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**PREPARATION, CHARACTERIZATION AND EXAMINATION
OF SOME NANOMATERIALS AS FREE RADICALS
SCAVENGERS IN SOME ANIMALS DISEASES**

A Thesis Submitted

By

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List of contents

<i>Contents</i>	<i>Page</i>
<i>Introduction</i>	1
<i>Review of Literatures</i>	3
Application of nanomaterials in biomedicine as free radicals scavengers	3
Preparation of nanomaterials	36
Characterization of nanomaterials	42
<i>Materials and Methods</i>	65
Synthesis of yttria NPs and characterization of synthesized particles	65
Characterization	65
Grafting polymeric shell on surface of synthesized yttria NPs and characterization of core shell polymerized NPs	67
Detection of cytopathic effect of polymerized NPs and survival rate	69
<i>In vivo</i> detection of toxicity, biocompatibility and antioxidant efficacy of nanoparticles	72
Statistical analysis	79
<i>Results</i>	81
<i>Discussion</i>	149
<i>Summary and Conclusions</i>	182
<i>References</i>	191
<i>Arabic summary</i>	1

List of Tables

<i>Table</i>	<i>Page</i>
1. Preparation of Y₂O₃ by coprecipitation method by aqueous urea	54
2. Preparation of Y₂O₃ by Hydrolysis assisted coprecipitation	55
3. Preparation of Y₂O₃ by coprecipitation method aqueous ammonia and ammonium hydrocarbonate	56
4. Preparation of Y₂O₃ by coprecipitation method Coconut water based sol-gel synthesis (proteic sol- gel route)	57
5. Preparation of Y₂O₃ by coprecipitation method AHC and Urea	58
6. Preparation of ceria nanoparticles by Sodium hydroxide precipitation method	59
7. Room temperature coprecipitation of nanocrystalline CeO₂ and Gadolinium doped Ceria	60
8. Egg white synthesis of metal oxides nanoparticles	61
9. Preparation of ceria nanoparticles by ammonia precipitation method	62
10. Preparation of Y₂O₃ by co precipitation method AHC and ammonium sulfate	63
11. Tools of characterization for nanoparticles	64
12. Experimental design 1 for detection the toxicity of NPs	73
13. Experimental design 2 for detection the biocompatibility and bio distribution of NPs	74
14. Distribution of Fluorescence NPs capped by AA and EGMP at toxic doses in tissues	99
15. Distribution of Fluorescence NPs capped by EGMP at low doses in tissues	100
16. Biochemical profile of oxidative biomarkers of control group and heat stressed group	101
17. Biochemical profile of oxidative biomarkers of heat stressed group and prophylactic group	102
18. Biochemical profile of oxidative biomarkers of heat stressed groups with and without NPS injection	103

List of Figures

<i>Figure</i>	<i>Page</i>
1. Reactive oxygen species	51
Reaction (1): Dismutation of superoxide radicals	51
Reaction (2): Fenton reaction	51
2. Antioxidant defense system	52
3. Biokinetics of nanomaterials	53
4. Schematic illustration for synthesis of yttria NPs by Co-precipitation method	80
5. TGA analysis of the yttria precursor	104
6. XRD pattern of yttria nanoparticles	104
7. (a) The TEM image shows crystallinity of the yttria nanoparticles annealed at 450°C, (b) The TEM image shows morphological shape of the yttria nanoparticles, (c) The TEM image shows high degree of agglomeration of the yttria nanoparticles	105
8. Pattern of DLS of yttria nanoparticles	106
9. Pattern of Zetapotential of yttria nanoparticles	106
10. Changes in the substrate and product concentration in time for catalyst reaction	107
11. Changes in the substrate and product concentration in time for reaction without NPs	107
12. Changes in the substrate and product concentration in time for reaction with NP (- - -) and without NP (__) after 1 hour, 24 hours and 48hours	108
13. Changes in the colour of solution prepared for catalyst reaction without yttria NPS (left) and with NPS (Right) within 2hrs (a) and 8hrs (b) of reaction beginning.	108

