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Molecular Diagnostic Strategies for the Detection of Toxicological Changes in Animals: A Review

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ABSTRACT

Rapid industrialization and successful green revolution have introduced a wide array of chemicals into our environment; some of these chemicals entered in ecosystem; gets accumulated and exert serious health and ecological problems. These toxic substances can enter the food chain and emphasize pathological changes which damage either cell, organ or system (circulatory, immune, respiratory, digestive, nervous, reproductive and musculo-skeletal *etc.*) by altering structure and/or function of biological components; DNA, RNA, proteins, lipids and carbohydrates. This review article provides certain molecular diagnostic techniques used for their robust and accurate detection at molecular level. Investigations conducted during 2020-2021 where various review and research articles were surveyed and then extracted to enlist congestive datum for rapid detection of toxicological changes in animals. In our investigations we concluded that toxic substances present in our environment affects health of animals by altering structure and functioning of biomolecules and their concerned system. These cytological and systemic changes can be detected with the help of molecular diagnostic techniques including dideoxysequencing, pyrosequencing, allele specific RT-PCR, CRISPER/Cas, genotyping and microarrays *etc.* present collection of data will provide congestive information for rapid toxicological detection at molecular level.

Key words: Biological components, Molecular diagnosis, Toxicant, Toxicological changes.

Capacity of a chemical to cause injury in living organisms depends upon the concentration of the chemical and the duration of exposure, is called toxicity (Pandey *et al.* 2012; Sharma, 2013). A toxicant is a chemical substance which demonstrates the potential to induce cancer produces long term diseases or injuries, affects health adversely, produces acute discomfort or endangers the life of humans or animals through exposure via the respiratory tract, skin, eyes, mouth *etc* (Pandey *et al.* 2012). Toxicity of a toxic substance affected by some major factors *i.e.* its solubility, persistence, bioaccumulation, biomagnifications and chemical interactions.

Environmental Protection Agency (EPA) is established to regulate the safety of industrial chemicals and to make awareness and protection towards the environment and human health. It also possess a list of hazardous chemical substances which has been reported since 1987 and now converted into Toxic release Inventory (TRI) programme maintaining TRI chemical list presently having 767 individual chemicals and 33 chemical categories within it (https://www.epa.gov/toxics-release-inventory-tri-program/tri-listed-chemicals).

Applications of these substances with reference to exposure towards human civilization has long history as lead has been utilized in building materials, water pipes and pigments for grazing ceramics since at least 5000 years while ancient Rome utilize it to sweeten old wine which results in grams of consumption by an individual per day. Mercury was significantly utilized by Romans as a remedy for tooth pain and syphilis. These hazardous substances has been known for a long time still there exposure is continues and is increasing in some areas like mercury is still utilized in gold mining in many parts of Latin America, lead is used in a variety of consumer and industrial products, arsenic is serving in wood preservation, tetraethyl lead works as additive in petrol. After knowing and also being aware towards consequences of such toxic substances developed countries has started to decrease their emission significantly (Jarup, 2003).

Inadequate testing of chemicals to which the public is exposed presents a serious public health risk. The embryonic period or developing fetus and infants are remarkably more susceptible towards environmental hazards. Toxic exposures to certain potential hazardous chemical pollutants during these windows of increase susceptibility may lead to disease or disability in infant, children and across the entire span of human life. Subsequently legal systems maintaining post and pre market laws found function poorly in identification and prevention against subclinical toxic effects and can result in long term harm hereby legal approaches will be needed for to preventing subclinical effects and their consequences in children.

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Categories of toxic substances

According to International Resister of Potentially Toxic Chemical of the UNEP (United Nations Environmental Programme), there are four million known chemicals present in the word today and 30,000 new chemical compounds are added to this list every year. On the basis of their occurrence in the environment these toxic substances can be classified into four groups: toxicants in air, toxicants in water, toxicants in contaminating food and others (Pandey *et al.* 2012). Some major potent health hazards are listed in Table 1.

