CHAPTER 3

Influence of FGF-2 on the antioxidant status in tissues during various stages of tail regeneration in gekkonid lizard, *Hemidactylus flaviviridis.*

INTRODUCTION

The process of epimorphic regeneration has been widely studied in Urodele amphibians (Boilly *et al*, 1991, Ohuchi *et al.,* 1994, Nye *et al*, 2003) In Urodeles, immediately after amputation the process of healing of the wound begins. Within first hour after amputation, epithelial cells begin to migrate as a sheet to cover the exposed mesenchymal tissues (Bryant *et al.,* 2002) Along with this, a large number of damaged and injured cells become apoptotic and are cleared from the site of amputation Further, the amputated site becomes inflammated and the healing process starts. Besides, within few days, there is genesis of a population of undifferentiated, proliferating cells that are able to restore the lost appendage All these early events of epimorphic regeneration are accompanied by the production of free radicals or reactive oxygen species (ROS), which are by-products of metabolic processes During the early inflammatory phase of wound healing, polymorphonuclear leukocytes and macrophages infiltrate the wounded tissue. Once activated, they produce large amounts of reactive oxygen species (ROS) to kill bacteria and prevent infection as has been observed by Cho *et al.,* (2001) and Weiss (1989) Although this process is beneficial, increased levels of ROS can inhibit cell migration and proliferation and can even cause tissue damage by creating DNA strand breaks and depleting NAD stores (Schacter *et al.,* 1988; Schraufstatter *et al*, 1986; Weiss, 1989). In extreme cases this leads to severe damage and necrosis (Duvall and Wyllie, 1986), while less severe oxidative overload causes lipid peroxidation and alterations of metabolic pathways (Slater, 1984, 1987) While inflammation is responsible for elaboration of cytokines and growth factors that subsequently trigger the healing process, leukocytes are also potent sources of tissue degrading enzymes and enzymes contributing to generation of reactive oxygen metabolites

Hence, the cells possess an arsenal of defense and repair mechanisms to deal with the potentially dangerous ROS to which they are continuously exposed as by-products of oxidative metabolism (Halliwell, 1984) These defense mechanisms include many antioxidant enzymes and vitamins (Sies, 1993), but the major antioxidant enzymes are

superoxide dismutase (SOD) which reduces O_2 to H_2O_2 ,and catalase (CAT) which reduces H_2O_2 to H_2O. Apart from enzymes, there are small molecules such as glutathione, which also help in detoxifying ROS Further, glutathione may react directly with ROS and also serve as an electron donor for glutathione peroxidase, which reduces hydrogen and lipid peroxides (Meister, 1988, Gaetani *et al.,* 1989). In addition, most growth factors and cytokines generate ROS at or near plasma membrane, which become target for peroxidation process. By and large ROS are considered detrimental for the cells, but recent research has stated that they can also be beneficial (Chandel *et al.,* 1999, Finkel, 1998)

