EXPLORING ASSOCIATED SIGNALLING PATHWAYS IN THE DEVELOPMENT OF SKIN CANCER AND NEUROLOGICAL DISORDERS

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biomedical Sciences

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MASTER THESIS WORK

We hereby recommend that the dissertation prepared under our supervision by: **Ms. Musawira Iftikhar** Registration number # **0000170993** Titled: **"Exploring associated signaling pathways in the development of Skin cancer and Neurological disorder"** be accepted in partial fulfillment of the requirements for the award of **MS Biomedical Sciences** degree. (Grade_____)

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I would like to dedicate this to my parents who have always provided me with love and unconditional support.

ABSTRACT

Neuro degenerative, progressive disease affecting the basal ganglia. Parkinson's disease affects the nerve cells in the brain that produce dopamine

Release of dopamine is associated with alpha-synuclein, Alpha-synuclein is a protein that is abundant in the human brain. Parkinson's is associated with voluntary motor control, procedural learning, eye movements, cognitive and emotional functions. A growing number of evidences suggest that people with Parkinson's disease (PD) have a decreased risk of almost all cancers. However, the incidence of melanoma is strikingly higher in patients with PD than that in general population. An association between Parkinson disease (PD) and cancer has long been suspected, but whether the association is with the dopaminergic treatments or with the disease itself remains a question

Keywords: Parkinson‟s disease (PD), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), Reduced glutathione level (GSH), Dopamine (DA).

ABBRIVATIONS

6-OHDA	6-Hydroxydopamine
$CHCl_3$	Chloroform or Trichloro methane
DA	Dopamine
DAT	Dopamine Transporter
SH	Reduced Glutathione Level
HCl	Hypohloric acid
Dopamine	L-DOPA
MAO-B	Monoamine Oxidase type B Inhibitors
Min	minute
Ml	milli liter
MPDP1	1-Methyl-4-Phenyl-2, 3-Dihydropyridinium
MPP+	1-methyl-4-phenylpyridinium
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
g	gram
NaOH	Sodium Hydroxide
NH3	Ammonia
NIH	National Institute of Health
Nm	Nano-meters
No	Nitric Oxide

MDA	Malonaldehyde
SOD	Super Oxide dismutase
DNTB	Ellmen’s Reagent
PBS	Phosphate-Buffered Saline
ROS	Reactive Oxygen Specie
TCL	Trichloroacetic acid
TH	Tyrosine Hydroxylase
UV	Ultra-violet
w/v	Weight/ Volume
µg	micro gram
µl	microliter

TABLE OF CONTENT

List of Figures

List of Tables

CHAPTER 1: INTRODUCTION

Parkinson's disease is the second most common neurodegenerative disease that is cause by the degeneration of dopaminergic neurons in the substantial nigra as a result there is depletion in the dopamine level.(Ran et al., 2017)

Parkinson‟s disease (PD) is characterized by bradykinesia (slowness of motion), rigidity (stiffness), resting tremor(Meyer et al., 2015) the major factors involve are the environmental factors like UV radiations, mutations, and age factor recently skin cancer is also associated with the Parkinson's disease. And Parkinson's disease is also considered as the risk factor of skin cancer that may be due to the common underlying mechanisms and genes involve in the progress of both the diseases.(Orozco-Arroyave, Arias-Londoño, Bonilla, Gonzalez-Rátiva, & Nöth, 2014)

The main genes involve in the progression of Parkinson's disease are alpha synuclein, LRRK2, Parkin, DJ1, Pten, PINK1 and UCHLI.(Soldner et al., 2016) and the genes involve in skin cancer are PTCH1 along with BRaf mutations and LRRK2 (Gly2019ser)(Zhao, Yang, Yu, Liu, & Yuan, 2014)In both the diseases the accumulation of Reactive oxidative stress (ROS) occurs and enhance the disease.(Wang et al., 2015)

Parkinson's disease is disease that effect the central nervous system effecting 1 or 2 individuals per 1000 people at any time.1% of all population is affected at the age

of 60 by Parkinson's disease but with time its prevalence is emerging at the age of 40. The prevalence of Parkinson's disease is affected by many factors that include age, environmental factors and behavioral factors may add to the prevalence of Parkinson's disease this observation is strongly supported by the observation that 90% of the Parkinson's disease is caused by unidentified genetic mutations that is in 15% cases. Genetic mutation is also a risk factor for PD other than that certain infections like hepatitis, helicobacter pylori, influenza and other infections of central nervous system(Ascherio & Schwarzschild, 2016; Fang et al., 2012) recently cancer is also considered as a major risk factor for parkinsonism the common feature hat is shared in both the disease is the atypical effects on cellular proliferation such as in Parkinson's the dopaminergic neuronal cell death accours in the substantia nigra of mid brain while cancer is described as the uncontrolled cellular proliferation forming masses of tissue called the tumors that can metastasize from one tissue to another.

CHAPTER 2: LITERATURE REVIEW

2.1: Parkinson's disease

In 1817 James Parkinson initially describe Parkinson's disease as ''Essay on the shaking palsy'' latter on it was called Parkinson's disease(Przedborski, 2017) Parkinson's disease (PD) is a progressive loss of dopaminergic neurons in substantia nigra part of mid brain, the clinical manifestations of Parkinson's disease include rigidity, resting tremor, postural instability and bradykinesia. Patients also experience several non-motor symptoms, due to involvement of central and peripheral organ systems.(Minguez-Castellanos et al., 2007) PD could also be described as the presence of inclusions of Lewy bodies (LBs) in the substantia nigra resulting in the loss of pigmented dopaminergic neurons. LBs are constructed of filaments measuring 10-15 nm in diameter. The accumulation of LBs are due to the mutations in a-synuclein gene that results in the misfolding's of a-synuclein protein and furthermore the accumulation of alpha synuclein (Spillantini, Crowther, Jakes, Hasegawa, & Goedert, 1998) loss of neurons in the substantia nigra leads to the reduction in voluntary movements.(Pringsheim, Jette, Frolkis, & Steeves, 2014) a-synuclein has the phosphorylation and oxidative modification (Spencer et al., 2013)and a unusual property of that make it resistive to proteases that could solubilize them. It is a protein abundantly present in the presynaptic terminal of neuron.(Adamowicz et al., 2016)

According to neuro pathology of PD there are cytoplasmic inclusions of alpha synuclein in the dopaminergic neurons commonly called as lewy bodies. they have structure like amyloid fibrils.(Burré, 2015) Lewy bodies are formed by cortical type lewy bodies and pale bodies. Cortical type lewy bodies have hyaline appearance through H and E staining in the amygdala and cortical region while the pale bodies are the immune reactive inclusions of pale staining's of neuronal cytoplasmic inclusions. both inclusions could be early form of classical lewy bodies that leads to the accumulation of alpha synuclein in the dopaminergic neuron which is the first indication of parkinsonism(Allen Reish & Standaert, 2015). With addition to that alpha synuclein accumulation are also found in the mid brain and basal ganglia along with the lewy bodies abnormal alpha synuclein filaments are present in neuronal cell process so called lewy neurites. (Adamowicz et al., 2016)

The gradual loss of dopaminergic neurons in the substantia nigra leads to the depletion of dopamine production and distribution. Modification in the mitochondrial functioning and proteasomal operations ad deformed production of alpha-syncline resulting in the elevated level of oxidative stress and level of glutathione (Mori et al., 2017)

Overactivation of Inflammatory factors such as prostaglandins, TNF-, IL-1, and some free radicals such as superoxide and NO are also involved in the neuro pathophysiology of Parkinson's disease. These are pro-inflammatory substances

mediated by microglia cells for the normal functioning of cells. The activation of these factors is firmly controlled to avoid overactivation harmful neurotoxic effects. Over activation of these factors has also been linked to the neurodegenerative diseases(Guillemin & Brew, 2004)

In 15-20 percent of cases the patient has familial history of Parkinson's disease. This evidently support that some genes might be involve in the prevalence of Parkinson's disease however, causative mutations have been only identified in rear cases usually of early onset. Some genes that might be involved in the prevalence Parkinson's disease are alpha synuclein, it is found in the presynaptic nerve terminal it helps in the release of dopamine a neurotransmitter, mutation in this gene causes the accretion of lewy bodies that is a recognized biomarker for Parkinson's disease. The other genes include LRRK2 gene which is involve in neural toxicity, Parkin gene is mutated in region 6q 25-27 is also known as a biomarker for Parkinson's disease.(Lücking et al., 2000) DJ1 mutation initiates the oxidative stress inside the cell that results in mitochondrial dysfunction(Hering et al., 2004). PINK1, Pten and UCHLI are other genes that are involve in the provocation of Parkinson's disease.(Scott, Dawson, & Dawson, 2017)

Parkinson's disease is the second most accruing neurodegenerating disease that effect the central nervous system effecting 1 or 2 individuals per 1000 people at any

time.1% of all population is affected at the age of 60 by Parkinson's disease but with time its prevalence is emerging at the age of 40.