14. The particle size obtained by DLS measurement as a function of time over 1week between Polymerized AA NPS with and without sonication	109
15. Pattern of Zetapotential of polymerized yttria nanoparticles capped by AA	109
16. The TEM image shows morphological shape of the core shell polymerized yttria NPs capped by AA	100
17. The behavior of polymerized AANPS is checked by DLS measurement as a function of time in synthetic media as BSA	100
18. The particle size obtained by DLS measurement as a function of time over 1week between Polymerized AA and EGMP capped NPS with sonication	111
19. Pattern of Zetapotential of polymerized yttria nanoparticles capped by EGMP	111
20. The TEM image shows morphological shape of the core shell polymerized yttria NPs capped by EGMP	112
21. The fluorescence intensity of YNPS without polymerization and NPS capped with AA	112
22. Cytopathic effect of polymerized NPs capped by AA on hepatocyte cell line at concentration (10-700 µg/ml) 72hrs post incubation	113
23. Cytopathic effect of polymerized NPs capped by EGMP on hepatocyte cell line at concentration (10-700 µg/ml) 72hrs post incubation	114
24. Distribution of fluorescein labeled AA NPs in hepatocyte cell culture line at different concentrations: (b), 10µg/ml; (c), 100µg/ml; (D), 250µg/ml; (E), 700µg/ml compared with blank (a)	115
25. Distribution of fluorescein labeled EGMP NPs in hepatocyte cell culture line at different concentrations: (b), 10µg/ml; (c), 100µg/ml;	116

(D), 250µg/ml; (E), 700µg/ml compared with blank (a)	
26. The survival rate of cell line incubated with AA NPs at concentrations ranged from 1.25µg/ml to 100µg/ml by MTT assay	117
27. The survival rate of cell line incubated with EGMP NPs at concentrations ranged from 10µg/ml to 1800µg/ml by MTT assay	117
28. Control group, liver showing normal lobular architecture with central vein (CV) and radiating hepatic cell cords. H&E. X 200.	118
29. Liver showing mild sinusoidal cells activation (head arrow). H&E. X 200	118
30. Liver showing mild hydropic degeneration of hepatocytes. H&E. X 200.	118
31. Liver showing mild portal fibroblasts and oval cells hyperplasia (arrow). H&E. X 400.	118
32. Liver showing mild mononuclear cells infiltrations (arrow) mostly macrophages as well as mild hydropic degeneration of hepatocytes. H&E. X 200.	119
33. Liver showing diffuse vacuolation of hepatocytes. H&E. X 200	119
34. Liver showing mild hemorrhage. H&E. X 200.	119
35. Liver showing severe vacuolation of hepatocytes as well as well demarcated new bile ducts in the portal areas (head arrow) together with fibroblasts proliferation (arrow) and mononuclear cells infiltrations. H&E. X 200.	119
36. Liver showing hepatic cell necrosis (arrow) as well as sever vacuolation of hepatocytes. H&E. X 200.	120
37. Liver showing increase in mitotic figures (arrow). H&E. X 400.	120
38. Liver showing intralobular fibroblasts and oval cells hyperplasia (arrow). H&E. X 200.	120
39. Liver showing mild vacuolation of hepatocytes. H&E. X 200.	120