Pathological changes followed by exposure to toxicants

Toxic substances can enter the food chain where they become accumulated and cause pathological changes (Pant and Singh, 1983). What so ever is the toxicant, it damages the tissues when enters the body. This damage is majorly in the form of oxidation of proteins, lipids and other biochemical molecules inside the cell and is governed by oxidation or generation of free radicals *via* toxicants. Injury can be assessed in particular organ, system, any specific type of cell, certain molecule and also in whole person (or animal). Systematic injury weaved hazardous responses into the circulatory (blood, cardiovascular system), immune (immune cells and their activity against certain pathogens and substances), respiratory (lungs and respiratory tract), renal (kidney, urinary system), endocrine (hormones, respective glands and their secretions), digestive (gastrointestinal tract), muscolo-skeletal, reproductive and central nervous systems (brain and neurons). At molecular level, these substances found to interfere with normal protein function (including structure), cell communication (effects upon primary and secondary messenger), metabolic energy

Table 1: Major categories of toxicants which potentially cause health hazards.

Class	Toxicants	Effects	Reference
Heavy metals	Arsenic Cadmium Chromium Lead Nickel Manganese Mercury	Perforation of nasal septum, respiratory cancer, peripheral neuropathy skin cancer, proteinuria, glucosuria, osteomalasia, emphysema, ulcer, aminoaciduria, encephalopathy, anemia, central nervous system disorders, chronic rhinitis and sinusitis, visual defects and EEG changes, pneumoconiosis.	(Alissa and Ferns, 2011)
Pesticides	Fungicides-triazines, hexachloro benzene Herbicides-phenoxy acids, triazoles, Rodenticides-arsenic, zinc phosphide Fumigants-HCN, HCHO, CH_3 BR Molluscicides-metaldehyde, carbamate, ethylene oxide Nematocides-aliphatic halogen compounds, organophosphates, carbamates	Anxiety, anorexia, anemia, tremor, lethargy, ataxia, headache, hyper excitability, dizziness, muscle twitching, myoclonic jerking, insomnia, irritability, arthralgia, slurred speech, blurred vision, loss of memory, decrease of sperm count.	(Mostafalou and Abdollahi, 2017)
Fertilizer	Ammonia Nitrite and Nitrate	Methaemoglobinaemia, blue coloration of skin, damage in respiratory and vascular system.	(Sharma and Singhvi, 2017)
Automobile emission	Carbon mono oxide Sulphur dioxides Nitrogen oxides Hydrocarbons Lead	Difficulty in breathing, irritation of mucous membrane, eyes, respiratory tract, formation of carboxyhemoglobin, anemia, peripheral vascular diseases, emphysema, infection in liver and kidney.	(Buckeridge *et al.* 2002)
Food additives	Antioxidants Dyes and colorings Emulsifiers Flavorings and taste enhancers Sweeteners Preservatives and antimicrobials Stabilizers	Dermatologic, gastrointestinal, respiratory, musculoskeletal, neurologic, cardiovascular.	(Wilson and Bahna, 2005)
Radioactive substances	Electromagnetic radiations Particulate radiations	Somatic effects-leukemia, eye contracts, malignant tumors, cardiovascular disorders, cancer, Genetic effects-lethal effects on egg and embryo, rate of mutation.	(https://www.epa.gov/radiation/radiation-health-effects)

inhibition (effects on building and breakdown of substances), or xenobiotic (foreign substance or increased concentration of particular substance) enzyme inhibition or induction, alteration in the normal function of DNA-RNA, central dogma (replication, transcription, translation), genes and their products and specific cytoplasmic and nuclear receptor binding (Table 2). Fig 1 explains hypothetical classification of toxicological changes under the influence of toxicants.

Cytological changes

Plenary of toxic agents primarily manifest their effects at cellular level indicated by earliest cellular responses and indications of the body's encounter with any toxicant.

Cellular and structural changes

Toxicants can affect the cells by two ways either by cell injury or by cell death which further regulated *via* either spectrum of events started with injury to end up with death, can specific to determinant cells or may be common to all distressed cells within certain organ or system. In case of lethal injury, it manifests inhibition in metabolic energy synthesis (ATP synthesis), disruption in authenticity of plasma membrane or uncertainty in essential growth factors however cell death occurs only in the occasion of uncompensated cell injury.