The prevalence of Parkinson's disease is affected by many factors that include age, environmental factors and behavioral factors may add to the prevalence of Parkinson's disease this observation is strongly supported by the observation that 90% of the Parkinson's disease is caused by unidentified genetic mutations that is in 15% cases. Genetic mutation is also a risk factor for PD other than that certain infections like hepatitis, helicobacter pylori, influenza and other infections of central nervous system(Ascherio & Schwarzschild, 2016; Fang et al., 2012) recently cancer is also considered as a major risk factor for parkinsonism the common feature hat is shared in both the disease is the atypical effects on cellular proliferation such as in Parkinson's the dopaminergic neuronal cell death accours in the substantia nigra of mid brain while cancer is described as the uncontrolled cellular proliferation forming masses of tissue called the tumors that can metastasize from one tissue to another.(Inzelberg, Flash, Friedman, & Azizi, 2016)

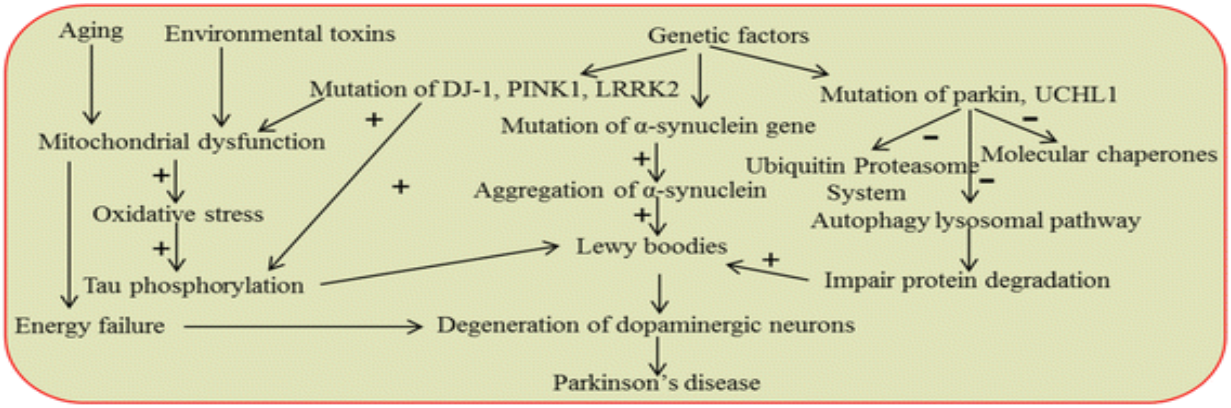


Figure 2.1: Risk factors associated with Parkinson's disease

2.2: Skin cancer

skin cancer could be described as the uncontrolled division of the skin cells. Skin is the largest organ of human body with immune function as well. It is constantly exposed to physical such as sun rays, biological like microbes and other disease-causing agents, and environmental assaults. That include the UV radiation, X-rays, cigarette smoke, automobile emissions, industrial soot, and water contaminations.(Esteva et al., 2017) Skin cancer is a multifactorial disease. These factors can lead the skin to cause the mutation in the genes regulating the cell cycle and trigger the initiation and progression of aggressive cancer. the incidence of skin cancer is increasing day by day. (Neagu et al., 2016)

Skin cancer is mainly categorized as melanoma and non-melanoma. Melanoma being the cancer of melanin cells and non-melanoma include basal cell carcinoma and squamous cell carcinomas.

Melanoma skin cancer arises from the melanin cells, if there is any uncontrolled cell division in melanin it results in melanoma. Melanoma appear as a lump or bump anywhere on the skin the lump may itch, ulcerate or start bleeding in the later stages

The other two types of skin cancer are commonly called as non-melanoma skin cancer.

Basal cell carcinoma is associated with the other lesion of skin spearing as shiny luster due to the small blood vessels present. BCC grows slowly and can destroy the tissues but is unlikely to spread to other parts of body. Basal cell carcinoma accounts for the 32% cases of cancer in the world.

The other type of non-melanoma skin cancer is squamous cell carcinoma. This type of skin cancer is also known as epidermoid carcinoma. it arises from the squamous cells. Squamous cell carcinoma mostly occurs in lungs, thyroid gland, esophagus and vagina.

Many epidemiological evidences suggest that the patients with Parkinson’s disease have lower risk of developing any cancer except skin cancer. PD and cancer seem

unlike at first glance, but reportedly there is increased prevalence of skin cancer in PD patients and vice versa(Bajaj, Driver, & Schernhammer, 2010)

It was initially reported in 1972, when a PD patient developed melanoma after using levodopa a therapeutic drug for PD and then more than 50 incidence were reported the issue was further investigated and concluded that levodopa was not involve in development of melanoma the risk of PD was already present in such patients, it may be due to the possibility of similar genetic profile that could contribute to the prevalence of melanoma in PD patients and vice versa. (Liu, Gao, Lu, & Chen, 2011)

It has been suggested that there is no risk of developing most kind of cancers in PD comparatively to a general population however high risk of skin cancer, thyroid cancer ad breast cancer has been associated with PD in which skin cancer is significantly observed.(Feng, Cai, & Chen, 2015; Schwid et al., 2010)

This indicates that a negative interaction is strongly associated between PD and skin cancer, according to epidemiological studies there is up to 4-fold increase of melanoma and non-melanoma(Ferreira et al., 2010) skin cancer in PD patients and the phenomena seems to be bidirectional, in skin cancer patients the chances of PD increases by 2 folds.(Olsen, Friis, & Frederiksen, 2006) The underlying mechanism and interaction between Parkinson's and melanoma is yet to be discovered but some suggested reasons could be genetics such as mutation the same genes, dysfunction

in the melanin and neuro melanin pathways, tyrosine metabolism, pathology of alpha synuclein, problems regarding cell cycle.(Devine, Plun-Favreau, & Wood, 2011; Hernández, 2009) interestingly the genes involved in developing PD also shows a role in regulating cell cycle, an important factor in developing cancer and cancer is caused due to un controlled cell cycle. Some tumor suppressors genes and oncogenes are also reportedly involved in developing PD. So, in light of this knowledge we can only predict that there are some interlinked genes that promote their interaction and ca cancer in PD patients and vice versa.(Robinson, 2010)

The aim of our study is to test the hypothesis for the presence of any biomarker interaction, common genes and genetic pathway, biomolecular defects in nervous system for the evidence of any interaction between PD and skin cancer.

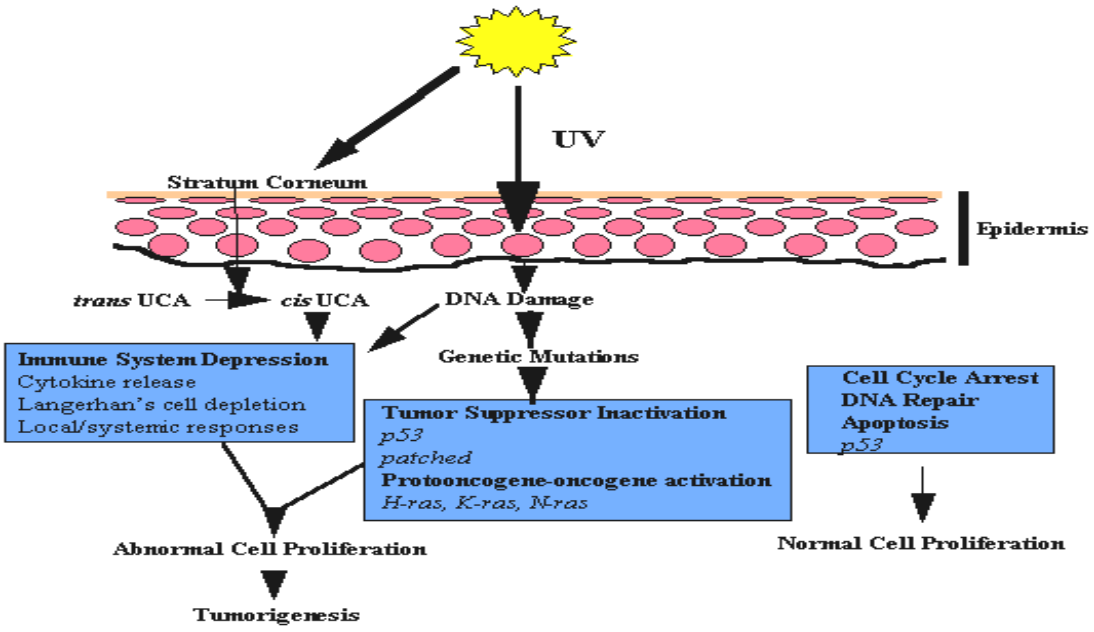


Figure 2.2: events involve in the pervasiveness of skin cancer

2.3 MPTP as a neuro toxin, induced Parkinson's models:

MPTP is a neurotoxin that can cause neuro degenerated disease.We can create acute models of Parkinson's by MPTP for four weeks. This will develop Parkinson's like symptoms. 1-methyl 4-phenyl 1, 2, 3, 6-tetrahydropyridine (MPTP) MPTP toxicity is mediated by energy crisis and oxidative stress. MPTP can efficiently cross the blood brain barrier and enhance the melatonin dependent oxidative stress and symptoms of parkinsons disease. The alteration of melatonin elicits the entry of certain neurotoxins into the cell. (He, Uchida, Megumi, Tsuge, & Nakayama, 2015)When MPTP is injected it is first converted into 1-methyl-4- 7 phenyl-2, 3-dihydropyridinium (MPDP1) and the converted into its active compound 1-methyl-4-phenylpyridinium (MPP1), its toxic form through oxidation. Increased concentration of MPP1 in dopaminergic neuron impair the mitochondrial functioning that results in the increase level of oxidative stress in the form of (ROS). (Yang et al., 2018)