40. Brain showing normal histological picture. H&E. X 200.	121
41. Brain showing mild neuronal degeneration and necrosis (arrow). H&E. X 200.	121
42. Brain showing neuronal degeneration and necrosis (arrow). H&E. X 200.	121
43. Brain showing neuronal degeneration, necrosis and neuronophagia (arrow). H&E. X 400.	121
44. Brain showing gliosis. H&E. X 200.	122
45. Spleen showing normal histological picture. H&E. X 200.	122
46. Spleen showing lymphoid hyperplasia. H&E. X 200.	122
47. Kidney showing normal histological picture. H&E. X 200.	122
48. Liver showing internalization of fluorescein labeled NPs, group 1, 12hrs after inoculation (arrow) X 40.	123
49. Liver showing normal architecture of control negative group X 40.	123
50. Liver showing internalization of fluorescein labeled NPs, group 2, 24hrs after inoculation (arrow) X 40.	123
51. Liver showing internalization of fluorescein labeled NPs, group 2, 72hrs after inoculation (arrows) X 40.	123
52. Liver showing internalization of fluorescein labeled NPs, group 3, 24hrs after inoculation (arrows) X 40.	124
53. Liver showing internalization of fluorescein labeled NPs, group 3, 72hrs after inoculation (arrows) X 40.	124
54. Liver showing internalization of fluorescein labeled NPs, group 4, 24hrs after inoculation (arrows) X 40.	124
55. Liver showing internalization of fluorescein labeled NPs, group 4, 72hrs after inoculation (arrows) X 40.	124
56. Liver showing internalization of fluorescein labeled NPs, group 5,	125

12hrs after inoculation (arrows) X 40.	
57. Brain showing normal architecture of control negative group X 40.	125
58. Brain showing internalization of fluorescein labeled NPs, group 1, 12hrs after inoculation (arrows) X 40.	125
59. Brain showing internalization of fluorescein labeled NPs, group 1, 24hrs after inoculation (arrow) X 40.	125
60. Brain showing internalization of fluorescein labeled NPs, group 1, 72hrs after inoculation (arrow) X 40.	126
61. Brain showing internalization of fluorescein labeled NPs, group 2, 72hrs after inoculation (arrows) X 40.	126
62. Brain showing internalization of fluorescein labeled NPs, group 2, 10d after inoculation (arrows) X 40.	126
63. Brain showing internalization of fluorescein labeled NPs, group 4, 12hrs after inoculation (arrows) X 40	126
64. Brain showing internalization of fluorescein labeled NPs, group 4, 24hrs after inoculation (arrow) X 40.	127
65. Brain showing internalization of fluorescein labeled NPs, group 5, 12hrs after inoculation (arrows) X 40.	127
66. Brain showing internalization of fluorescein labeled NPs, group 5, 24hrs after inoculation (arrows) X 40.	127
67. Kidney showing normal architecture of control negative group X 40.	127
68. Spleen showing internalization of fluorescein labeled NPs, group 1, 12hrs after inoculation (arrows) X 40.	128
69. Spleen showing internalization of fluorescein labeled NPs, group 1, 24hrs after inoculation (arrows) X 40.	128
70. Spleen showing internalization of fluorescein labeled NPs, group 1, 72hrs after inoculation (arrows) X 40.	128

VII

71. Spleen showing internalization of fluorescein labeled NPs, group 2, 12hrs after inoculation (arrows) X 40.	128
72. Spleen showing internalization of fluorescein labeled NPs, group 2, 24hrs after inoculation (arrows) X 40.	129
73. Spleen showing internalization of fluorescein labeled NPs, group 2, 72hrs after inoculation (arrows) X 40.	129
74. Spleen showing internalization of fluorescein labeled NPs, group 3, 12hrs after inoculation (arrows) X 40.	129
75. Spleen showing internalization of fluorescein labeled NPs, group 3, 24hrs after inoculation (arrows) X 40.	129
76. Spleen showing internalization of fluorescein labeled NPs, group 3, 72hrs after inoculation (arrows) X 40.	130
77. Spleen showing internalization of fluorescein labeled NPs, group 4, 12hrs after inoculation (arrows) X 40.	130
78. Spleen showing internalization of fluorescein labeled NPs, group 4, 24hrs after inoculation (arrows) X 40.	130
79. Spleen showing internalization of fluorescein labeled NPs, group 4, 72hrs after inoculation (arrows) X 40.	130
80. Spleen showing internalization of fluorescein labeled NPs, group 5, 12hrs after inoculation (arrows) X 40.	131
81. Spleen showing internalization of fluorescein labeled NPs, group 5, 24hrs after inoculation (arrows) X 40.	131
82. Spleen showing internalization of fluorescein labeled NPs, group 5, 72hrs after inoculation (arrow) X 40.	131
83. Liver showing sinusoidal cells activation. H&E. X 200.	132
84. Liver showing portal fibroblasts hyperplasia as well as mononuclear cells infiltrations mostly macrophages. H&E. X 200.	132
85. Liver showing portal fibroblasts and oval cells hyperplasia as well as mononuclear cells infiltrations. H&E. X 200.	132

