أخلاقيات البحث العلمى

ETHICS IN MEDICAL RESEARCH

What is research:

- Performing a methodical study in order to prove a hypothesis or answer a specific question.
- Finding a definitive answer is the central goal of any experimental process.

Chronic Addiction to Tramadol and Withdrawal Effect on the Spermatogenesis and Testicular Tissues in Adult Male Albino Rats

Marwan Abdel-Latif Ibrahim[a] Alaa-Eldin Salah-Eldin[a, b]

[a]Department of Biology, College of Science, Majmaah University, Majmaah, Al-Zulfi, Saudi Arabia; [b]Department of Zoology, Faculty of Science, University of Aswan, Aswan, Egypt

Abstract

Aim: The present study aimed to elucidate the effects of tramadol on the testicular functions of adult male rats due to the chronic usage of tramadol and the effect of its withdrawal. ***Method:*** Adult male albino rats were classified into the following 3 groups: (I) a control administered with normal saline and (II) tramadol-treated rats (40 mg/kg b.w. orally) for 21 successive days; and (III) like the rats in the second group but kept for 4 weeks after the last tramadol dose to study the effect of tramadol withdrawal. At the end of the experimental period, blood was collected and specimens from testis were taken for histopathological, biochemical, and molecular studies. A reverse transcription-polymerized chain reaction after RNA extraction from specimens was detected for the anti-apoptotic and pro-apoptotic genes in testicular tissues. Also, malondialdehyde (MDA) was measured in tissues homogenate and antioxidant enzymes activities were evaluated. ***Results:*** The results of this study demonstrated histological changes in testicular tissues in groups II and III com-

pared to the control group, accompanied with increased apoptotic index and proved by increased B-cell lymphoma-2 (Bcl-2) associated-X-protein and caspase-3 expression, whereas anti-apoptotic Bcl-2 markedly decreased. Moreover, in tramadol-abused and -withdrawal groups, the MDA level increased, while the antioxidant enzymes activity decreased and revealed oxidative stress, indicating that tramadol is harmful at the cellular level and can induce apoptotic changes in testicular tissues. The withdrawal effect showed signs of improvement, but it did not return to normal levels. ***Conclusions:*** It could be concluded that the administration of tramadol causes abnormalities on testicular tissues associated with oxidative stress, which confirmed the risk of increased oxidative stress on testicular tissues due to tramadol

Introduction

Tramadol administration efficiently alleviates pain in acute and chronic conditions [1]. In many countries, tramadol has been placed into Schedule III or IV of the federal Controlled Substances Act. It was classified as a controlled substance in the same category as codeine and

although there is little information about the reproductive effects of tramadol in males. The present work was conducted to investigate the possible effects of tramadol administration on rat testicles tissues and spermatogenesis. Therefore, we focus on the effect of tramadol addict and withdrawal on the oxidative stress and apoptotic genes in rats' testis.

Materials and Methods

Chemicals and Animals Treatments

Tramadol tablets containing 225 mg tramadol hydrochloride and obtained from the October Pharma Co. (Giza, Egypt) was given orally for 21 successive days at a dose equivalent to 40 mg/kg body weight. Kits for; LPO, reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT); were purchased from Biodiagonistic Co. All other chemicals were of the highest quality. The experiment was carried out on 30 male adult Wistar albino rats, weighing 150–170 g (4 months old) at the beginning of the study. Rats were reared 1 week before the study and kept at room with controlled light at photoperiods of 12-h/12-h light/dark cycle and a temperature around 28 ± 2 °C with a relative humidity of 50 ± 5%. The animals had unlimited access to water and rats chow ad libitum.