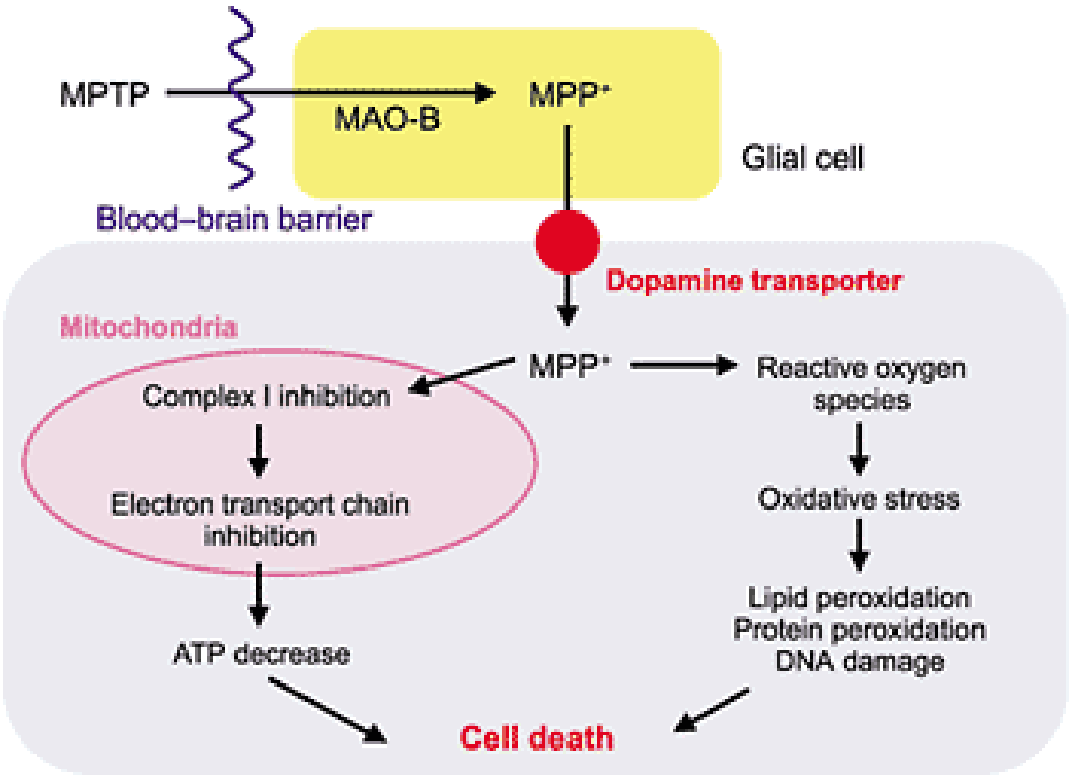


Figure 2.3: Neuro toxicity of MPTP

2.4: DMBA induced skin cancer

We can develop an in vivo model of skin cancer by the tropical application of DMBA as an initiator and the tropical application of croton oil as a promotor This allows for a greatly accelerated rate of tumor growth, making skin cancer studies possible I animal models. 7,12-dimethylbenz[a]anthracene (DMBA), is an immunosuppress or as well as a potent organ-specific carcinogen(Zhou, Young, Loch-Caruso, & Shikanov, 2018)

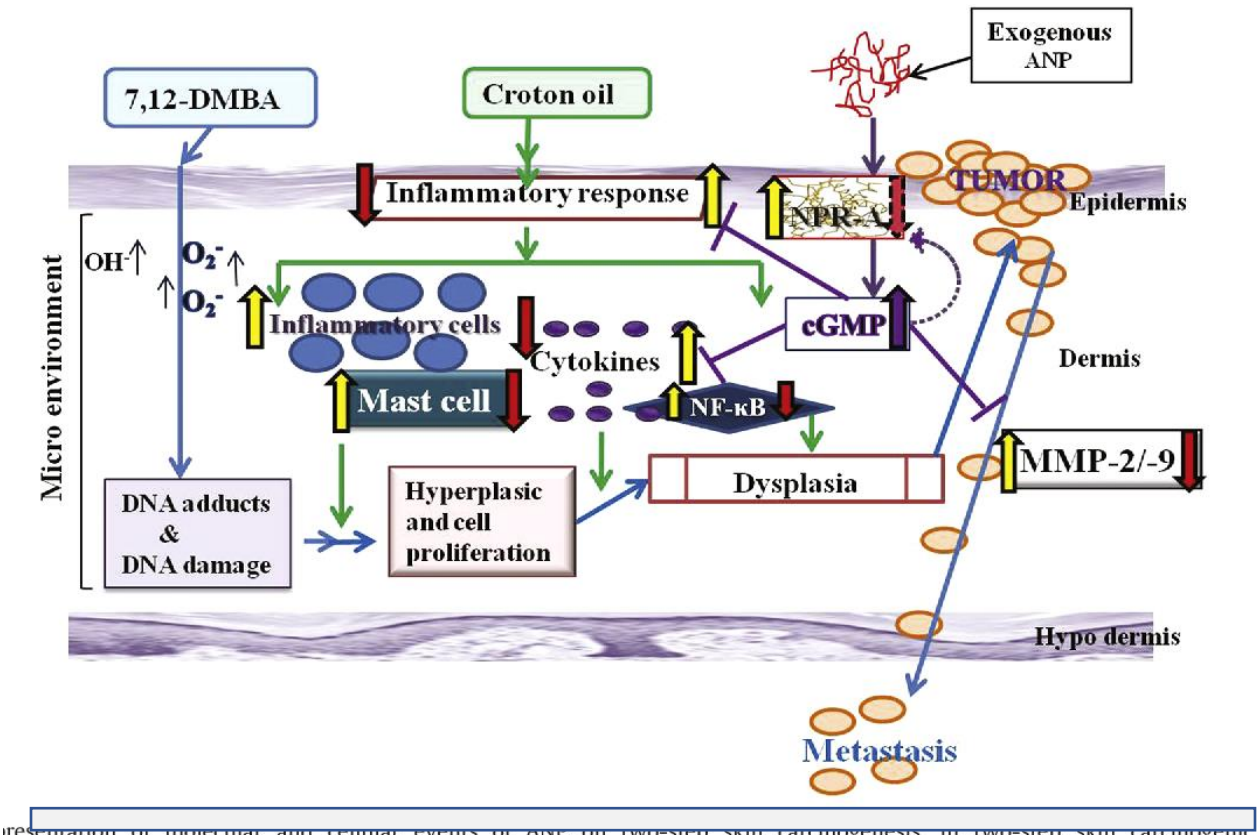


Figure 2.4: DMBA induced skin cancer in mice model

CHAPTER 3: METHODOLOGY

3.1: Chemicals and reagents

1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine hydrochloride(MPTP), 7,12 Di methyl benza anthracene (DMBA), and croton oil, anesthesia (calamine and xylase)

3.2: Animals:

Animals were purchased from National Institute of health sciences (NIH)

3.3: developing models for the study

Groups	**No of animals**	**IP injections**	**Dose**	**No of days**
control	5	Normal saline	**–**	**–**
DMBA	5	7,12 Di methyl benza anthracene	30mg/kg	
MPTP	5	1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine	30mg/kg	
DMBA+MPTP	5	7,12 Di methyl benza anthracene+1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine	30mg/kg	

Table 3.1: development of models

3.3.1: developing MPTP models

MPTP models were prepared by intraperitoneal injections of 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine MPTP which is a neurotoxin that helps in developing acute models of MPTP.

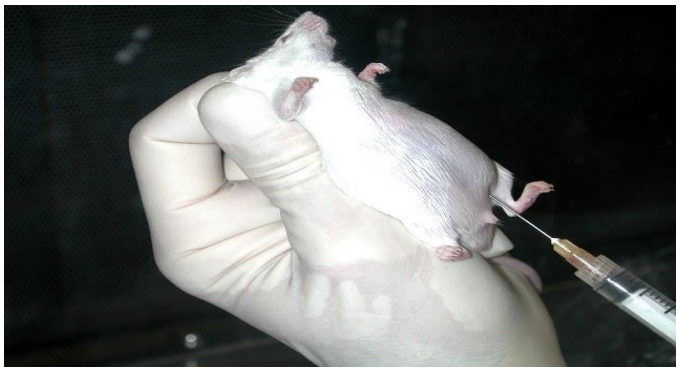


Figure 3.1: Intraperitoneal MPTP injection to mice model

3.3.2: developing DMBA models

skin cancer models were prepared by the tropical application of DMBA is a cancer initiator and croton oil as a promotor. They alter the cell cycle of ells and develop skin cancer in mice

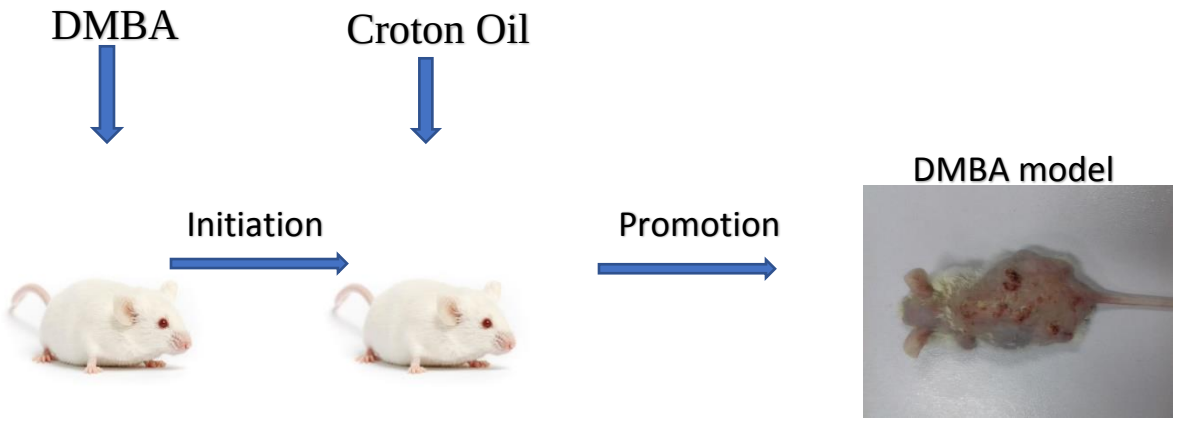


Figure 3.2: Developing DMBA model

3.3.3: DMBA+MPTP group:

Mice of this group consists of both Parkinson's and skin cancer through the intra peritoneal injections of MPTP and tropical application of DMBA

3.4: Behavior analysis

3.4.1: Horizontal bar test

Horizontal bar test is performed to check akinesia which is a common symptom in Parkinson's disease for that we used a horizontal bar of length 15cm and width of 4.66mm. to perform the test we hold the mice through its tail and place it on the bench in front of the setup, once the mice is relaxed slide it quickly backwards and then rapidly rise it towards the horizontal bar and let it grasp the bar with its forelimbs only and then quickly release the tail. Count the time in which the mice use its hind limbs to grab the bar and score the mice accordingly.

Figure 3.3: horizontal bar test

3.4.2: Pole test

Pole test was performed to confirm bradykinesia, this procedure includes a pole of length 26cm and width 2.8cm. to perform the procedure correctly the mice were placed at one end of the pole and the time in which it reaches the end of pole is recorded using stop watch and score accordingly. The time in which the mice descend to the end is called locomotor activity time

Figure 3.4: pole test

3.4.3: Swim test

To check the motor impairment swim test was performed to carry out the procedure a tub was used that was 40cm in length, 28cm wide and 20cm deep the level of the water was maintained up to 14 cm at 27-28 °C. animals were placed in the tub to check the swimming aptitude so that their motor abilities could be observed each mice was placed for 5 mi and time of mobility was deducted from total time to calculate immobility time.

Figure 3.5: swim test

3.5: Weight analysis

Weights of the mice were measured each week using a digital weight machine

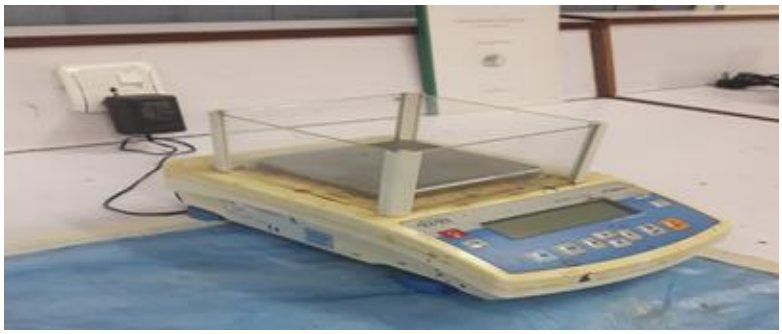


Figure 3.6: weight analysis

3.6: Tumor analysis

Tumors started appearing in week 4 and week 5 onwards tumors were measured till the end of the experiment.

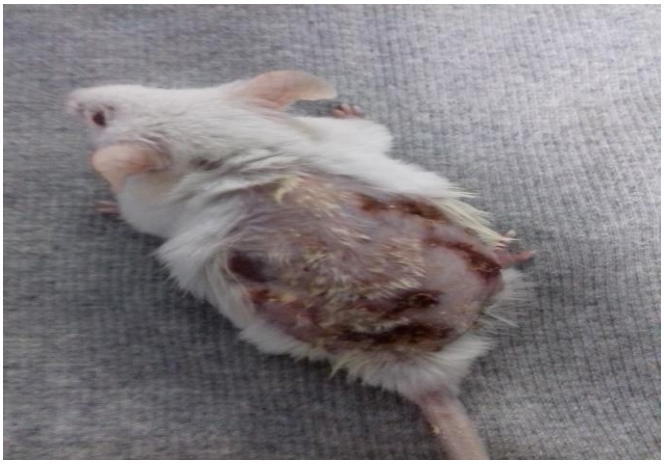


Figure 3.7: Tumor Analysis

3.6.1: Tumor number

we manually counted the tumor numbers and take the average within the group.

3.6.2: Tumor size

tumor size was measured using digital Vernier caliper and by using this Formula: $(sh\ ort\ length)$ ^2*$(long\ length)$^2/2 average size of tumors is calculated.

3.7: Sample collection

After the end of 13th week all the mice were sacrificed. First the mice were injected with Anesthesia and the cardia perfusion was performed using phosphate buffer saline (PBS). cardiac perfusion is important for the collection of brain samples. we divided each group into two subgroups, among them one group samples were stored at -80 directly and the other subgroup was fixed in the paraformaldehyde for histopathological analysis. Both the brain and skin samples are collected to study interaction between parkinsons and skin cancer.

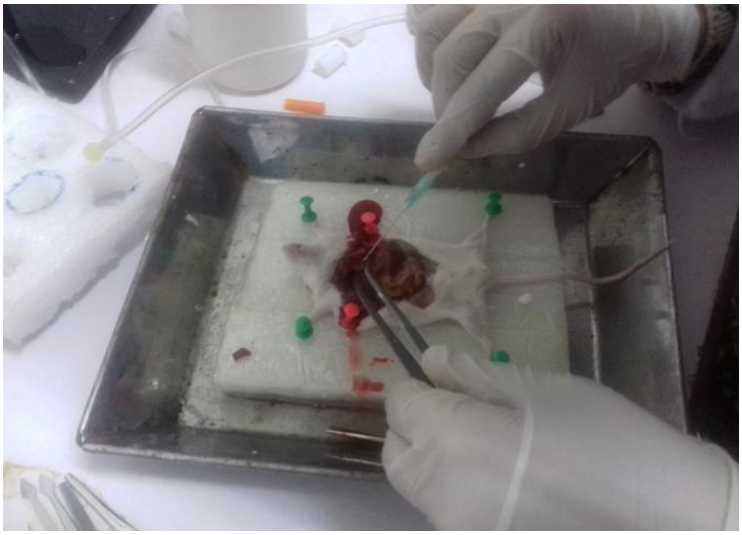


Figure 3.8: Sample collection

3.8: Histopathology

Normal brain and skin tissues were harvested and fixed in paraformaldehyde. These fixed tissues were embedded in paraffin were cut into 4mm sections on the slide and then stained with for hematoxylin and eosin (HE) staining. Histopathology was performed for the skin tissues and brain tissues of all the samples and tumor samples of the DMBA and MPTP+DMBA. Samples were fixed in 4% paraformaldehyde. The tissues embedded in paraffin were cut into 4mm section of histology slides and staining was performed using H and E staining.

3.9: Biochemical assay

3.9.1: Different biochemical assay

After the mice are sacrifices and tissues (substantia nigra) are harvested for different biochemical assays. Before performing each biochemical assay, tissues samples are homogenized in rippa buffer. Homogenized samples are centrifuge at 15000 rpm for 15 min to collect the supernatant to perform on Superoxide dismutase (SOD), Reduced glutathione (GSH), and Lipid peroxidation (LPO).

3.9.2: Lipid peroxidation

Thiobarbituric acid is produced as a byproduct of lipid peroxidation.as a result of reactive oxidative stress the lipid portion that is present in the cell membrane is converted into the product called malondialdehyde(MDA) and appear as a red adduct. Lipid peroxidation by reactive oxidative stress is known to be involve in Parkinson’s and skin cancer.

500ul of the tissue samples with 500ul of 10% TCA in a centrifuge tube for 10 min at 3000 rpm. to 500ul supernatant of the supernatant add 500ul of 0.67% TBA,

after mixing them the sample in boiling water bath for15 min. the absorbance of the TBA-TCA-homogenate is checked on 532nm

Collect supernatant of homogenized sample
Add 500ul of TCA to the sample
Centrifuge at 3000 rpm
To 500ul supernatant add 500ul of TBA
Vortex the sample and keep in boiling water for 15min
Check the absorbance at 532nm

Table 3.2: TBARS assay

3.9.3: Supper oxide dismutase

Super oxide dismutase is an anti-oxidant enzyme. The level of sod enzyme is high in disease condition is high due to the oxidative stress produced as a result of abnormal cell cycle or cell death.to find the sod activity 0.1ml of EDTA buffer of ph. 8 is added to 2.8ml of phosphate buffer solution and 0.1ml of 20 Mm pyragallol is added to the reaction at the end 0.1ml of sample is added and incubate for 15-30 min. check the absorbance at 420nm

0.1ml of sample
0.1ml of EDTA buffer
2.8ml of phosphate buffer solution
20 Mm pyragallol
absorbance at 420nm

Table 3.3: SOD activity

3.9.4: Reduced Glutathione assay

0.5 ml of homogenate is mixed with 2ml of 5% TCA (1.5/30ml DMSO). centrifuge the final volume and separate the supernatant then add 0.5ml elman's reagent that is dissolved in 1% of sodium citrate and 3 ml of phosphate buffer ph. 8.