Besides having robust protective mechanism, mitochondria is also vulnerable to toxicant exposure due to genetic deficiencies in the mitochondrial processes and leads to mtDNA mutations (Meyer and Bess, 2012). Secretary activity of golgi apparatus ceased under the stress of certain toxicants they can also disrupt the intracellular traffic and cause morphological alterations in its plasma membrane and physiology also. Recently, researchers have been suggested to focus on plasma membrane and its components for cancer screening and chemotherapy (Erdemli, 2016).

Changes in biological molecules

Toxicants such as heavy metals and pesticides can generate reactive oxygen species (ROS) in the form of free radicals which damage biomolecules; lipids, proteins, carbohydrates and DNA by directly bind to sulfhydryl and amino groups in case of proteins, breaking bonds and strands in DNA, affect the structure of carbohydrates by scavenging activity further resulting in structural and functional modifications (Letelier *et al.* 2005; Valko *et al.* 2005).

Changes in immune system

Immunotoxicity may be the consequence due to either direct and/or indirect effect of the xenobiotics or host's immunological response towards respective metabolite and/or compound (Bahadar *et al.* 2014).

Toxic effects may occur in certain critical functions:

- Development and expansion of different stem cell populations.
- Lymphoid and myeloid cell proliferation with supportive tissues in which these cells get mature and function.
- During functioning of antigen-presenting cells and macrophages.
- Regulatory function of T-helper and T-suppressor cells.
- Production of various cytokines or interleukins.
- Complement regulation and activation.
- Functioning of cytotoxic T cells.
- Functioning of Natural killer (NK) cells.
- Chemotoxic and cytotoxic functions of Macrophage and polymorphonuclear leukocyte.

Changes in reproductive system

Hazardous substances like DBP [Di (n-butyl) phthalate], on exposure acted as a reproductive toxicant may alter the anatomy and/or functioning of development of system in males and females both, affect their reproductive behaviors and respective hormonal functioning via affecting

Table 2: Human diseases associated with occupational exposure of toxicants.

Organ/system	Effects	Toxicants	Reference
Cardiovascular system	Heart disease	Carbon monoxide, lead, cobalt cadmium	(Alissa and Ferns, 2011)
Respiratory system	Nasal disease	Isopropyl alcohol	(McKay, 2014)
	Lung cancer	Radon, silica, nickel, arsenic, chromium, mustard gas	
	Hypersensitivity	Beryllium, isocyanates	
	Irritation	Ammonia, sulfur oxide, formaldehyde	
Nervous system	Peripheral neuropathies	Acrylamide, mercury, lead, arsenic, DDT	(Moser *et al.* 2008)
	Central nervous system depression	Alcohol, ketones, aldehydes, solvents	
Urinary system	Toxicity	Mercury, lead, glycol ethers	National research
	Bladder cancer	Naphthylamines, benzidine	council, 1995
Reproductive system	Male infertility	Lead, phthalate, plasticizers	(Rim, 2017)
	Female infertility	Cadmium, lea	
	Tetratogenesis	Mercury, polychlorinated biphenyls	
Hematopoietic system	Leukemia folliculitis and acneiformdermatosis	Benzene, radon, uranium Polychlorinated biphenyls, dioxins, herbicides	(Pyszel *et al.* 2005)
Skin	Cancer	Ultraviolet radiation	
Gastrointestinal tract	Liver angiosarcoma	Vinyl chloride	(Gelberg, 2018)

secretions of pituitary gland, hypothalamus, gonads and germ cells, influence fertility, pregnancy and the duration of the functioning of reproductive system (Mathur and D'cruz, 2011) (Table 2).

Changes in cardiovascular system

Toxicants especially heavy metals affecting the circulatory system increase the risk cardiovascular diseases, atherosclerosis, myocardial infarction, hypertension and coronary disfunctioning (Jennrich, 2012). Oxidized low-density lipoproteins (LDL) are predictors of the level of mercury within the body and responsible for the development of acute coronary insufficiency (Meisinger *et al.,* 2005) and atherosclerosis when found to accumulate in atherosclerotic lesions (Salvayre *et al.,* 2002). Mercury is also responsible for the inactivation of an enzyme, "paraoxonase" that lowers the LDL oxidation process and exerting toxic effect on cardiovascular system (Fernandes *et al.,* 2012).