Results

Behavioral Observations and the RWs of Testes and Seminal Vesicles

Tramadol-treated animals displayed behavioral alterations. They essentially appeared with hyperactivity and increased excitability when compared to control animals, which exhibited a normal behavior. In the tramadol-

Table 3. Effect of tramadol treatment on MDA concentrations and CAT, SOD, and reduced GSH activities in blood and testes of the control and treated groups

Measurements	Rat groups					
	blood			testis		
	control	tramadol	withdrawal	control	tramadol	withdrawal
MDA, μmol/mg protein	52.31±1.58	103.9±6.33*	108.4±5.10*	0.44±0.059	0.69±0.049*	0.61±0.037*
CAT, U/g protein	1.92±0.066	0.83±0.11*	0.85±0.12*	23.32±0.95	18.88±1.7*	19.68±1.9*
SOD, U/mg protein	255.5±3.44	210.3±7.11*	214.5±7.7*	2.75±0.49	1.32±0.31*	1.22±0.41*
GSH, U/mg protein	1.77±0.067	0.97±0.057*	1.14±0.060*	16.13±0.88	11.12±0.61*	12.02±0.51*

Values are mean ± SE of 10 animals in each group.
* Values are significantly different ($p \leq 0.05$) compared to control.
MDA, malondialdehyde; CAT, catalase; SOD, superoxide dismutase; GSH, glutathione.

Discussion

Tramadol is a centrally acting analgesic with weak opioid action, which efficiently alleviates pain in acute and chronic conditions [1]. The addiction resulted in physical and psychological dependence. Rats of trama-

Ethical Approval

M.A.I. declares that all applicable international, national, and/or institutional guidelines for the care and use of animals were followed. A.S. declares that all applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Disclosure Statement

M.A.I. has received a research grant from the Deanship of scientific research at Majmaah University, Saudi Arabia (grant number 52/37). A.S. has received a research grant from the Deanship of scientific research at Majmaah University, Saudi Arabia (grant number 52/37).

Funding Source

This study was funded by the Deanship of scientific research at Majmaah University, Saudi Arabia (grant number 52/37).

References

1 Scott LJ, Perry CM: Tramadol: a review of its use in perioperative pain. Drugs 2000;60:139–176.

2 Rafati A, Yasini SM, Dashti-Rahmatabadi MH, Pakdel S, Norani F: Tramadol dependence rate as compared with morphine in rats. World J Med Sci 2006;1:40–43.

3 Tjaderborn M, Jonsson AK, Hagg S, Ahlner J: Fatal unintentional intoxications with tramadol during 1995-2005. Forensic Sci Int 2007;173:107–111.

4 Garrett PM: Tramadol overdose and serotonin syndrome manifesting as acute right heart dysfunction. Anaesth Intensive Care 2004;32:575–577.

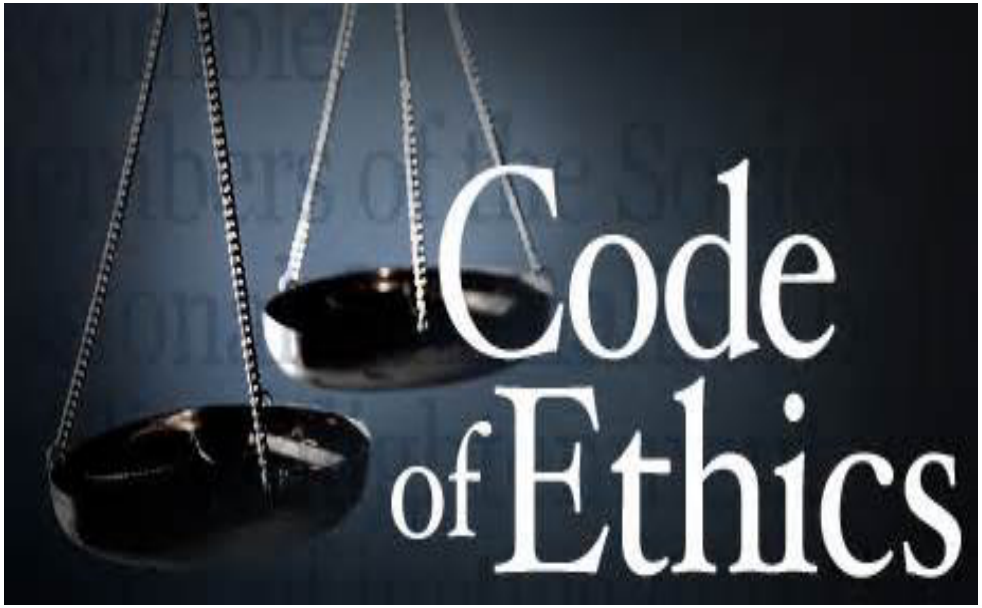
5 Daubin C, Quentin C, Goullé JP, Guillotin D, Lehoux P, Lepage O, Charbonneau P: Refractory shock and asystole related to tramadol overdose. Clin Toxicol (Phila) 2007;45:961–964.