0.5ml of homogenate
Add 2ml of TCA to homogenate
0.5ml of Elman's reagent
3ml of phosphate buffer saline
Absorbance at 420nm

Table 3.4: GSH assay

CHAPTER 4: RESULTS

4.1: BEHAVIOR ANALYSIS:

Study plan: we aim to determine the association between Parkinson's disease and skin cancer. We had developed MPTP models, DMBA models and MPTP+ DMBA models. First group were of the healthy mice the second group were MPTP models with Parkinson's disease. Parkinson's disease was induced using intra paratonial MPTP injection each day in week two. The DMBA group had skin cancer, these models were developed using tropical application of DMBA twice in the second week followed by croton oil thrice from week three till week thirteen. The fourth group contained of both skin cancer and Parkinson's disease and these models were developed by injecting fife doses of MPTP and 2 doses of DMBA in week followed by the tropical application of croton oil

Behavioral analysis tests
Horizontal Bar Test
Pole Test
Swim Test

Table 4.1: behavior analysis

4.1.1: Horizontal bar test

Horizontal bar test is conducted to observe the motor abilities in the mice. horizontal bar test mice were scored according to the time they took to use their hind limbs for grabbing the bar as we had 4 groups, so the control and DMBA scored high in the motor disability test because they were not suffering from parkinsonism. MPTP scored less because of the induction of Parkinson's. MPTP plus DMBA group scored very less because of the parkinsons and skin cancer and these results support our hypothesis skin cancer support Parkinson's and vise verse.

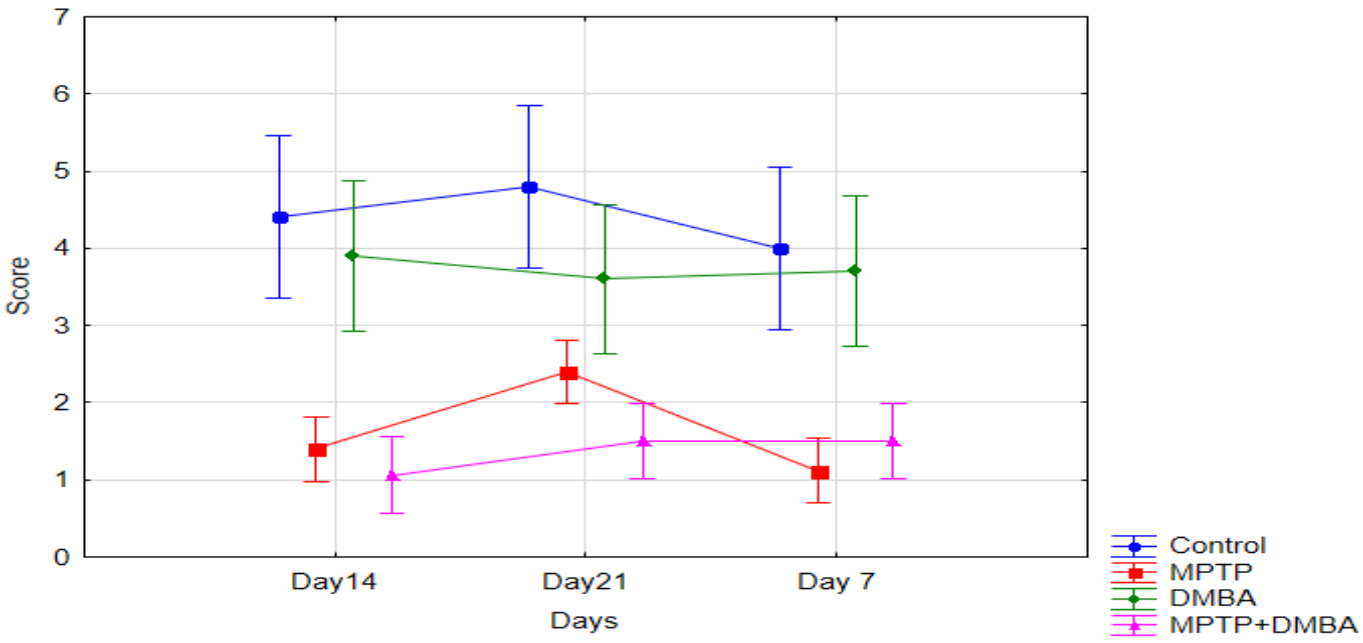


Figure 4.1: Horizontal bar Test

4.1.2: Pole test

Pole test was performed for the Symptoms of bradykinesia. The groups were scored relative to the time the reach the ground. Control and DMBA group showed better results than the MPTP and in DMBA+MPTP group the mice took much time to descend

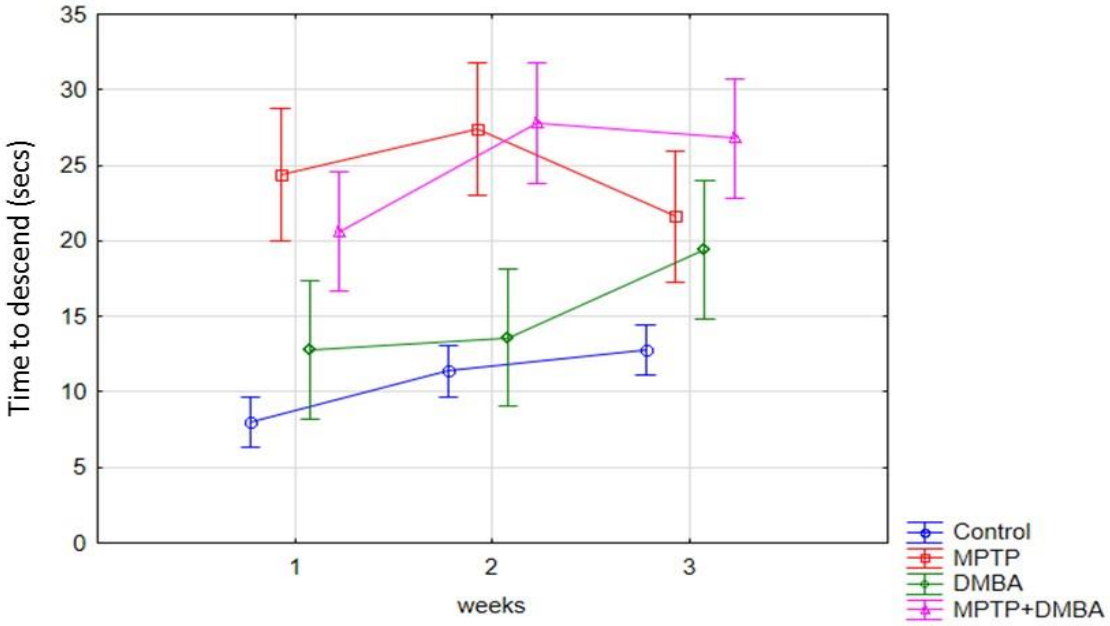


Figure 4.2: Pole Test

4.1.3: Swim Test

Swim test showed the motor impairment in the mice control was used as a standard. MPTP showed motor impairment as the had more immobility time. MPTP +DMBA showed the elevated level of motor impairment because they had immobility time more than MPTP that support our hypothesis that Parkinson's disease is boosted by the effect of skin cancer .

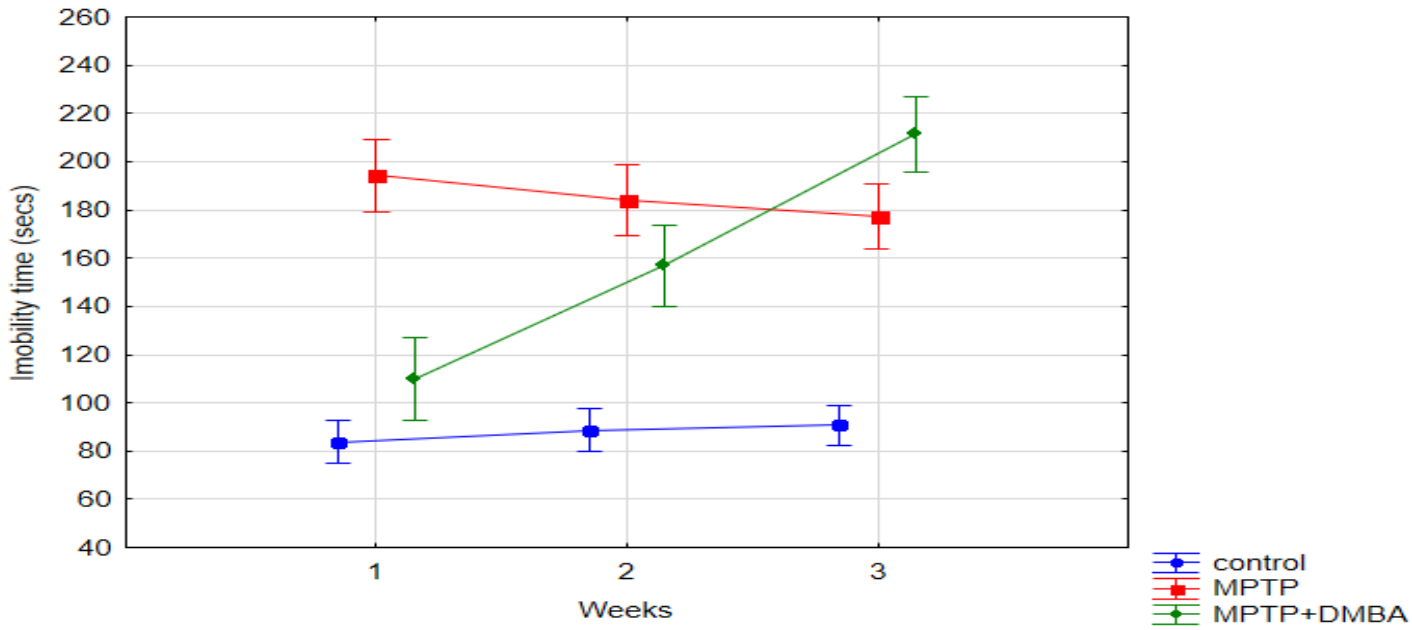


Figure 4.3: Swim Test

4.2: WEIGHT EVALUATION

4.2.1: Weight evaluations

Weight evaluation were performed every week till the end of experiment with the help of weight machine. A slight increase and decrease was found in the weight of control group

4.2.2: Tumor evaluation

Tumors were evaluated from week 5 and onwards once the tumors start appearing. Tumor evaluation was done by calculating the average number of tumor as well as average size of tumor.

