigofera tinctoria* L. (Family: Fabaceae). Cytologia 2016; 81:1–6.
16. Ghosh B, Mallick T, Ghosh A, Datta AK, Pramanik A. Taxonomical, anatomical, cytological and palynological assessment of a germplasm of *Indigofera tinctoria* L. (Fabaceae) – an ayurvedic plant. Int J Res Ayurveda Pharm 2016b; 7:90–95.
17. Das D, Datta AK, Kumbhakar DV, Ghosh B, Pramanik A, Gupta S. Conditional optimisation of wet chemical synthesis for pioneered ZnO nanostructures. Nano-Structures & Nano-Objects 2017; 9:26–30.
18. Konzak CF, Nilan RA, Wagner J, Foster RJ. Efficient chemical mutagenesis. Rad Bot (Suppl.) 1965; 5:49–70.
19. Gaul H. Mutation in plant breeding. Rad bot 1964; 4:155–252.
20. Walther F. Effectiveness of mutagen treatments in ionizing radiation in barley. Induced mutation in plants. Proc. symp. On the nature, induction and utilization of mutations in plants. IAEA-FAO. Pullman wash 1969; 261–270.
21. Marks GE. An acetocarmine glycerol jelly for use in pollen fertility counts. Stain Technol 1954; 29:277.

Cite this article as:

Bapi Ghosh et al. Nanoparticles mediated phenotypic mutation in *Indigofera tinctoria* L. (Family: Fabaceae). Int. J. Res. Ayurveda Pharm. 2017;8(Suppl 2):290-295 <http://dx.doi.org/10.7897/2277-4343.082129>

Source of support: Nil, Conflict of interest: None Declared

Disclaimer: IJRAP is solely owned by Moksha Publishing House - A non-profit publishing house, dedicated to publish quality research, while every effort has been taken to verify the accuracy of the content published in our Journal. IJRAP cannot accept any responsibility or liability for the site content and articles published. The views expressed in articles by our contributing authors are not necessarily those of IJRAP editor or editorial board members.