86. Liver showing mild mixed fatty change and hydropic degeneration of hepatocytes together with very mild sinusoidal cells activation and mononuclear cells infiltrations. H&E.X 200.	132
87. Liver showing moderate mixed fatty and hydropic degeneration of hepatocytes, portal fibroblasts, oval cells hyperplasia and mononuclear cells infiltrations. H&E. X 200.	133
88. Liver showing normal lobular architecture with central vein and radiating hepatic cell cords. H&E. X 200.	133
89. Liver showing sinusoidal cells activation. H&E. X 200.	133
90. Brain showing normal histological picture. H&E. X 200.	133
91. Kidney showing normal histological picture. H&E. X 200.	134
92. Spleen showing normal histological picture. H&E. X 200.	134
93. Liver showing normal architecture of control negative group X 40.	135
94. Liver showing internalization of fluorescein labeled NPs, group 8, 1hr after inoculation (arrows) X 40.	135
95. Liver showing mild internalization of fluorescein labeled NPs, group 8, 24hrs after inoculation (arrows) X 40.	135
96. Liver showing internalization of fluorescein labeled NPs, group 8, 72hrs after inoculation (arrows) X 40.	135
97. Brain showing normal architecture of control negative group X 40.	136
98. Brain showing internalization of fluorescein labeled NPs, group 6, 24hrs after inoculation (arrows) X 40.	136
99. Brain showing mild internalization of fluorescein labeled NPs, group 6, 72hrs after inoculation (arrows) X 40.	136
100. Brain showing internalization of fluorescein labeled NPs, group 6, 10d. after inoculation (arrow) X 40.	136
101. Brain showing internalization of fluorescein labeled NPs, group	137

9, 24hrs after inoculation (arrows) X 40.	
102. Brain showing mild internalization of fluorescein labeled NPs, group 9, 72hrs after inoculation (arrow) X 40.	137
103. Brain showing internalization of fluorescein labeled NPs, group 9, 10d. after inoculation (arrow) X 40.	137
104. Brain showing internalization of fluorescein labeled NPs, group 7, 1hr after inoculation (arrows) X 40.	138
105. Brain showing internalization of fluorescein labeled NPs, group 7, 24hrs after inoculation (arrow) X 40.	138
106. Brain showing mild internalization of fluorescein labeled NPs, group 7, 72hrs after inoculation (arrows) X 40.	138
107. Brain showing internalization of fluorescein labeled NPs, group 7, 10d. after inoculation (arrow) X 40.	138
108. Brain showing internalization of fluorescein labeled NPs, group 8, 1hr after inoculation (arrow) X 40.	139
109. Brain showing internalization of fluorescein labeled NPs, group 8, 24hrs after inoculation (arrow) X 40.	139
110. Brain showing mild internalization of fluorescein labeled NPs, group 8, 72hrs after inoculation (arrows) X 40.	139
111. Brain showing internalization of fluorescein labeled NPs, group 8, 10d. after inoculation (arrows) X 40.	139
112. Brain showing internalization of fluorescein labeled NPs, group 10, 1hr after inoculation (arrows) X 40.	140
113. Brain showing internalization of fluorescein labeled NPs, group 10, 24hrs after inoculation (arrows) X 40.	140
114. Brain showing mild internalization of fluorescein labeled NPs, group 10, 72hrs after inoculation (arrows) X 40.	140
115. Brain showing internalization of fluorescein labeled NPs, group 10, 10d. after inoculation (arrow) X 40.	140