4.2.3: Average number of tumor

Average number of tumor was calculated by counting the number of tumor on each mouse and the finding the average within the group, so we will get average number of tumor in a group. The graph clearly shows that the number of tumor in MPTP+DMBA is more then DMBA that support our hypothesis that Parkinson's disease boost skin cancer and vice versa.

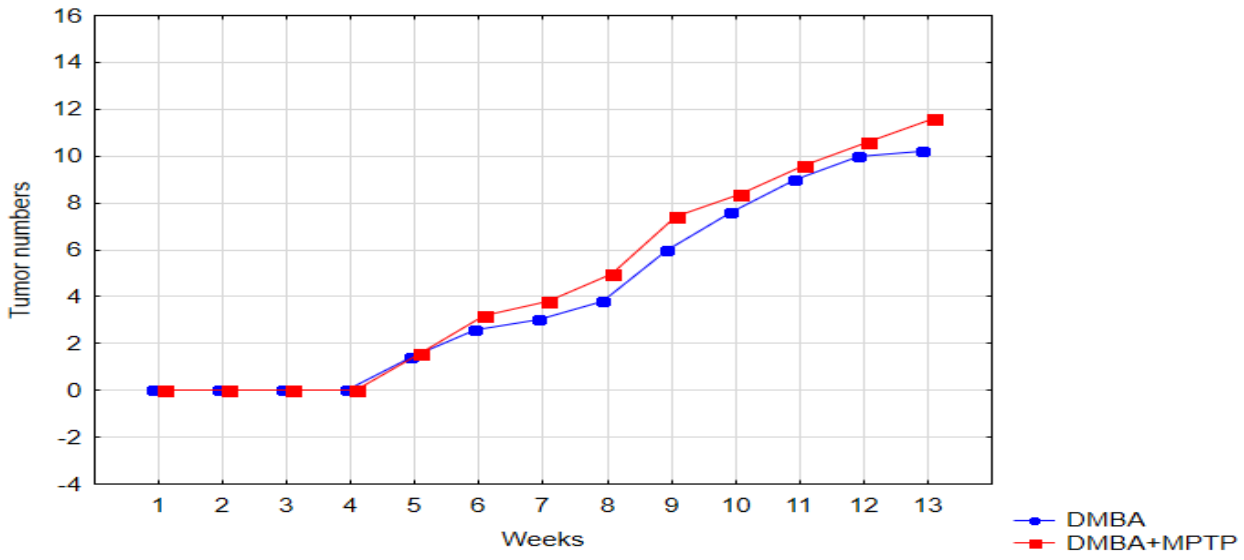


Figure 4.4: average numbers of Tumors

4.2.4: Average size of tumor

Average size of tumor is calculated by taking the size of each tumor using digital Vernier caliper of each mice in a group and then taking the average within the group to compare it with other groups.in the graph we can see that the size average size of tumor is also larger in MPTP+DMBA group that means skin cancer has developed an interaction with Parkinson's disease which is boosting the efficacy of tumor size in skin cancer as compare to skin cancer alone.

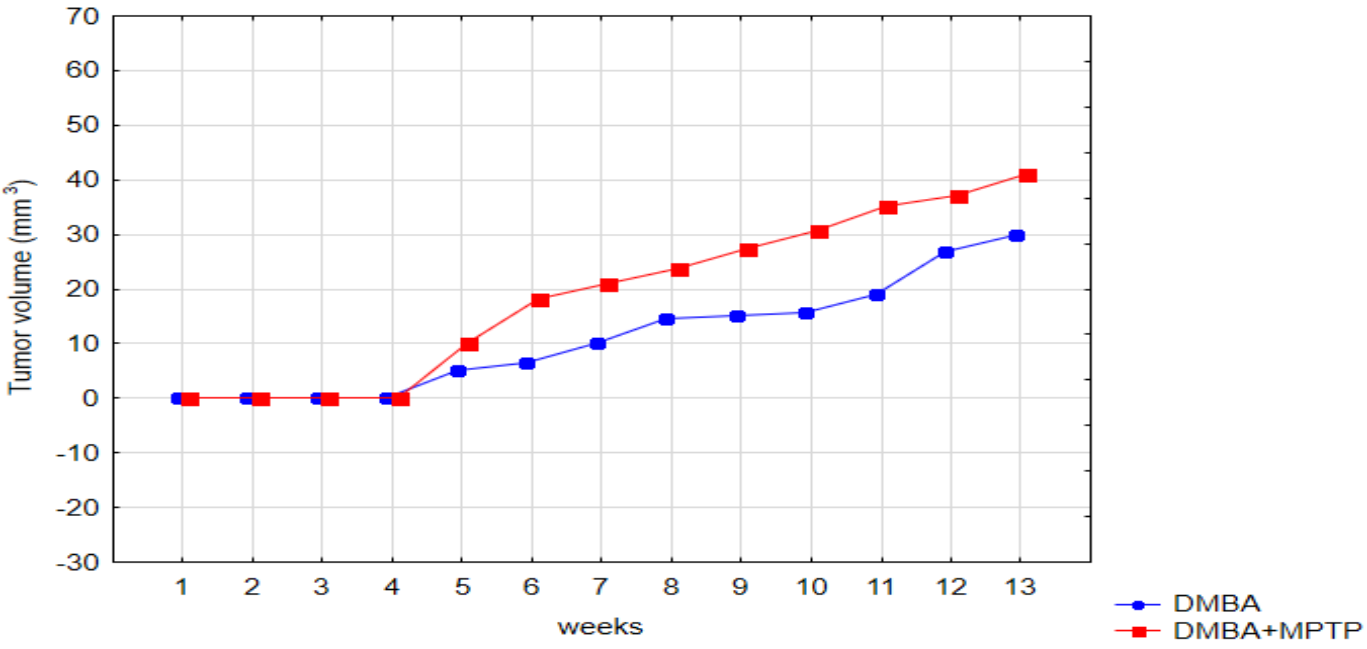


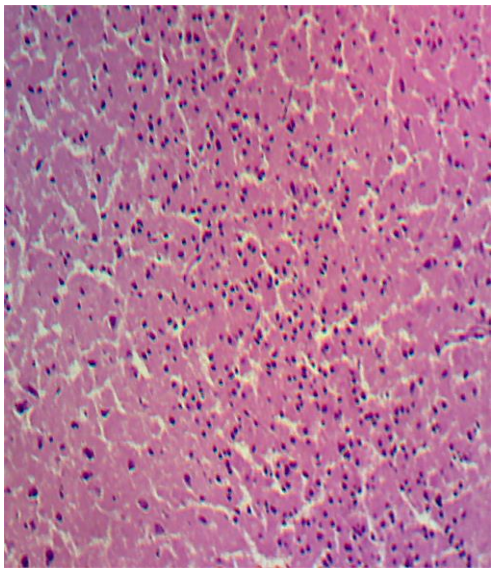
Figure 4.5: average size of Tumors

4.4: Histopathology

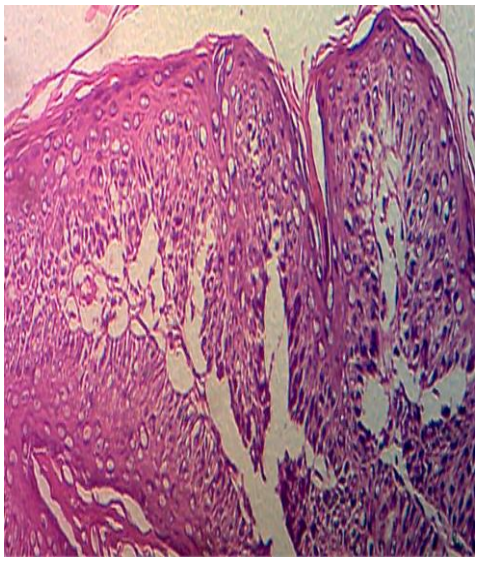
Histopathological analysis shows the stained cross section of the tissues that were fixed on the slide

4.4.1: Histopathology of control mice

Histopathology for the brain and skin tissues were performed. The crosection intersection of healthy mice showed normal physiology of tissues of brain and skin.