And so, as against the notion that ROS are harmful for the cells, accumulating evidence from *in vitro* studies suggests that ROS are not only injurious by-products of cellular metabolism but also essential participants in cell signaling and regulation (Pryor *et al.,* 1991; Finkel, 1998a) However, a significant event to occur during epimorphic regeneration in amphibians is the reorganization of matrix. This reorganization requires dissolution of cell-cell adhesion and clearance of dead and damaged cells by apoptosis. All these processes are accompanied by production of ROS In relation to this, the mitochondrial ROS mediate cell signaling, particularly with regard to the regulation of apoptosis in Jurkat human T cells and peripheral blood lymphocytes (Banki *et al* , 1999), in endothelail cells (Li *et al* , 1999), and other cell lines (Cai and Jones, 1998, Chandel *et al.,* 1999a, Von Harsdorf *et al.,* 1999; Lee *et al.,* 2000). Further, a number of growth factors that bind with receptor tyrosine kinases (RTKs), for example, FGF-2, have been shown to generate intracellular ROS essential for mitogenic signaling. Similarly, in mammalian system, the oxidases associated with plasma membrane generate the oxidants via growth factors and/or cytokines (Meier *et al.,* 1989, Satriano *et al.,* 1993; Griendling *et al.,* 1994, Krieger-Brauer *et al.,* 1995, Sundaresan *et al.,* 1995; Thannickal and Fanburg, 1995) Besides, arachidonic acid metabolism which is an intermediary in the FGF-2 signaling, particularly involving the lipooxygenase (LOX) pathway, leads to leukotriene synthesis, that has been reported to generate ROS in human platelets (Singh *et al.,* 1981), in macrophages (Lim, *et al* , 1983), in JB-6 cells (Nakamura *et al.,*1985), and in kidney (Baud and Ardaillou, 1986) This pathway is in turn involved in the FGF-2 induced vascular endothelial cell proliferation *in vitro* (Dethlefsen *et al* , 1994) Thus, during healing of the wound and subsequent stages of epimorphic regeneration, the process of angiogenesis is mediated partly by FGF-2, involving arachidonic acid metabolism However, another characteristic happening in the initial events in the epimorphic regeneration is the rapid proliferation of cells

Recently, ROS have gained significant attention because of their role in cell signaling involved in apoptosis and/or proliferation. Early events of epimorphic regeneration include apoptosis of damaged cells, healing of the wound and the subsequent proliferation of

pleuripotent blastemal cells Further, the FGF-2 signaling pathway involves PGE2 synthesis, which is yet another source of ROS production *in vitro* (Kawaguchi *et al.,* 1995, Kage *et al.,* 1999, Majima *et al.,* 2000) All these events are associated with over production of ROS Moreover, several reports have established relation between FGF-2 and ROS (Yang and Bono, 1997; Kage *et al* , 1999) Moreover, FGF-2 is known to evoke antioxidant enzymes to take care of the excess ROS production (Yong-Fang and Yong-Jie, 2001) The major antioxidant enzymes that come into action as a first line of defense are SOD and CAT In addition to this, glutathione is also an important molecule that plays role in detoxification of ROS Similarly, since ROS are produced near plasma membrane which is prone to peroxidation and hence level of malondialdehyde (MDA) is considered an important indicator of the extent of lipid peroxidation (Borrello *et al.,* 1985) Therefore, it was thought worth studying the influence of extraneous FGF-2 on the antioxidant enzymes in the tissues during tail regeneration in lizard, *Hemidactylus flaviviridis.*

MATERIAL AND METHODS

A total of ninety lizards of both the sexes were selected and acclimated in the laboratory, at 30 ± 2^0 C, for a week prior to experiments. The animals were divided into six groups of fifteen animals each and were treated as follows:

Group I· The animals of this group were injected intraperitoneally with 0 6% saline and served as control for all the experimental groups.
Group II· Animals were given FGF-2 intraperitoneally (25µg/kg b wt)
Group III: Lizards were administered antiFGF-2 intraperitoneally (25mg/kg b wt.)
Group IV· Animals were injected with FGF-2 *in loco* (12 5 µg/kg b wt)
Group V These animals were injected with antiFGF-2 *in loco (*12 5 mg/kg b wt.)

All the drugs were prepared in 0 6% saline, fresh before use Each of the drugs was administered at a dosage of 0 05ml/animal intraperitoneally while the *in loco* injection was given at a dose of 0 025ml/ animal The treatment in each group started four days prior to amputation and was continued till the animals reached differentiation stage The drugs were administered every alternate day

From each group, five animals, which attained the WE stage on the same day, were selected and sacrificed. The superoxide dismutase (SOD), catalase (CAT) activity and levels of reduced glutathione (GSH) and malondialdehyde (MDA) were estimated in blood and tissues *viz.* liver, kidney and intestine of the animals Further, the animals, which attained BL

stage on the same day, in all the groups, were selected and sacrificed. The antioxidant enzymes' activities as well as GSH and MDA levels were determined in blood and tissues. Finally, animals in all the groups, which attained DF stage simultaneously, were sacrificed to determine the SOD and CAT activities in blood and tissues The levels of GSH and MDA were also quantified in blood, liver, kidney and intestine.