6 Mugunthan N, Davoren P: Danger of hypoglycemia due to acute tramadol

When scientists started get attention about ethics?:

- **It started after the abuse of human lives during Holocaust.**
- **Nuremberg Code: is a set of research ethics principles for human experimentation set as a result of the subsequent Nuremberg trials at the end of the Second World War..**
- **Declaration of Helsinki (1964).**

HISTORICAL EVENTS AND DEVELOPMENT OF CODE OF ETHICS



1. NAZI MEDICAL EXPERIMENTS (1933-1945)

- Unethical activities included sterilisation (castration), euthanasia, mutilating surgeries and numerous medical experiments in Nazi concentration camps against prisoners in war.

- “Sterilised Jews whom Nazis considered as racial enemies”

-

(Horrific experiments were carried out in concentration camps

by fascist doctors in Germany and Japan during the 1939-45 war)

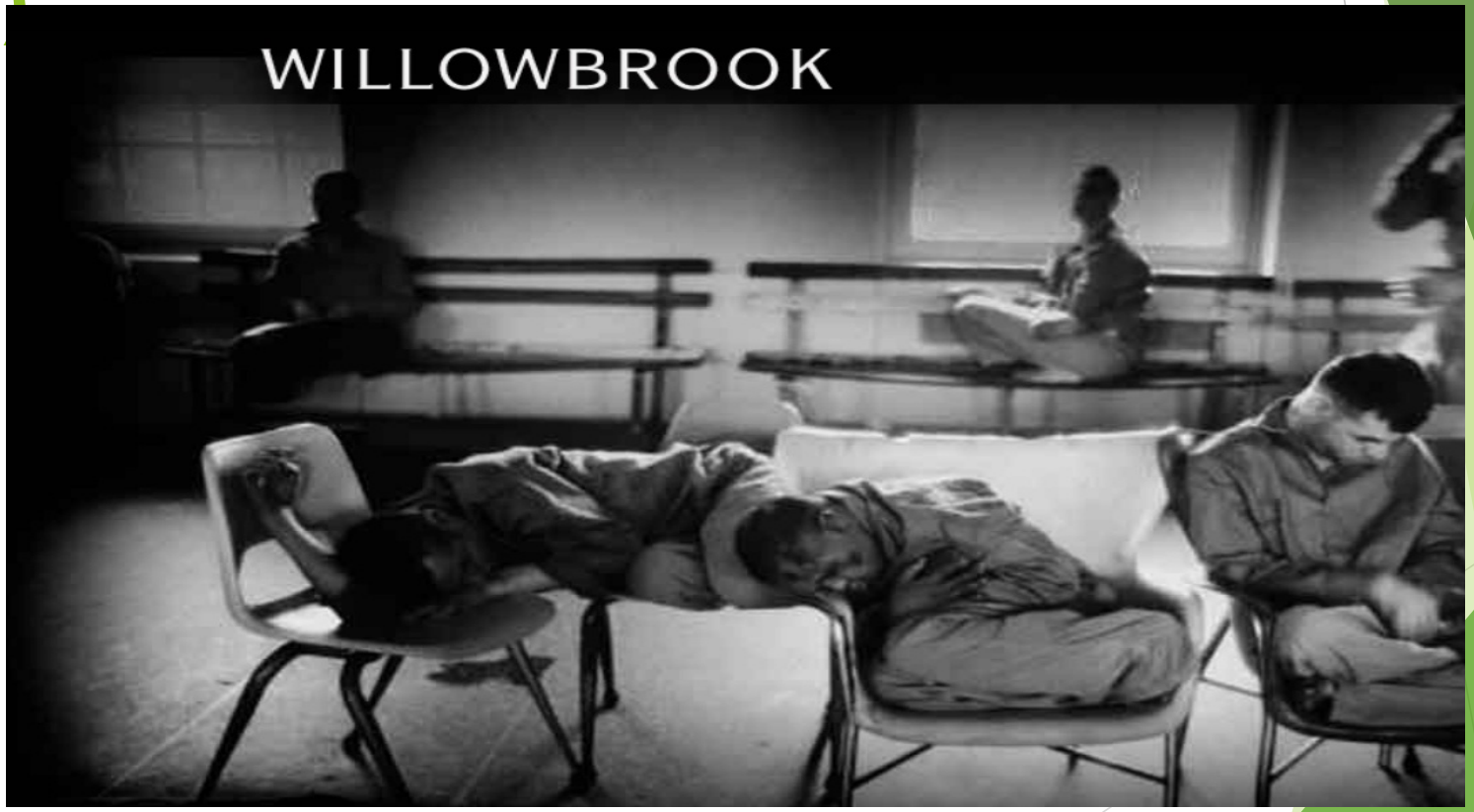
- ***Medical experiments involved exposing to high altitudes, freezing temperature, malaria, poisons, typhus fever, untested drugs and surgery without anaesthesia***
- ***Selection of subjects was racially based***
- ***Subjects had no opportunity to refuse the participation.***