116. Spleen showing internalization of fluorescein labeled NPs, group 7, 1hr after inoculation (arrow) X 40.	141
117. Spleen showing internalization of fluorescein labeled NPs, group 8, 24hrs after inoculation (arrow) X 40.	141
118. Spleen showing mild internalization of fluorescein labeled NPs, group 8, 72hrs after inoculation (arrow) X 40.	141
119. Spleen showing internalization of fluorescein labeled NPs, group 9, 1hr after inoculation (arrows) X 40.	141
120. Spleen showing internalization of fluorescein labeled NPs, group 10, 1hr after inoculation (arrows) X 40.	142
121. Spleen showing internalization of fluorescein labeled NPs, group 10, 24hrs after inoculation (arrows) X 40.	142
122. Liver showing thrombus with high mononuclear cells infiltrations (arrow) of heat stressed group. H&E. X 200.	143
123. Liver showing diffuse hemorrhage with hemosiderin pigment (arrow) of heat stressed group. H&E. X 200.	143
124. Liver showing mild mononuclear cell infiltration of heat stressed group injected with NPs. H&E. X 400.	143
125. Liver showing very mild congestion and less inflammation of prophylactic group. H&E. X 200.	143
126. Biochemical profile of enzymatic antioxidants biomarkers of control group and heat stressed group.	144
127. Biochemical profile of oxidative byproducts of control group and heat stressed group.	144
128. Concentrations of TAC and 8-OHdG of control group and heat stressed group.	145
129. Biochemical profile of enzymatic antioxidants biomarkers of heat stressed group and prophylactic group.	145
130. Biochemical profile of oxidative byproducts of heat stressed	146

group and prophylactic group.	
131. Concentrations of TAC and 8-OHdG of heat stressed group and prophylactic group.	146
132. Biochemical profile of enzymatic antioxidants biomarkers of heat stressed groups with and without NPS injection 2, 24 and 48hrs post injection.	147
133. Biochemical profile of oxidative byproducts of heat stressed groups with and without NPS injection 2, 24 and 48hrs post injection	147
134. Concentrations of TAC and 8-OHdG of heat stressed groups with and without NPS injection 2, 24 and 48hrs post injection.	148

SUMMARY AND CONCLUSION

Synthesis of nanoparticles with unique characters able to overcome the disadvantages of traditional antioxidants as well as being safe on cells when applied *in vitro* and *vivo* is the main aim of this study. Novel ultrafine spherical yttrium oxide nanoparticles with particle size of 7.78 nm and average particle size 149.5 nm with surface charge of +29.4 were successfully synthesized by a low temperature coprecipitation method. Synthesized NPs characters like thermal profile, particle size, crystalline structure, morphological characters and surface charge were revealed by variable characterization tools as TGA, XRD, DLS, ZP and TEM. Also, the catalytic activity of yttria NPs was examined towards oxidation reaction of hydroquinone to benzoquinone in the presence of the hydrogen peroxide solution which revealed powerful highly specific catalytic activity extend to 48hrs. Synthesized NPs showed degree of agglomeration which will only effect on its antioxidant efficacy but also increase toxicity, so some of attempts performed to solve the problem of agglomeration and increase hydrophilicity of particles.

Grafting polymeric shell on surface of NPs by AA and EGMP polymers considered the most successful trials to produce hydrophilic well dispersed yttria NPs stable for one week and valid for wide range of biomedical applications, N-fluorescein Acrylamide added to shell in polymerization mixture as fluorescent material help in tracking the NPS *in vitro* and *vivo* as novel approach in diagnosis of NPs in cell culture and animal tissues.