STATISTICAL ANALYSIS:

The data was analyzed by One Way ANOVA with Duncan's Multiple Range Test. The values are expressed as Mean ± SE A 'p' value of 0 05 or less was considered statistically significant.

RESULTS

Effect of FGF-2 on antioxidant status:

WH stage: The levels of reduced glutathione were significantly higher ($p \leq 0.01$) in both the experimental groups (IP and *in loco* treatments) compared to control group (Table 3 1, Figure 3 1) However, the levels of GSH were higher in blood than in tissues, in control as well as experimental groups The extent of tissue damage was significantly lower in experimental groups as is evident from the lower levels of MDA in blood and tissues (Figure 3 2). The activity of SOD and catalase was found to be significantly elevated ($p \leq 0.01$) in animals of experimental group as compared to animals of control group (Table 3 2, Figures 3 3, 3.4)

BL stage: FGF-2 treatment was found to elevate the GSH levels significantly as compared to control group, while the MDA levels were significantly decreased ($p \leq 0.01$) (Table 3.3, Figures 3 5, 3 6). The SOD and CAT activities showed significant increases ($p \leq 0.01$) in experimental animals in comparison to control group (Table 3 4, Figures 3 7, 3 8).

DF stage: The GSH and MDA levels of control and experimental groups were comparable during DF stage (Table 3 5, Figures 3.9, 3 10) However, the SOD activity was found to be significantly higher ($p \leq 0.01$) in blood and liver of experimental animals, while in kidney and intestine the difference in the enzyme activity between treated and control animals, was not statistically significant (Figure 3.11) Similarly, CAT activity was not significantly altered in intestine of treated animals, while in blood, liver and kidney, it was significantly elevated ($p \leq 0.05$) (Table 3.6, Figure 3 12)

Effect of antiFGF-2 on antioxidant status:

WH stage: The levels of GSH were significantly lowered ($p \leq 0.01$) in antiFGF-2 treated groups (both IP and *in loco* treatment), while there was a significant increase ($p \leq 0.01$) in the MDA levels in experimental group (Table 3.7, Figures 3.13, 3.14) The activity of the two enzymes, SOD and CAT was found to be significantly lowered ($p \leq 0.01$) in antiFGF-2 treated lizards (Table 3.8, Figures 3.15, 3.16).

BL stage: Treatment with antiFGF-2 decreased levels of GSH significantly ($p \leq 0.01$), while MDA levels were elevated as compared to control group (Table 3.9; Figures 3.17, 3.18) Further, the activity of the enzymes, SOD and CAT, was found to be significantly lowered ($p \leq 0.01$) in experimental animals compared to control animals (Table 3.10; Figures 3.19, 3.20)

DF stage: Administration of antiFGF-2 showed no influence on the GSH levels in tissues of regenerating tail, while MDA levels were found to be higher as compared to control group (Table 3.11, Figures 3.21, 3.22) The SOD activity showed a significant decrease ($p \leq 0.05$) in blood, liver and intestine of experimental animals treated intraperitoneally than control animals (Table 3.12, Figure 3.23), while those treated *in loco* showed a still more significant decrease ($p \leq 0.01$) in SOD activity in blood and liver only. Similarly, CAT activity was significantly lowered ($p \leq 0.01$) in blood and liver in both, IP and *in loco*, treatments (Figure 3.24)