Changes in respiratory system

Long term exposure toward large amount of hazardous substances may overwhelm the respiratory system to protect and repair itself in unfavorable conditions which results in impaired lung functions sometimes. In chronic bronchitis, number of secretary cells in bronchitis tree increased and produce an excess of mucus. In condition of emphysema (shortness of breath), network of muscle fibers (collagen and elastin) disrupted that is supposed to support the lung in breathing. Severe condition involving constriction of smooth muscles will lead to development of asthma in milder stimuli than do healthy people (McKay, 2014).

Molecular diagnostic methods for the detection of changes in protein

Nucleic acid amplification techniques involves genotyping, DNA fingerprinting at sub-species-level, quantitative detection and molecular resistance testing. Particular

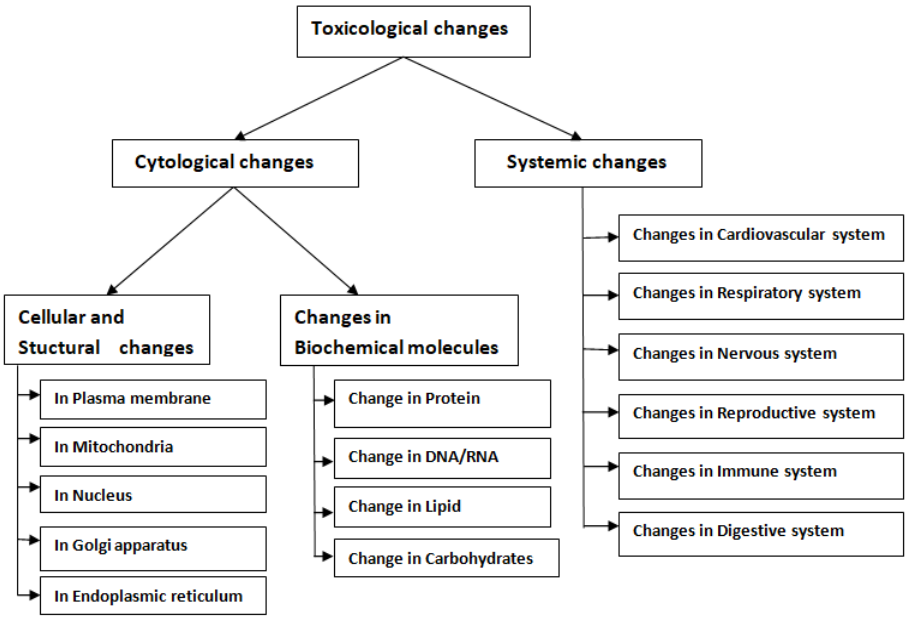

Fig 1: Classification of toxicological changes.

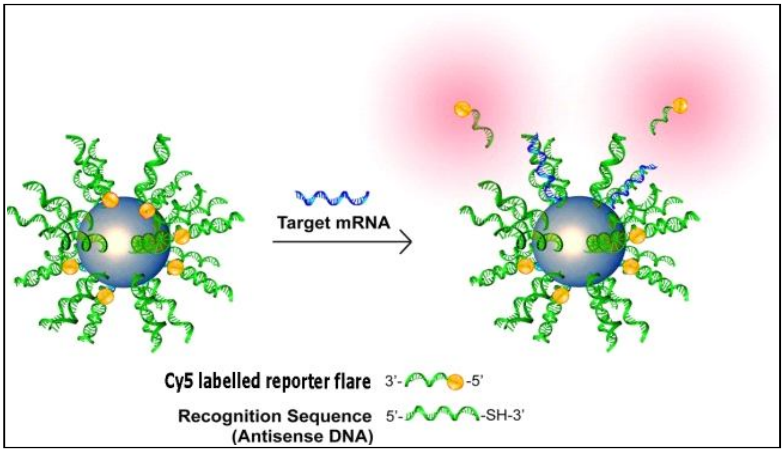

Fig 2: Nanoflares for detection of intracellular mRNA.