The cytopathic effect and survival rate of polymerized NPs *in vitro* on 96-well plate of mouse normal hepatocyte cell line

Summary and Conclusion

with four replicate for each concentration of two polymerized NPS with AA and EGMP at wide range of serial dilution for each, NPS capped by AA (1.25-1000 $\mu\text{g/ml}$ PBS) and NPS capped by EGMP (10-1800 $\mu\text{g/ml}$ PBS), in this assay, the particles capped by AA showed cytotoxicity at low concentration of 10 $\mu\text{g/ml}$ at which loss its normal spindle shape of control cells being to sloughed and detached over time of examination. On the other hand, the particles capped by EGMP didn't show toxicity even at highest concentration 1800 $\mu\text{g/ml}$ that cells keep the same morphological characters compared with control ones.

24hrs post incubation, cell line plate which incubated with two polymerized NPS with AA and EGMP observed with fluorescence microscope detecting green internalized particles representing the penetration of cell membrane and well distribution of particles intracellular. The survival rate of incubated cell culture with two polymerized NPs with AA and EGMP 24hrs post incubation calculated by MTT assay, survival rate of cell line incubated with AA ranged from 99.9 to 100% at concentration ranged from 1.25 to 8 $\mu\text{g/ml}$ and ranged from 1.67 to 0.59% at concentration ranged from 10 to 1000 $\mu\text{g/ml}$ but survival rate of cell line incubated with EGMP ranged from 99.5 to 99.8% at concentration ranged from 10 to 1800 $\mu\text{g/ml}$.

Depending on *in vitro* results which help in trendy determining of toxic and safe doses, two experiments applied *in vivo*, one to detect toxicity of NPs capped by AA and EGMP at high concentration and another to detect the biocompatibility and bio distribution of EGMP capped NPs. At the first trial, high toxic doses (10, 30 and 50mg EGMP

Summary and Conclusion

capped NPs group 1-3) and (2 and 1mg AA capped NPs group 4 and 5) administered intravenous via tail vein for five groups of Sprague-Dawley rats weighed 150g Bwt each consisted of 7 animals and one control group, tissue specimens were collected from all experimental NPs groups after scarifying the animals from targeted organs like liver, spleen, kidney and brain collected at time interval 12, 24, 72hrs and 10d after injection of NPs for histopathological examination as well as fluorescence imaging of NPs in tissues.

The liver is known to be a major immunological organ affecting systemic responses in animals in response to xenobiotics, Adverse changes were detected in liver ranged from mild changes as congestion, sinusoidal cell activation and hydropic degeneration in group 1, 2 and 4 to moderate degree in group 3 ended with severe changes in group 5, 12hrs post injection and portal fibroblasts and oval cells hyperplasia was observed in a mild degree in group 1, 4, 5 72hrs post inoculation, the morphological changes begin 12hrs after injection extending 24 and 72hrs declined 10days to be mild changes in most of experimental groups. The differences in hepatic response between EGMP and AA capped NPs not only dose dependent but also the solubility and hydrophilicity effect on the toxicity and bio distribution of NPs, our study noticed decline on severity of lesions even at high toxic doses which referred to surface modification of NPs by core shell polymerization as key factor assess in reducing extension of adverse effects overtime of *in vivo* experimentations of NPs.

Our study able to record adverse changes in brain architecture after high toxic doses injected, adverse changes were detected in brain ranged from very mild neuronal

Summary and Conclusion

degeneration and necrosis was observed in group 1 to mild degree in group 2 and group 3 and in severe degree in group 4 with focal neuronophagia and one hemorrhagic area were observed in group 3. Also, mild degree of neurophagia and gliosis in group 1 and 2 and in severe degree in group 3, 4 and 5 with severe sub meningeal hemorrhage and congestion was observed in group 4 and mild acute meningitis was observed in group 5 extended 72hrs and 10days post injection, the severity of response between EGMP and AA capped NPs is clear that severe sub meningeal hemorrhage was observed in group 4 matched with the effect of solubility and hydrophilicity on the toxicity of NPs. The spleen was normal in all experimental groups 12hrs post injection and showed lymphocytic cells depletion varied from mild to moderate degree in all experimental groups 24hrs and 72hrs post inoculation respectively to be mild lymphoid hyperplasia 10days post inoculation in all experimental groups as well as no pathological changes observed in kidney.