DISCUSSION

The present study has indicated significant alterations in tissue antioxidant profile during the process of tail regeneration in wall lizard, *Hemidactylus flaviviridis* The production of ROS was estimated indirectly at three crucial stages of tail regeneration *viz.* WE stage, BL stage and DF stage The formation of a functional WE is a very crucial and rate limiting step for the regeneration of tail Several processes occur simultaneously to ensure accurate formation of WE, one such process is the healing of wound. Wound healing is a complex and intricate process, which initiates in response to injury that restores the function and integrity of damaged tissue The healing involves continuous cell–cell and cell–matrix interactions that allow the process to proceed in overlapping phases comprising inflammation, proliferation and remodeling Alterations in any phase of wound healing can contribute to defective healing as reviewed by Clark (1993) Moreover, Martin (1996) reported that ROS are

produced in response to injury, which impedes the healing process by causing damage to cellular membranes, DNA, proteins and lipids. However, cells possess defense mechanisms against these ROS, which include antioxidant enzymes like SOD, CAT and other indicators of the damage caused to the tissues due to ROS, such as levels of reduced glutathione (GSH) and malondialdehyde (MDA) The current study showed that administration of FGF-2 increased the activities of SOD and CAT in blood, liver, kidney and intestine at the WE and BL stages of tail regeneration as compared to controls, while activities of theses enzymes were significantly inhibited after administration of antiFGF-2 at both the stages Since, SOD, which scavenges oxygen radicals or inhibits lipid peroxidation, is adapted to be induced/increased under oxygen toxicity in humans (Niwa, 1989), the observed increase in SOD activity in the regenerated wound tissue during early stages of tail regeneration might be due to enhanced level of superoxide radicals (O_2) in the lizards treated with FGF-2 Further, SOD is an inducible enzyme and its activity depends on O_2 concentration Injury causes recruitment of neutrophils and macrophages at the site of injury, which in turn produces ROS in mammals (Steiling *et al.,* 1999). A similar mechanism of ROS genesis at the site of amputation cannot be ruled out and that subsequently might have resulted into enhanced activity of SOD Thus, the elevated activity of SOD might have contributed to the early healing of the wound in FGF-2 treated animals, as has also been reported by several investigators SOD accelerates the healing of the wound in pigs (Weinstein *et al.,* 1989), in rats (Foschi *et al.,* 1990, Silaeva *et al.,* 1990) and also in culture (Slater, 1984, Martin, 1996) Additionally, few studies have also shown that FGF-2 increases the activity of SOD in rats (Mattson *et al.,* 1995; Hou *et al.,* 1997)

Furthermore, the delayed healing, observed in antiFGF-2 treated lizards, could be due to the elevated levels of ROS, which might have got accumulated due to inadequate detoxification of ROS by the decreased activity of the antioxidant enzymes In addition, it has also been shown that oxygen free radicals play an important role in delaying ischemic wound healing in rats (Trabucchi *et al.,* 1988, Senel *et al.,* 1997) Moreover, altered status of antioxidants has been reported to delay healing of wound in diabetic and aged rats (Hallberg *et al*, 1996; Mezzett *et al.,* 1996, Rasik and Shukla, 2000) Nonetheless, in FGF-2 supplemented lizards the healing process was accelerated, which could be due to the detoxification of ROS through elevated activities of antioxidant enzymes An increase in SOD activity in FGF-2 treated lizards during early events of tail regeneration was accompanied by an increase in CAT activity in tissues Catalase is the primary defense mechanism against oxidative stress resulting from low concentration of H_2O_2 as reported by Masaki *et al.,* (1998) in human fibroblasts and degrades H_2O_2 to water. However, the results were entirely opposing for the animals administered with antiFGF-2. Similarly, Bray *et al*, (1974) have also demonstrated that the decrease in the SOD activity in pancreas is associated with the decrease in the CAT

activity and H_2O_2 levels become elevated due to the decrease of CAT activity which in turn inhibit SOD activity Also, previous studies have reported that mucosal SOD activity decreases during ischemia and the decrease in SOD activity was related to CAT activity in rats (Tanaka *et al.,* 1993; Kaçmaz *et al.,* 1999; Tashima *et al.,* 2000, Ustündag *et al.,* 2000) Thus, the activities of SOD and CAT were found to be closely associated in the present study as well In addition, during DF stage the activity of SOD was elevated only in the blood and liver, while that of CAT was higher in the blood, liver and kidney of the FGF-2 treated animals Conversely, in the antiFGF-2 treated animals, the SOD and CAT activity was lowered in blood and liver at the DF stage Moreover, results suggested that the activities of both the enzymes decreased from WE to DF stage, from which it could be postulated that the generation of free radicals is maximum immediately after amputation and during healing of the wound. However, later on the production of ROS decreases and returns to the basal level. Another indicator of the generation of ROS is the formation of GSH moieties, which are involved in the detoxification of the ROS