sequences of nucleic acids or proteins having affinity for respective target molecule are also known as aptamers and serves as diagnostic tool (Ruiz *et al.* 2018). Many toxicants alter the conformation or structure of protein and affect their functionality. The efficient function of all protein depends upon their 3D structure that is the main target of toxicants. For example MeHgfirst binds with cell membrane proteins and then interfere with corresponding cellular processes (Atchison and Hare, 1994). On the exposure of stressful conditions like high temperature or toxic substances cells start synthesis of heat shock protein or stress proteins which is esteemed as weapon in cell's armamentarium against recovery from physical and chemical insult by environment (Mahmood *et al.* 2014; Kumar *et al.* 2017).

eNOS is a dual-purpose enzyme, known to produce sulfate and nitric oxide when bound to membrane or in cytoplasm respectively hypothesize to have dual damaging role when overused in high-SPF sunscreens that the overuse of sunscreen (Seneff *et al.,* 2015). There are various environmental toxicants like mercury, arsenic, cadmium, glyphosate and lead that supposed to disrupt the activity of CYP enzymes. However all of certain environmental pollutants stimulate their pathological effects at molecular and/or sub-cellular level leads to change in potential biomarkers which may provide earlier signals for prospective injury at particular region. These biomarkers make efforts to provide adaptive response to prevent damage induced by stressors either in the form of biomarkers of exposure or biomarker of effects (Ryan and Hightower, 1996).

DNA based protein detection

DNA based protein detection technique utilize the advantage of signal amplification when protein binds antibody is combined with DNA. In proximity extension assay (PEA) antigen specific antibodies are conjugated with complimentary oligonucleotide which produces PCR target sequence. DNA barcode is quantified by standard microfluidic qPCR. PEA used to analyze prognostic markers in cancer and inflammation.

Mass spectrometry

Immunoprecipitation, immunocapture, Capture by anti-peptide antibodies (SISCAPA) and mass spectrometric immunoassay (MSIA) are alternative methods that provide greater selectivity and specificity for low abundant proteins as dedicated specific peptide or protein targets. Mass spectrometry is powerful technique used for the detection of metabolites, proteins, lipids and carbohydrates in addition with post transcriptional modifications and characterization of specific metabolic isoforms of chemical interactions. Validation of biomarkers by mass spectrometry includes targeted proteomics experiments which are featured using triple quadrupole mass spectrometers in multiple reaction monitoring (MRM) and selective reaction monitoring (SRM) applied for small molecule analysis that can be used in bottom-up proteomics. Data-dependent and data-independent analysis (DDA and DIA) based MS approaches usually utilizes commercially available mass spectrometers; ion traps, Quadruploe Time of Flight (Q-TOFs), Ion Mobility Spectrometry(IMS-TOF MS), Orbitraps, Fourie-transform (FT-MS) and LC-MALDI MS/MS for Data-Dependent Acquisition and compilation of proteomics data particularly for discovery-based strategies.

Microarray for protein detection

Protein microarray has ability to perform rapid, multiplex protein biomarker analysis in biological fluid like plasma, serum, urine which provides multiplex detection of frequent targets in single sample. Although their applications are limited by high risk of assay cross reactivity in comparison to DNA microarray (Lee *et al.,* 2008). In this technique proteins are immobilized on solid surface such as membranes, microscopic slides, beads or microtitre plates. Nitrocellulose film slides are widely used solid surface for microarray technique. Some emerging microarray techniques involve nucleic acid aptamer microarrays (DNA aptamer microarray, RNA aptamer microarray), lysate microarrays, peptide and small molecule microarray.

Alternatives or some other emerging technologies

Alternative protein binders, alternative protein separation, Photonic and plasmonic resonating structure arrays, Multi-omics analysis platforms, miniaturized sensors, Protein sequencing are some emerging powerful and robust techniques utilized for the molecular detection of protein biomarkers.