- **Mistreatment of human subjects in Nazi experiments led to the development of Nuremberg Code (1947)**

International code of ethics NUREMBERG CODE- 1947

- **Nuremberg Code contains 10 guidelines**
- **Voluntary consent**
- **Withdrawal of subjects from study is possible**
- **Protection of subjects from physical and mental suffering, injury, disability, and death.**
- **The balance of benefits and risks in the study.**

2. WILLOW-BROOK STUDY (1950-1970)

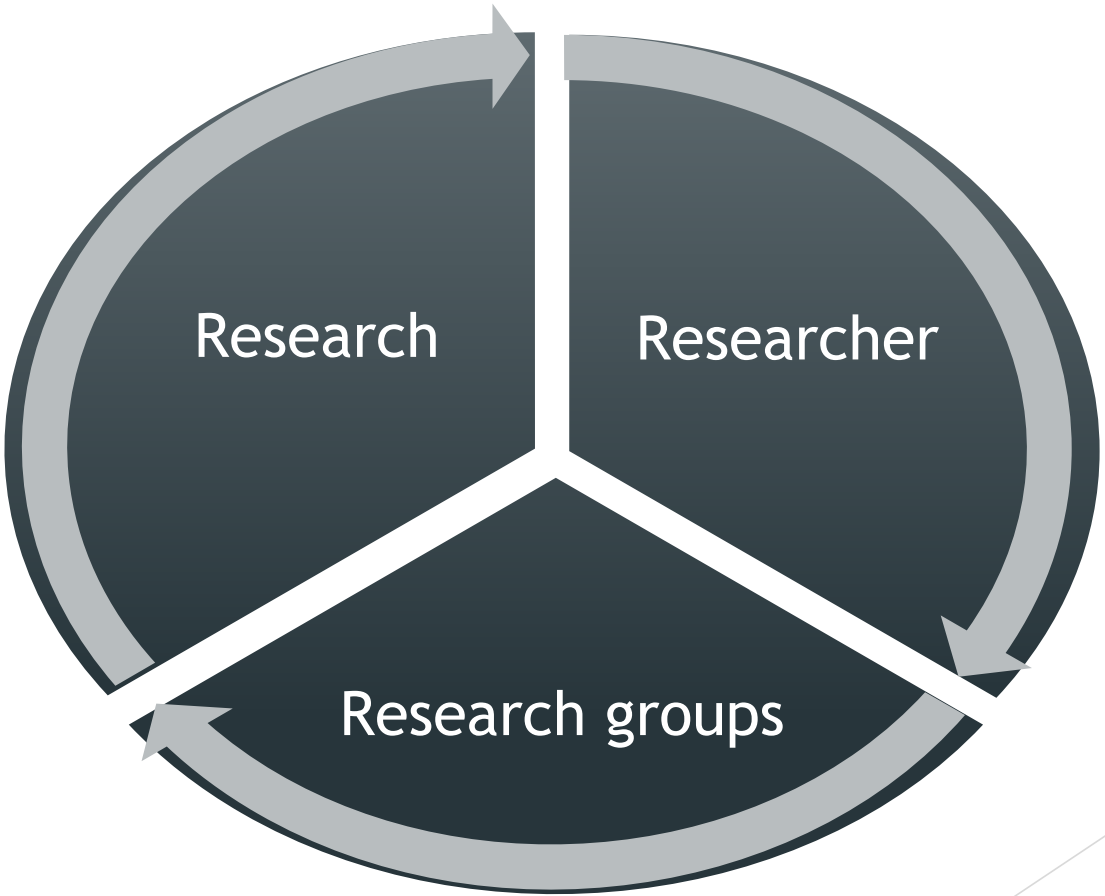


- **Research on hepatitis by Dr. Krugman at Willowbrook among mentally retarded children**
- **The researcher also wanted to determine the effectiveness of gamma globulin injections as protection against hepatitis.**
- **Early subjects were fed extracts of stool from infected individuals**
- **Later subjects received injections of purified virus**
- **Parents were forced to give permission for the child to be a subject.**

DECLARATION OF HELSINKI (1964)

- Greater care can be exercised to protect subjects from harm
- Strong, independent justification for exposing a healthy volunteer to substantial risk of harm
- Investigators must protect life and health of research subjects

Items of research




Ethics concerned the research:
Researcher
Research
Target group

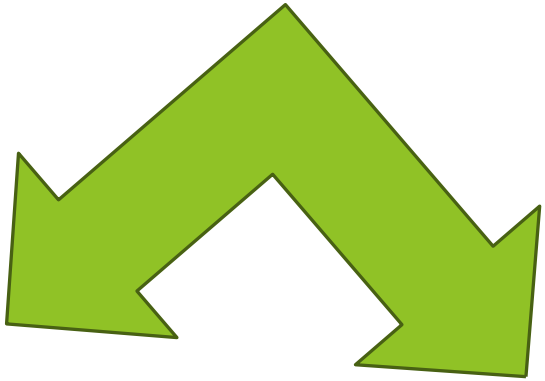
Ethics concerned the research itself:

- Follow scientific guidelines in the research.
- The research provides better alternative for the approved and used one.
- Follow the instructions as regard the environment.
- Don not violate religious rules.

The researcher should be:

- Well qualified and highly specialized.
- Collect all available data regarding the point of research.
- Follow scientific stages of the research.
- Respect research groups.
- Do not plagiarize the work of other research.

The research group: it is divided into



Animals

Human

subgroups
children
pregnant
prisoners

Animals:

- Select the suitable number (not more not less to give significant statistical results).
- Suitable species.
- Do not perform more than one experiment on the same animal.
- Use the least harmful maneuver with animals.
- Use suitable food and care for the animals.
- If the experiment involves killing the animal, use non-painful technique.

human

- Perform the experiment on animals before start to be tested on human.
- Obtain written **consent**:
  - Written in clear, simple language and must be explained to the volunteer by the research.
  - Contains research title, aim, procedures or steps, any side effects, and benefits of the research.
- All the volunteer to withdraw from the research at any time without threats or punish.