It is well known that the major endogenous thiol-antioxidant in biological system is reduced glutathione (GSH). An increase in the GSH profile in the FGF-2 treated lizards has been found to be accompanied by a decrease in lipid peroxidation in the tissues at the WE and BL stages of tail regeneration Elevated levels of GSH in FGF-2 treated lizards during early events of tail regeneration provide protection to the regenerate from the undue damage to the tissues On the other hand, in antiFGF-2 treated animals, the GSH levels were found to be significantly lowered during WE and BL stages of tail regeneration. It has been reported that glutathione brings about detoxification of ROS in two ways, firstly by reacting directly with ROS and secondly by serving as an electron donor for glutathione peroxidase, which further reduces hydrogen and lipid peroxides (Gaetani *et al.,* 1989; Meister *et al.,* 1988), and the strongest protection against H_2O_2 to cells is provided by glutathione, the main intracellular reducing agent (Fernandez- Checa *et al.,* 1998). Furthermore, during DF stage the GSH levels were found to be comparable in the experimental and control animals. It might be concluded from the above results that the rise in GSH levels might be relative to the rate of proliferation, as proliferation is associated with the increase in the metabolic activities and a subsequent production of ROS In the early events when the rate of proliferation was higher, the GSH levels were higher, but as the proliferative activities were slowed down, the GSH levels were also diminished as seen during the DF stage. Similarly, Hou *et al.,* (1997) have also demonstrated that cell cultures treated with FGF-2 show higher levels of GSH, and inhibition of cell proliferation prevents the rise in GSH in FGF-2-treated cultures. Further, glial cells stimulated by FGF-2 up-regulate the antioxidant defenses and thus, support cell survival during oxidative stress Depletion of glutathione leads to an exponential increase in the levels of reactive oxygen species and a subsequent increase in

intracellular calcium levels, which is highly harmful for the cells (Maher *et al.,* 1999) Similarly, Yang and de Bono (1997) have also reported that FGF-2 causes an increase in intracellular reduced glutathione concentration All these defense systems against ROS protect the cells from the damage However, the levels of MDA, which is the most commonly used indicator of lipid peroxidation, were found to be significantly increased in lizards treated with antiFGF-2 at WE, BL and DF stages, and thus might have caused increased cell membrane damage This might have interfered with the cell signaling and thus could be the possible cause for the delayed regeneration of tail in the early phases in antiFGF-2 treated animals. However, lower levels of MDA in FGF-2 supplemented animals at WE and BL stages, might have proved advantageous as there could have been less damage to the plasma membrane of the cells due to ROS. Hence, such protected cells might have proceeded through the normal proliferative activities and contributed to the increase in the rate of regeneration of the tail. Also, the activities of antioxidant enzymes and levels of MDA might have been related as stated by Murphy (2001), who observed that insufficient superoxide dismutase (SOD) activity can lead to lipid peroxidation and cellular damage However, during DF stage in the FGF-2 treated animals the MDA levels were comparable with those of control animals.