Control Brain

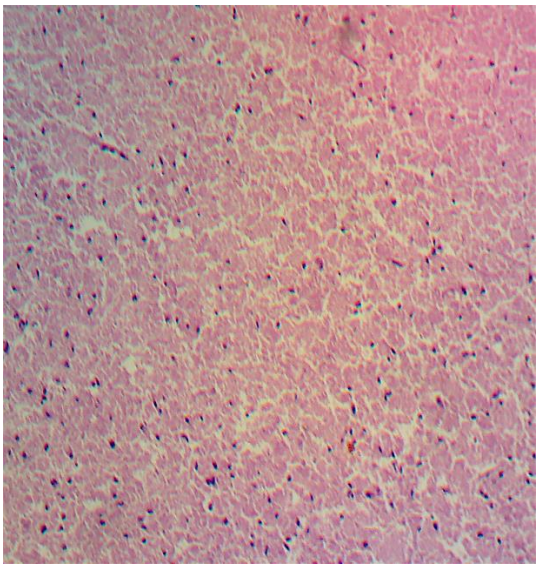


Control Skin

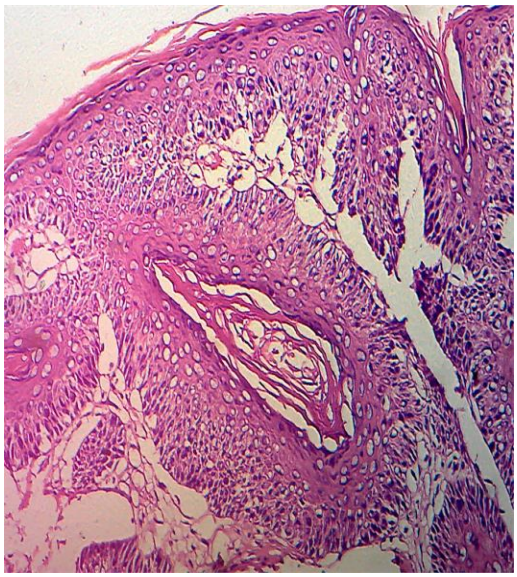
Figure 4.6: Histopathology of control Group

4.4.2: Histopathology of DMBA group

Histopathology of DMBA group was performed for Brain and skin samples the brain tissues showed normal substantia nigra, as the brain of these mice was not infected but the skin samples showed prominent dysplasia, extensive autolysis, superfacial ulceration, hyperkeratosis, focal papilloma and Destruction of cells.



DMBA Brain

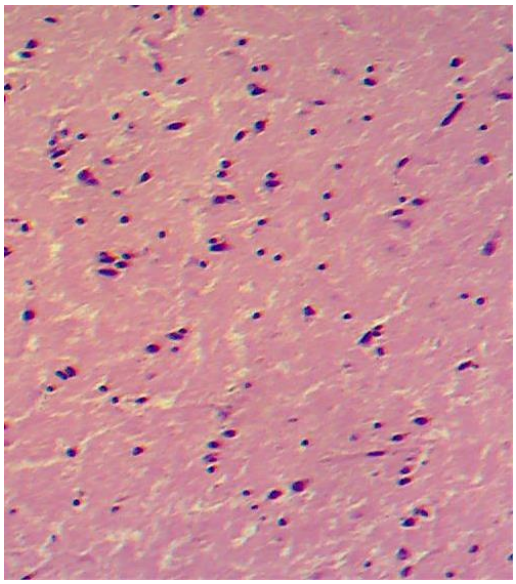


DMBA Skin

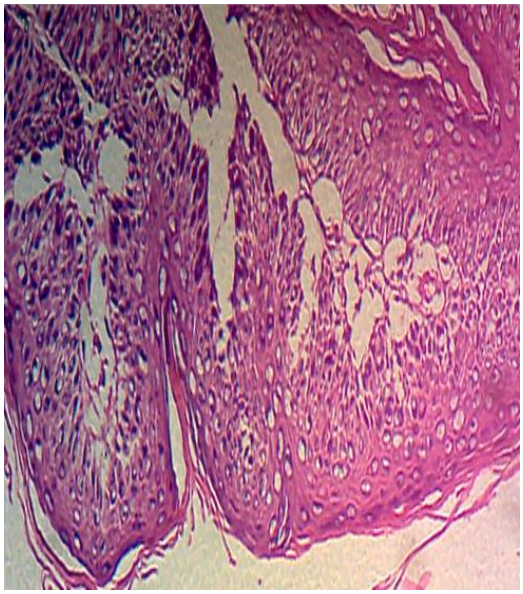
Figure 4.7: Histopathology of DMBA Group

4.4.3: Histopathology of MPTP group

Brain of MPTP group showed clear diseased condition in histopathology of neurons of substantia nigra of MPTP injected models appeared shrunken, extensive autolysis was observed, and inflammation was also observed in these tissues. But in the skin tissues there was no significant change observed as they were not exposed to DMBA.



MPTP Brain

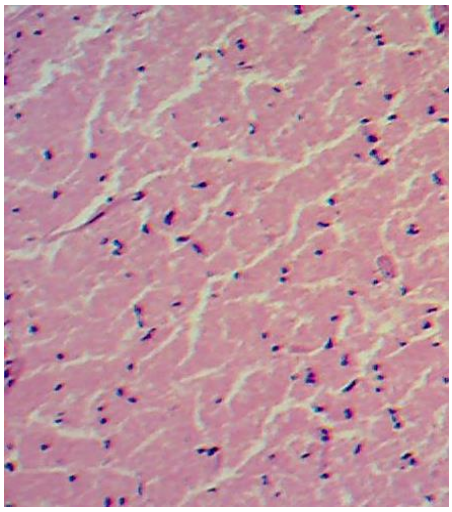


MPTP Skin

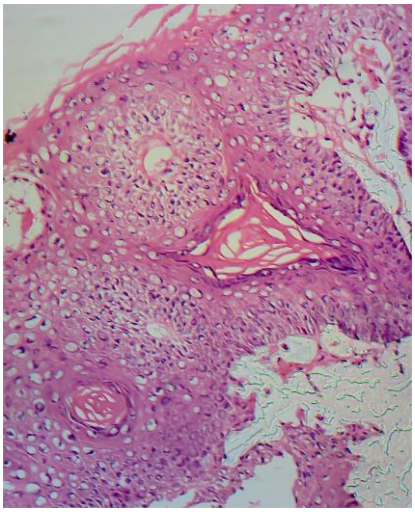
Figure 4.8: Histopathology of MPTP Group

4.4.4: Histopathology of MPTP + DMBA

In the fourth group the models were introduced both to the MPTP and DMBA. So, both their brain and skin were affected. There was sever inflammation in the neurons of substantia nigra and major autolysis and more destruction then the MPTP group alone and the results of histopathology of skin also showed more server destruction as compare to DMBA group. The tumors were enlarged and sever autolysis and hyperkeratosis was observed.



DMBA +MPTP Brain



DMBA+MPTP skin

Figure 4.9: histopathology of DMBA+MPTP Group

4.5: Biochemical assay

4.5.1: Lipid peroxidation

Lipid peroxidation assay was done for both skin and brain the bar graphs shows that the level of MDA which is a biproduct of lipid peroxidation is less in control healthy mice. In case of brain the concentration is increase in MPTP and very high in MPTP+DMBA. In case of skin tissues analysis, the level of MDA is high in DMBA and very elevated in MPTP+DMBA. This evidently elaborate the effect of

Parkinson's on skin cancer and vice versa

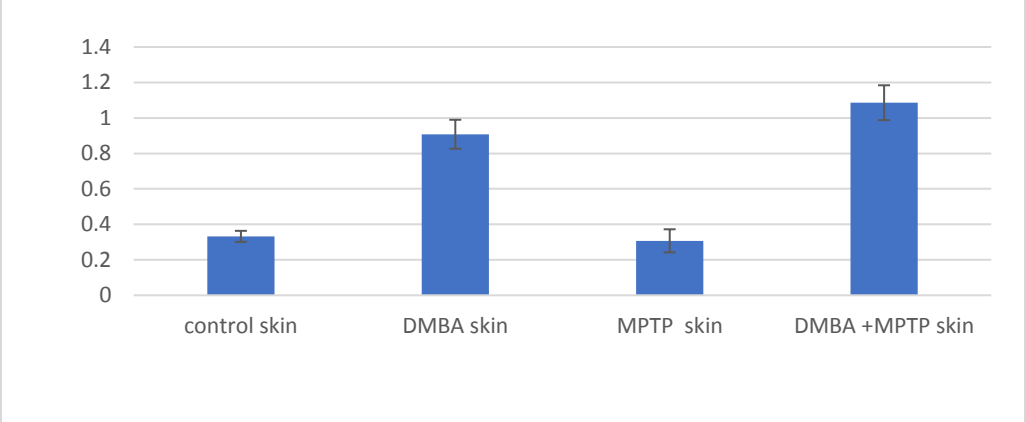


Figure 4.10: Tbars assay of skin

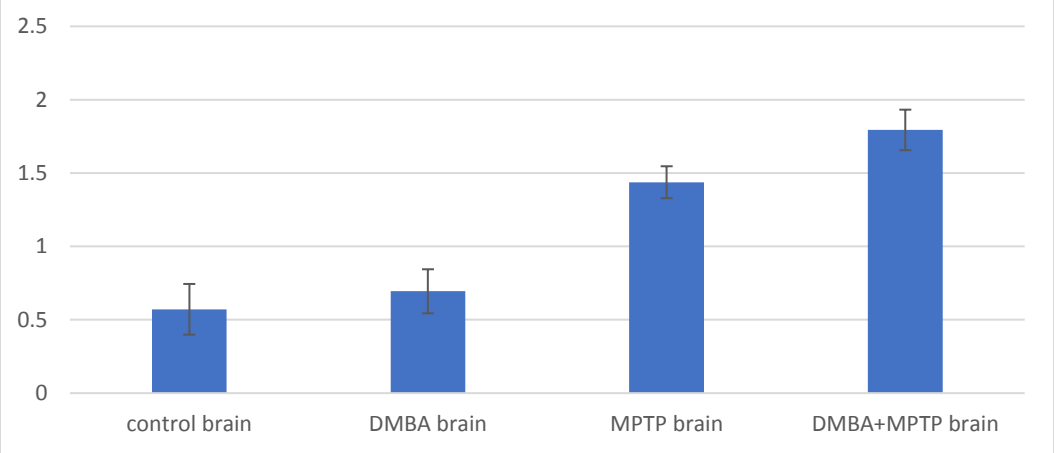


Figure 4.11: Tbars assay of Brain

4.5.2: Supper oxide dismutase

Sod activity is measured as unit per ml of protein. The biochemical analysis of control brain sample shows normal sod activity but in MPTP brain the insignificant sod activity has been noticed that shows the disease condition but in brain of MPTP+DMBA sod activity is insignificant that shows the effect of both disease on each other in case of skin tissue sod activity is decreased in DMBA group and MPTP+DMBA.