Intracellular localization and distribution of fluorescence labeled NPs in tissues was detected by fluorescence microscope 12, 24, 72hrs and 10days after NPs injection. NPs group1 (dose 10mg) were identified in liver specimens 12hrs after inoculation absent after that while in group 2 (dose 30mg) present 24 and 72hrs after injection absent before and after these times respectively, group 3 (dose 50mg) and 4 (dose 2mg) detected 12 and 24hrs post inoculation absent after that while in group 5 (dose 1mg) detected only 12hrs post inoculation not present after that , The NPs was detected in spleen tissues in all groups 12, 24 and 72hrs after inoculation absent after that. Our results revealed brain localization of injected NPs in group 1 was identified in brain tissues 12, 24

Summary and Conclusion

and 72hrs after inoculation absent after that while in group 2 not able to pass BBB before 72hrs and 10d , group 4 and 5, NPs detected 12 and 24hrs post inoculation absent after that. Those results showed that EGMP NPs group1 at lowest dose 10mg enter the brain earlier than group 2 at dose 30mg revealing dose differences affecting cellular uptake of NPs as well as solubility of NPs as in group 4 and 5 which not able to persist more than 24hrs post injection differing from EGMP capped NPs, fluorescence microscope investigation of the polymerized YNPs were completely absent in kidney tissues in all groups all over the time.

The second *in vivo* trial performed to determine the biocompatibility and biodistribution of polymeric nanomaterials based on EGMP only in low doses injected intravenous via tail vein for five groups of Sprague-Dawley rats weighed 150g Bwt each consisted of 7 animals, groups 6-10 at doses 0.02, 0.066, 1.0, 0.02 and 0.1mg respectively. The regular findings of liver immune response against any foreign materials were observed by light microscope mainly mononuclear cell infiltrations as macrophage ranged from mild degree in NPs group 6, 9, 7 and 10, and in a moderate degree in group 8 1hrs after injection extend in all experimental groups 24hrs post injection without significant variation in the degrees of severity between groups and finally decline to mild and very mild changes 72hrs and 10 days post injection as those results referred to high solubility and hydrophilicity of NPs as well as the biodistribution and biocompatibility depends mainly on dose of administration and surface characters of NPs.

Summary and Conclusion

Histopathological studies were conducted on brain, spleen and kidney sections harvested 1hr, 24,72hrs and 10 days post injection with EGMP capped NPs, all groups revealed no remarkable changes that it is the first study recorded no pathological effects at dose up to 1mg. Intracellular localization and distribution of fluorescence labeled EGMP was detected by fluorescence microscope 1hr, 24, 72hrs and 10days after NPs injection, NPs were absent in liver tissues in group 6, 7, 9 and 10 at all time intervals while present in group 8 at 1hr after inoculation but mildly present 24,72hrs after inoculation. The NPs was detected in spleen tissues in group 7 at low level 1hr after inoculation absent after that, group 8 NPs present mildly 24, 72hrs after inoculation absent before and after that while group 9 NPs mildly present 1hr after injection and 1hr, 24hrs in group 10 and completely absent in group 6, those records referred to greater extension of polymeric coating on surface of NPs resulted in avoiding ready recognition and lateness of uptake of circulating particles in blood by reticuloendothelial organs.

Our study success to record NPs localization in brain at all times intervals at all doses 1hr, 24, 72hrs persist till 10days post injection but the lowest dose (0.02mg) of group 1 detected 24hrs extend to 10days post injection, the lateness in reticuloendothelial uptake of injected NPs due to proper highly extended surface coating give high chance to NPs to remain longer time circulating in blood subsequently ability to pass to brain tissues. The polymerized YNPs with EGMP were completely absent in kidney tissues in all groups all over the time as the same results recorded in trial of toxicity detection of NPs.