In conclusion, it could be stated that the activity of SOD and CAT significantly increased in the tissues in the FGF-2 treated animals, while in antiFGF-2 treated animals the activity of these enzymes decreased at all the stages of tail regeneration Though, the GSH levels were higher in the FGF-2 treated animals at WE and BL stages, at DF stage however, the treated animals recorded only basal levels of GSH Conversely, the GSH levels were lowered in the antiFGF-2 treated animals at WE and BL stages, but were comparable at DF stage However, the MDA levels were decreased in FGF-2 treated animals at WE and BL stages, but the levels became comparable with control animals at DF stage. Furthermore, the animals administered with antiFGF-2 showed higher MDA levels at all the stages of tail regeneration Thus, FGF-2 could be an important growth factor helping cells to cope up with the oxidative stress Moreover, increased resistance to oxidative stress mediated by growth factors is likely to be biologically relevant, and may open new avenues for therapeutic protection against oxidative stress

TABLE 3 1. Levels of Reduced Glutathione (GSH) and Malondialdehyde (MDA) in tissues of FGF-2 treated and control wall lizards, *Hemidactylus flaviviridis*, at WE stage

Tissue	GSH (Blood- mg/dl of blood, Tissue- µg/g of tissue)		
	Control	IP FGF-2	IL FGF-2
Blood	307 218 ± 0 840[@]	380 833 ± 8 846**↑	370 343 ± 12 992**↑
Liver	450 025 ± 2.621	556 980 ± 19 139**↑	553 758 ± 15 177**↑
Kidney	433 265 ± 1 590	542.92 ± 12 336**↑	536 643 ± 13.365**↑
Intestine	416 968 ± 1 118	552 76 ± 14 31**↑	542 495 ± 16.113**↑

Tissue	MDA (Blood- n moles MDA formed/ g Hb; Tissue- n moles MDA formed/ g tissue)		
	Control	IP FGF-2	IL FGF-2
Blood	36 203 ± 1 023	25 278 ± 1 726**↓	26 78 ± 1 239**↓
Liver	24 8 ± 0 534	18 31 ± 0 651**↓	16 988 ± 0 783**↓
Kidney	24.233 ± 0 809	16 895 ± 1.211**↓	16.828 ± 0.850**↓
Intestine	23 965 ± 0 788	16 985 ± 0 929**↓	17 343 ± 0 948**↓

[@] Values are expressed as Mean ± SE, * p≤0 05, ** p≤0.01

TABLE 3 2 Superoxide dismutase (SOD) and Catalase (CAT) activities in tissues of (FGF-2 treated) and control wall lizards, at WE stage

Tissue	SOD (Blood- SOD units/g Hb/min, Tissue- SOD units/mg protein/min)		
	Control	IP FGF-2	IL FGF-2
Blood	262 923 ± 8.146[@]	428.085 ± 14.888**↑	439 205 ± 15 635**↑
Liver	0 397 ± 0.011	0.750 ± 0 019**↑	0 735 ± 0 019**↑
Kidney	0 325 ± 0 002	0 449 ± 0 013**↑	0 438 ± 0 014**↑
Intestine	0.308 ± 0.005	0 370 ± 0 009**↑	0.367 ± 0 007**↑

Tissue	CAT (Blood- K/g Hb Tissue- m moles H_2O_2 decomposed/sec/g tissue)		
	Control	IP FGF-2	IL FGF-2
Blood	1.853 ± 0 038	2 125 ± 0.072**↑	2 14 ± 0 066**↑
Liver	0.040 ± 0 002	0.066 ± 0 005**↑	0.069 ± 0 004**↑
Kidney	0 040 ± 0 002	0 075 ± 0 005**↑	0 074 ± 0 004**↑
Intestine	0 033 ± 0.002	0 065 ± 0 006**↑	0.066 ± 0 005**↑

[@] Values are expressed as Mean ± SE, * p≤0 05, ** p≤0.01

TABLE 3.3. Levels of Reduced Glutathione (GSH) and Malondialdehyde (MDA) in tissues in FGF-2 treated and control gekkonid lizards, at BL stage

Tissue	GSH (Blood- mg/dl of blood, Tissue- µg/g of tissue)		
	Control	IP FGF-2	IL FGF-2
Blood	282 038 ± 2.018[@]	364.188 ± 10 712**↑	373 475 ± 15 984**↑
Liver	448 808 ± 3.988	560.345 ± 10.199**↑	551.883 ± 12 692**↑
Kidney	417.468 ± 1.790	517 203 ± 10 883**↑	521 935 ± 13.366**↑
Intestine	327 355 ± 3 520	441 203 ± 11 937**↑	446 818 ± 13 670**↑