- Evaluation of the balance between the benefits and risk factors of the research.
- Suitable numbers of volunteers.
- Keep patients and volunteer’s data secret.
- Do best to minimize side effects and risk factors.
- Respect social and religious aspects of volunteers.
- Determine the point in which the research must stop.
- The main object of the volunteer is not gaining money.
- . If any harm will occur he/she will be compensated

Research on human :

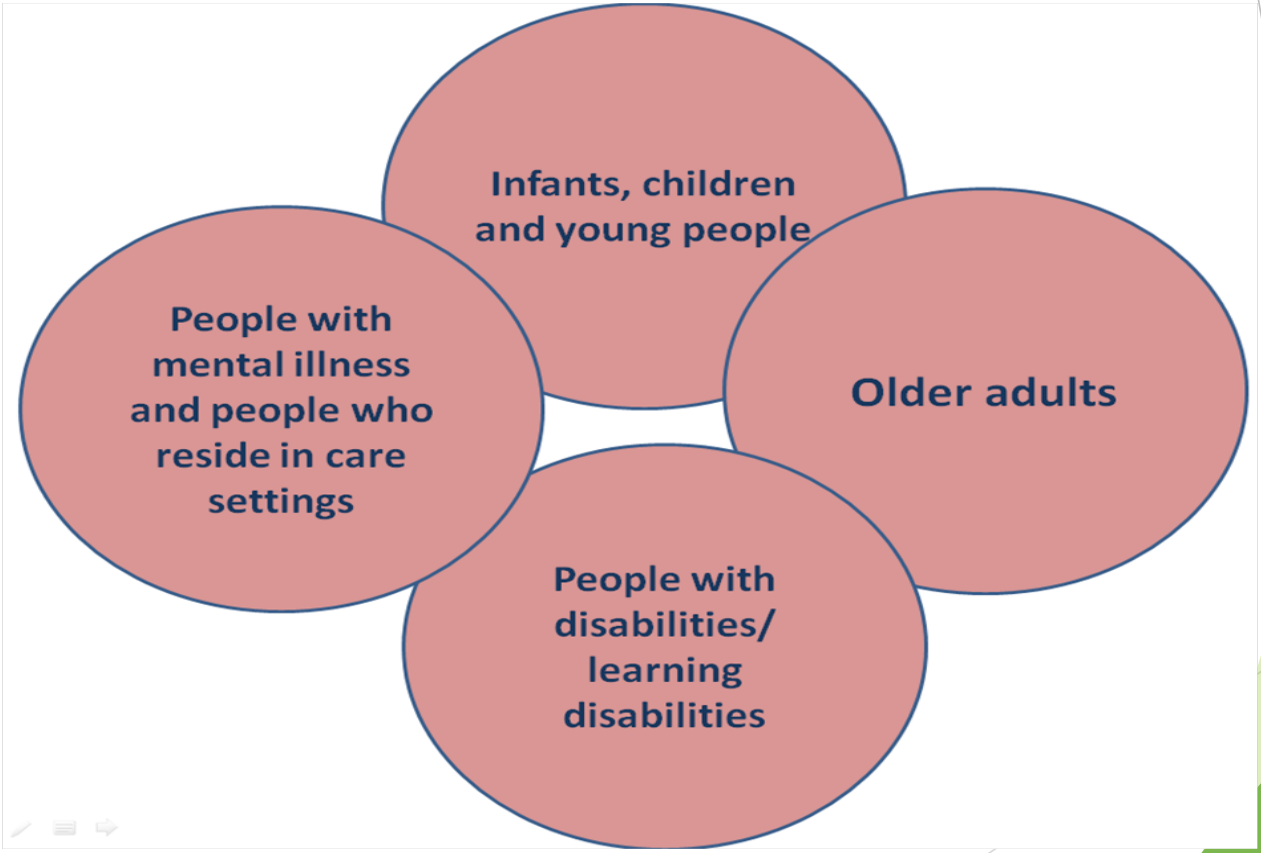
- **Prospective study:** Informed consent will be taken from patients. In case of incompetent patients the informed consent will be taken from the guardians.