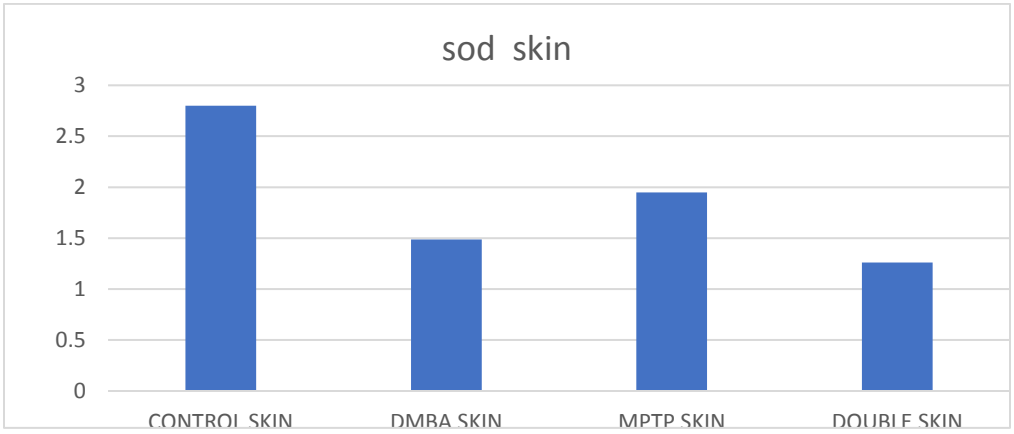


Figure 4.12: sod activity in skin

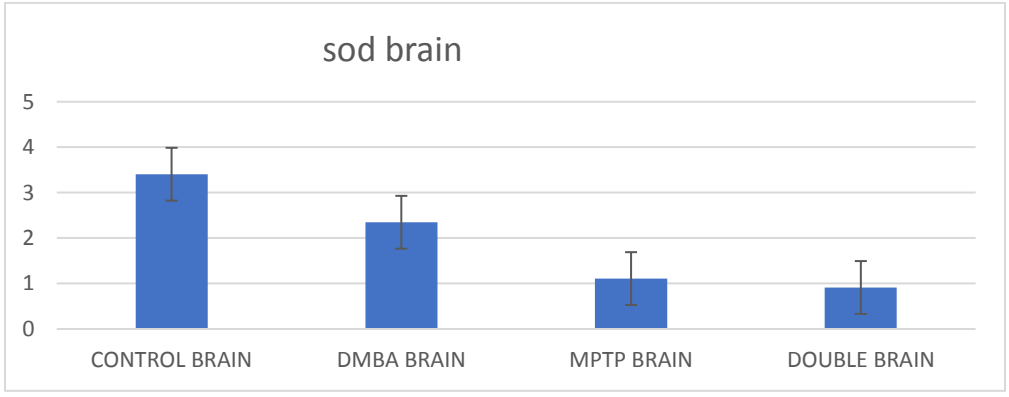


Figure 4.13: sod activity in brain

4.5.3: Reduced Glutathione assay

Glutathione is an anti-oxidant enzyme. GSH concertation of measured in Mm/mg of protein. Concentration of GSH is decreased in brain tissues of MPTP group and in skin tissues of DMBA group and decrease significantly in both the brain and skin tissues of MPTP+DMBA group which shows that both the disease boosts the effects of each other.

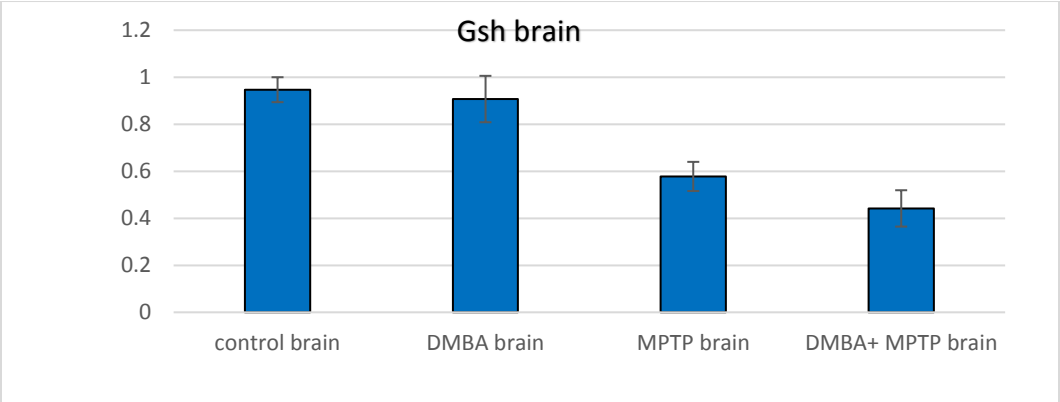


Figure 4.14: GSH activity of in brain

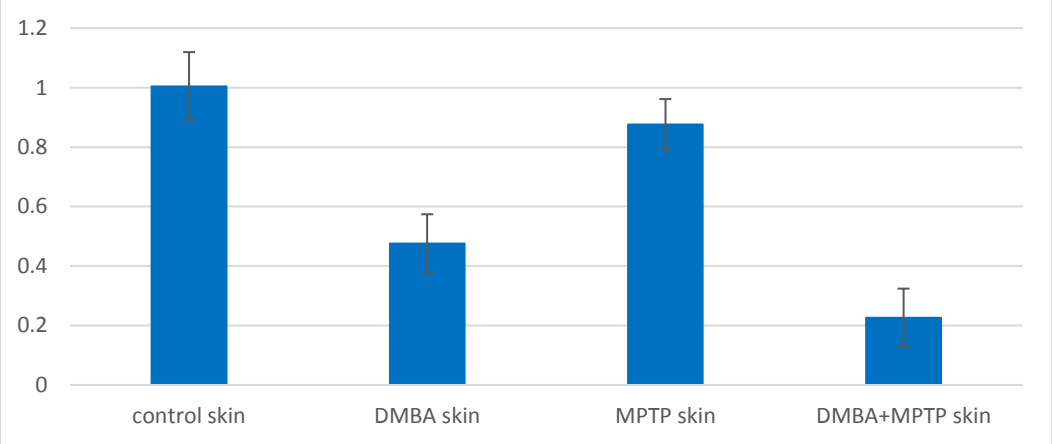


Figure 4.15: GSH activity of in skin

Chapter 5: Discussion

Parkinsons is known to be a neurological disorder characterized by tremors, postural instability and cognitive deficits in this condition the motor functioning is affected due to the loss of dopaminergic neuron. There are many risk factors associated to the prevalence of Parkinson's disease such as mutation, environmental factors, age factors, life style recent studies associate Parkinson's disease to skin cancer. There are some evidences in the literature. This debate goes for skin cancer as well. Apart from the genetic mutations and environmental factors, Parkinsons disease is considered as the major risk factor for skin cancer. It has been reported in the literature that certain drugs like levodopa that is used in the treatment of parkinsons can treger the initiation of skin cancer. There are some similar genes involve in the pathology of parkinsons disease and skin cancer. Expression of alpha synuclin is also reported in skin cancer that is the major gene involve in the development of parkinsons disease.

This experiment was conducted to perform a research on the interactions of neurological disorders and skin cancer we aimed to find out the major genes involve in the interaction the underlined mechanism and common pathways through which both the conditions are associated. Animal models were categorized into four groups.

The biochemical analysis shows that there is elevated level of oxidative stress in the brain tissues of MPTP group and in the skin tissues of DMBA group. The group with both MPTP and DMBA has oxidative stress more than each group separately that means both the conditions are associated and trigger the effect of each other. similarly, the histopathological analysis shows major destruction in the tissues of models with MPTP and DMBA, comparatively to the brain tissues of MPTP group and skin tissues of DMBA group.

Chapter 6: Conclusion

Through our study we can conclude that the combine effect of DMBA and MPTP on the mice models showed boosted skin cancer and augment degeneration of dopaminergic neuron resulting in worse Parkinson's disease. This reveals that both the disease is increasing the effects of each other. Significant increase in the cognitive abilities, performance of motor skills and encounter of the oxidative stress was observerd in the PD mice and intensification in the tumor volume and tumor number were seen in the DMBA injected mice.

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