Tissue	MDA (Blood- n moles MDA formed/ g Hb; Tissue- n moles MDA formed/ g tissue)		
	Control	IP FGF-2	IL FGF-2
Blood	25 458 ± 0 533	16.668 ± 0.652**↓	17.593 ± 0 627**↓
Liver	27 038 ± 0 598	16.665 ± 0 847**↓	16 265 ± 0.685**↓
Kidney	20.38 ± 0.861	14 843 ± 1 236**↓	15 605 ± 1 060**↓
Intestine	18 343 ± 0 552	11 095 ± 1 241**↓	9 828 ± 1 264**↓

[@] Values are expressed as Mean ± SE, * p≤0 05, ** p≤0 01

TABLE 3 4 Activities of Superoxide dismutase (SOD) and Catalase (CAT) in tissues at BL stage in normal and FGF-2 treated lizards

Tissue	SOD (Blood- SOD units/g Hb/min; Tissue- SOD units/mg protein/min)		
	Control	IP FGF-2	IL FGF-2
Blood	279 965 ± 3 637[@]	509 738 ± 10 804**↑	534 563 ± 8 132**↑
Liver	0.469 ± 0 006	0 898 ± 0 012**↑	0 879 ± 0.011**↑
Kidney	0 441 ± 0.006	0 660 ± 0 013**↑	0 676 ± 0.016**↑
Intestine	0 371 ± 0 004	0 671 ± 0.009**↑	0 629 ± 0.011**↑

Tissue	CAT (Blood- K/g Hb, Tissue- m moles H_2O_2 decomposed/sec/g tissue)		
	Control	IP FGF-2	IL FGF-2
Blood	1 793 ± 0 012	1 98 ± 0.046*↑	2 028 ± 0 053**↑
Liver	0 032 ± 0 002	0 064 ± 0 003**↑	0 065 ± 0 001**↑
Kidney	0.029 ± 0 001	0 058 ± 0 008*↑	0.053 ± 0 006*↑
Intestine	0 03 ± 0 001	0 059 ± 0.005**↑	0 056 ± 0.006**↑

[@] Values are expressed as Mean ± SE, * p≤0 05, ** p≤0 01

TABLE 3.5. Tissue levels of Reduced Glutathione (GSH) and Malondialdehyde (MDA) in tissues of FGF-2 treated and control gekkonod lizards at DF stage

Tissue	GSH (Blood- mg/dl of blood; Tissue- µg/g of tissue)		
	Control	IP FGF-2	IL FGF-2
Blood	262 288 ± 1.847[@]	259.535 ± 10 061	263 758 ± 11 465
Liver	418 09 ± 5 227	421.205 ± 18 674	420 543 ± 14 486
Kidney	372 233 ± 3 330	367 43 ± 8 033	368 728 ± 12.104
Intestine	364.73 ± 2.447	369 688 ± 8.314	362 978 ± 10 104

Tissue	MDA (Blood- n moles MDA formed/ g Hb, Tissue- n moles MDA formed/ g tissue)		
	Control	IP FGF-2	IL FGF-2
Blood	16.355 ± 0 761	11.885 ± 0.906	12 340 ± 0 943
Liver	15 248 ± 0.368	14 24 ± 1 260	13.72 ± 0.757
Kidney	14.883 ± 0 245	13 02 ± 2 21	13 98 ± 0.733
Intestine	12 58 ± 0 261	10 963 ± 1.448	11 875 ± 1 64

[@] Values are expressed as Mean ± SE, * p≤0 05, ** p≤0 01

TABLE 3 6· Activities of antioxidant enzymes, Superoxide dismutase (SOD) and Catalase (CAT), in control and FGF-2 treated lizards at DF stage