- **Retrospective study:** Confidentiality of records will be considered

- DNA / genomic material: Informed consent for DNA / genomic test and for research will be taken from patients. No further tests will be carried out except with further approval of committee and patients. If the samples will travel outside country the researcher will be responsible for transportation and security approval.

- All drugs used in the research are approved by the Ministry of Health

VULNERABLE SUBJECTS



Children:

- In addition to the roles that followed in adult ones; the following should be done:
- If the research is not applicable on adult, and this age group should gain the benefits of the results of the research.
- Consent is obtained from the guardians.

Pregnant and lactating women:

- Consent is obtained from wife and husband.
- Inability to perform the experiment in non-pregnant women.
- Benefits of the research focus on this group.
- No risk to the infant and children.

Prisoners:

- Prisoners must obtain full medical care.
- Consent.
- Do not use any collected data against the prisoners.
- Have the full rights as free person

When to stop the research

- It is impossible to reach the main aim of the research.
- Endanger the life of the participants.
- The risk of the research is much more than its benefits.

Ethics of stem cell reasearch

- Source: embryo or adult
- Consent of mother for cord blood should be taken
- Consent from couple in spare embryo should be taken
- Should be used in treatment only not for cloning

II. RESEARCH MISCONDUCT

Unethical publication

- FABRICATION
- FALSIFICATION
- PLAGIARISM

Research misconduct

- *Fabrication is making up data or results and recording or reporting them.*
- *Falsification is manipulating research materials, equipment, or processes, or changing or omitting data or* results.
- *Plagiarism is the use of another person's ideas, processes, results, or words without giving* appropriate credit and presenting them as your own.

PLAGIARISM CHECKER: soft wares

RESEARCH MISCONDUCT

- Was any type of enforcement or unnecessary influence used to recruit participants?
- Were the participants deceived in any way?
- Were appropriate informed consent procedures used?
- Were adequate steps taken to safeguard participant's privacy?

THERAPEUTIC MISCONCEPTION

- Research subject misinterpret and enrol in the study thinking it to be routine medical care

- Were vulnerable groups involved in research?

- Were groups omitted from the inquiry without a justifiable rationale?

Areas of Academic misconduct

1. Plagiarism
2. Fabrication and falsification
3. Non-publication of data
4. Faulty data-gathering procedures
5. Poor data storage and retention
6. Misleading authorship

Non-Publication of data

- Data not included in results because they don’t support the desired outcome

Data Gathering

- Collecting data from participants who are not complying with requirements of the study
- Using faulty equipment
- Treating participants inappropriately
- Recording data incorrectly
- Most important and most annoying.
- Always drop non-compliers.
- Treat subjects with respect and dignity.
- Record data inaccurately.
- Store data in a safe and private place for **3** years.

Authorship…

Misleading authorship—who should be an author?

- Technicians do not necessarily become joint authors.
- Authorship should involve only those who contribute directly.
- Discuss authorship before the project!

- Publication of the thesis
  - Should be regarded as the student's work
  - Students are listed as secondary authors
- Dual publication – a manuscript should only be published in a single journal
- Proper and complete referencing is an essential part of any physics research publication.
- Deliberate omission of an author or reference is unethical and unacceptable.

Research Implications

- protocol
- undertaking study
- interpretation
- making recommendations
- presenting your findings

There is a danger of reducing research subjects

to research objects

Human rights violations

Ethics of clinical trial & drug development

1. experimentation on animals at first (preclinical study).
2. Follow 4 phases:

- Phase I:Healthy volunteers (10-80)
- Phase II: Diseased (100-300)
- Phase III: Diseased (1000-3000)
- Phase IV: After license and marketing

Ethics of discarded tissue

- Discarded data, documents, records and specimens. (collected for purpose of diagnosis or treatment).
- all collections to have detailed records.
- all collections would have their own ethics committees.
- Informed consent should be taken.


Thank You!