Summary and Conclusion

The third *in vivo* trial performed to determine the antioxidant efficacy of EGMP capped YNPs, heat stressed model performed on total of 21 rats (heat stressed group) and exposed to 48°C and relative humidity 50±15% for 15 min two times at 5 min interval and assuring stress by measuring internal body temperature (39±1.5 °C) and then differentiated to two subgroups 1(heat stressed with NPs injection) and 2 (heat stressed without NPs injection), first one injected with 0.2mg of EGMP capped YNPs and another group not injected with NPs post heat stress exposure as well as another group consisted of 7 rats injected with 0.2mg of NPs pre heat stress exposure (prophylactic group) with the same conditions of heat stressed group. Different samples of blood, serum and tissue homogenate samples were collected at time intervals along the period of experiment to measure the oxidative biomarkers like enzymatic antioxidants (SODs, GPX, GST, GR and TAC) and oxidative byproducts (MDA, PC and 8-OHdG) and liver specimens histopathologically examined 2hrs post NPs injection, prophylactic group and heat stressed ones.

In heat stressed group, significant increase was recorded ($P>0.05$) in SODs activity in erythrocyte lysate and TAC concentration in serum as well as PC and MDA concentrations in tissue homogenate of liver and spleen and 8-OHdG in serum compared with control group however other enzymatic antioxidants as GST, GPX and GR activities erythrocyte lysates revealed significant decrease ($P<0.05$). In prophylactic group, significant decrease ($P<0.05$) in SODs activity and TAC revealed as well as PC and MDA in tissue homogenate of liver and spleen and 8-OHdG concentrations in

Summary and Conclusion

serum however other enzymatic antioxidants as GST, GPX and GR activities revealed significant increase ($P>0.05$).

Significant increase ($P>0.05$) in TAC concentration, GST, GPX and GR activities as well as significant decrease ($P<0.05$) in SODs activity, PC, MDA and 8-OHdG concentrations was recorded in heat stressed group with NPs injection 2, 24 and 48hrs post injection compared with heat stressed group without NPs injection at the same time intervals.

Histopathological findings revealed improvement of morphological characters of prophylactic group and heat stressed ones 2hrs post NPs injection compared with heat stressed ones.

The present study was concluded to

- Nanotechnology based theranostics for oxidative stress is successful alternative to traditional antioxidants, our study success in synthesis of unique yttria NPs with particle size of 7.78 nm and average particle size 149.5 nm with surface charge of +29.4 with high catalytic activity as those novel characters give the chance for wide range of biomedical applications.
- Core shell polymerization with EGMP polymer is successful novel approach for the first time to modify NPs characters, increase hydrophilicity, dispersity and control agglomeration over time, surface modification of synthesized NPs is the key factor in maximizing the beneficial effects rather than toxic ones especially *in vitro* and *in vivo* applications. Also, labeling of NPs with fluorescein is successful way in tracking of NPs in

Summary and Conclusion

tissues and considered cheap and safe alternative method of diagnosis.

- The cytopathic and survival rate of core shell AA and EGMP based YNPs were detected *in vitro* to be concluded that the difference in response between two NPs depending on the hydrophilicity and surface charge of NPs mainly.
- The morphological characters of NPs as surface characters, size, shape, surface charge as well as dose and route of administration are collectively factors not only effect on toxicity of NPs *in vivo* but also on biodistribution and compatibility of NPs as this study first recorded the ability of EGMP capped NPs to pass BBB as real challenge at all low doses up to 1mg with persistence up to 10days without pathological changes. Also, depending on those novel characters, lateness in reticuloendothelial uptake of circulating NPs in blood give the chance to enter other organs is achieved.
- The synthesized NPs success to perform double purposes, one prophylactic and another therapeutic antioxidant one on heat stressed lab animal model to overcome the wide limitations of traditional antioxidants and able to face the cascade reaction of free radicals generation and rescue cells from harmful effect.