Changes in DNA/RNA

Some chemicals have potential to directly react with DNA by metabolic activation. Genotoxic agents usually act on hydrogen bonds between the phosphodiester backbone of DNA and nitrogenous bases and sugars (producing abasic sites) in DNA which leads to increased level of DNA strand break (Simoniello *et al.,* 2009). miRNAs has differential expression in diverse conditions including diseases, injuries and normal physiological conditions. Level of miRNA-122 found to increase in the influence of certain toxic substances like acetaminophen, *d*-galactosamine, or ethanol (Zhang *et al.,* 2010). Copper has potential affect chromatin condensation and formation of adducts when binds with DNA (Benjamini and Hahochberg, 1995). Molecular diagnostic techniques evolved use of labeled probes (small pieces of DNA) that has potential to hybridize on its complementary strand to specific (target) gene which can reveal the rearrangements or/and location of genes on the chromosome that is visualized as colored sectors (fluorescence) on the chromosomes. Single-cell gel electrophoresis assay (commonly called the "comet" assay) permits the detection of DNA breakage within single cell therefore utilized to detect chromosomal damage.

CRISPER/Cas9

Clustered regularly interspaced short palindromic repeats (CRISPR) has found widespread application as gene editing tool that makes the recognition of DNA

sequences possible. CRISPER/Cas system can detect the nucleic acid, protein, metal ions, bacteria and small molecules and is potent a nucleic acid recognition tool which can bind to and cleave RNA in an RNA-programmable manner. Variety of toxic substance alters genetic material of organisms can specifically recognized by CRISPER/Cas system in case of point mutation and/or single nucleotide variant as it compare target sequence with CRISPER/Cas system (Zhou *et al.,* 2018).

PCR based approach

Real time PCR, Real time transcription PCR and Loop mediated-isothermal amplification (LAMP) is a robust and predictable technique that collecting the data and combining amplification and detection in a single step and gives output in real time as correlate PCR product with fluorescence intensity (Gupta *et al.,* 2017; Singh *et al.,* 2020). PCR based techniques target molecular markers as they shows accurate prognosis, response prediction and resistance to therapy. To detect the changes after toxicological exposure control group is compared with experimental sample after getting fluorescence monitoring of each cycle. mRNA expression can also be studied after reverse transcription (Bernard and Wittver, 2002).

Molecular detection of BRAF V600E mutation

BRAF gene encodes serine/threonine protein kinase responsible for the downstream process of RAS in the RAS-RAF-MEK-ERK signaling pathway (Ciardiello *et al.,* 2008). Mutations in this gene are responsible for approximately 8% of all human cancers especially 50% in melanoma and papillary carcinoma of thyroid. Usual patients of BRAF mutations do not respond to (immunoassay based treatment) anti-EGFR monoclonal antibody (mAb) treatment (Siena *et al.,* 2009). Therefore plenty of methods are evolved for their rapid and accurate diagnosis mainly include allele-specific real-time PCR, dideoxy sequencing, colorimetric Mutector assay, pyrosequencing, high resolution melting (HRM) analysis and COLD-PCR (Pinzani *et al.,* 2011). Huang and colleague develop Amplification Refractory Mutation System (ARMS)-PCR for detection of BRAF V600 mutation in formalin-fixed, paraffin-embedded (FFPE) tissue. Method contains 4 primers in a single PCR reaction tube and amplifies three products: amplification control, specific product and wild-type specific product. Fo-Ro (Forward and Reverse) primer pair generates a common fragment of 200 bp flanking the mutation site while Fo-Rimut (Reverse mutation identifying) primer pair generates the 144 bp fragment specific to BRAF V600E. This assay can detect 0.5% BRAF V600E allele in a wild-type background. The mutant and wild-type sequences can be distinguished by the difference in their fragment size. The mutant or wild-type primer compete with each other for binding to the limited templates as their amplification takes place in single reaction tube. The ARMS-PCR assay can be easily implemented by many researchers and clinical laboratories for BRAF V600E mutation testing (Huang and Zhang, 2013).

Molecular detection of DNA methylation in cells

DNA methylation played an important role in the epigenetic control of mammalian gene expression as well as and is required for X inactivation, genomics imprinting and silencing of retrotransposons and repetitive sequences. Degree of DNA methylation can be measured by several methods one of them focuses on the quantification of 5-methyl cytosines using reversed phase high performance liquid chromatography (RPHPLC), two dimensional thin layer chromatography (2D-TLC), high performance liquid chromatography mass spectrometry (HPLC-MS) and high performance capillary electrophoresis (HPCE) and liquid chromatography electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) (Stach *et al.,* 2003) whereas second group of technologies has focused on quantification of global 5-methylcytosine *via* the radio labeling of CpG sites, ELISA method, pyrosequencing, use of methyl-sensitive enzymes in COBRA method for example (King *et al.,* 2008).

Molecular detection of genotoxic effects

An alkaline version (pH>13) of comet assay is recommended for the detection of genotoxic effects because of its ability to provide detection of a broad spectrum of DNA lesions. Comet assays are convenient for assessing DNA damage in animals by measuring DNA damage in individual cell, require small number of cells for reaction and can be performed on all eukaryotic cell. Simoniello *et al.* (2009) evaluate an *in vivo* alkaline comet assay method for diagnosis of genotoxic effects in *P. lineatus* after exposer of sublethal concentration of Cypermethrin. Comet assay have been proved as a useful and more sensitive tool than cytogenetic technique for the measurement of relationship between DNA damage and exposure of organisms to genotoxic pollutants.

Nanoflares for intracellular mRNA detection

NanoFlares are spherical nucleic acid (SNA) have ability for transfection of cell followed by detection and quantification of RNA molecule. These spherical probes work on AuNP-based platform. This technique consist thiolated “recognition” antisense DNA which is adsorbed on to the spherical AuNP (Seferos *et al.,* 2020). When target binds to recognition site leads to release of reporter flare that generates a measurable fluorescence signal (Fig 2).

Molecular-beacon-modified AuNPs for intracellular mRNA detection

Traditional molecular beacons are combination of quencher and fluorophore pair conjugates with hairpin DNA that targets the specific mRNA. Proximity of the quencher to fluorophore founds to be very low in the absence of target molecule but when target mRNA binds, the hairpin opens, increasing the distance between the quencher and fluorophore to generate fluorescence “turn-on”. Advance technique utilizes AuNP-based systems where traditional molecular beacons are improved upon through the enhanced fluorescence quenching efficiency of AuNPs in comparison to organic dyes. Reproductive toxicants found to cause sperm DNA

fragmentation after exposure towards them and it can be observed during metaphase of cell division in embryo. Sperm Chromatin Structure DNA is viewed as the molecular precursor to detect DNA damage and can be observed under the light microscopy (Evenson and Wixon, 2005).

Change in lipid

In the absence of aspecific defense mechanism Reactive oxygen species (ROS) cause peroxidation of membrane polyunsaturated fatty acids (Halliwell and Gutteridge, 1984). Enhanced production of free radicals in the brain increases the level of thiobarbituric acid reactivity substances (TBARS) which served as an index of lipid peroxidation.

CONCLUSION

Exposure of toxic substances may lead to toxicological or pathological changes in organisms which can be diagnosed at molecular level with the help of certain molecular diagnostic techniques. These approaches provide early diagnosis; prevention and effectiveness of treatment for these changes at molecular level. There are numerous molecular diagnostic strategies present that can be used for faster, more accurate and reliable detection of pathological changes in the organisms. In spite of wide array of analytical developments that have come with toxicological exposure there are many issues that need to be improved. As regards hazardless of certain substances/chemicals researchers have developed microarray technologies that represent an advance in nucleic acid testing methods. Functioned magnetic promising nanotechnologies coupled with advance devices make rapid, accurate, portable, sensitive and easy-to-use diagnostic tools for early detection. These methods have some drawbacks also; immunoassays need specific antibodies that are costly, biosensor method needs highly pure sample preparation, PCR based methods need specific designed probes and labeling of isotope and fluorescence elements. There are need to develop some new, accurate, more reliable and cost adjustable approaches. However molecular biology techniques are almost revolutionary. Nowadays researchers have urged to for the need to define best biomarker to improve the field of biomarker research and their diagnostic methods. Further advances and development in modern biotechnology will have a promising and bright future.

Conflict of interest

No potential conflict of interest to declare.

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