Tissue	SOD (Blood- SOD units/g Hb/min; Tissue- SOD units/mg protein/min)		
	Control	IP FGF-2	IL FGF-2
Blood	252 92 ± 2 720[@]	349 753 ± 7 962**↑	344.303 ± 8 847**↑
Liver	0 410 ± 0 005	0 464 ± 0.007**↑	0 462 ± 0 009**↑
Kidney	0 388 ± 0 004	0 416 ± 0.012	0 421 ± 0 012
Intestine	0 335 ± 0 003	0 417 ± 0 010	0 416 ± 0 012

Tissue	CAT (Blood- K/g Hb, Tissue- m moles H_2O_2 decomposed/sec/g tissue)		
	Control	IP FGF-2	IL FGF-2
Blood	1 208 ± 0 015	1 83 ± 0 045**↑	1 698 ± 0 049**↑
Liver	0.021 ± 0.001	0 047 ± 0 007*↑	0.049 ± 0.006**↑
Kidney	0 021 ± 0 001	0.036 ± 0.004*↑	0 035 ± 0 004*↑
Intestine	0 021 ± 0 001	0 034 ± 0 005	0 033 ± 0.004

[@] Values are expressed as Mean ± SE, * p≤0 05, ** p≤0 01

TABLE 3 7. Difference in the Reduced Glutathione (GSH) and Malondialdehyde (MDA) levels in tissues of antiFGF-2 treated and control lizards at WE stage

Tissue	GSH (Blood- mg/dl of blood; Tissue- µg/g of tissue)		
	Control	IP FGF-2	IL FGF-2
Blood	313 08 ± 1.377[@]	291.12 ± 3 939**↓	291 658 ± 4 670**↓
Liver	438 34 ± 3 982	362.51 ± 12 890**↓	377 94 ± 17 682**↓
Kidney	432.178 ± 5.884	344 48 ± 12.789**↓	347 853 ± 9.633**↓
Intestine	415 64 ± 2.369	306.67 ± 13 458**↓	296.308 ± 12 792**↓

Tissue	MDA (Blood- n moles MDA formed/ g Hb, Tissue- n moles MDA formed/ g tissue)		
	Control	IP FGF-2	IL FGF-2
Blood	34.808 ± 0 979	48 463 ± 2 279**↑	49 196 ± 2 003**↑
Liver	28.403 ± 0 539	38.473 ± 1 433**↑	39 48 ± 1.355**↑
Kidney	22 313 ± 0 791	36 255 ± 1 058**↑	35 793 ± 1 150*↑
Intestine	20 77 ± 0.753	39 355 ± 1.095**↑	39.298 ± 1 310**↑

[@] Values are expressed as Mean ± SE, * p≤0 05, ** p≤0 01

TABLE 3 8 Superoxide dismutase (SOD) and Catalase (CAT) activities in the tissues of antiFGF-2 treated and control gekkonid lizards at WE stage.

Tissue	SOD (Blood- SOD units/g Hb/min; Tissue- SOD units/mg protein/min)		
	Control	IP FGF-2	IL FGF-2
Blood	297.258 ± 4 291[@]	146 04 ± 6 941**↓	165 315 ± 7.343**↓
Liver	0 480 ± 0 003	0 350 ± 0.003**↓	0 382 ± 0 004**↓
Kidney	0 381 ± 0.004	0 246 ± 0.005**↓	0 247 ± 0.004**↓
Intestine	0.276 ± 0 005	0.162 ± 0 007**↓	0.161 ± 0.006**↓

Tissue	CAT (Blood- K/g Hb, Tissue- m moles H_2O_2 decomposed/sec/g tissue)		
	Control	IP FGF-2	IL FGF-2
Blood	1.943 ± 0 016	1 465 ± 0 034**↓	1 47 ± 0 037**↓
Liver	0.037 ± 0 008	0.018 ± 0 001**↓	0 020 ± 0.001**↓
Kidney	0 029 ± 0 001	0.019 ± 0 002**↓	0 017 ± 0 002**↓
Intestine	0 024 ± 0 001	0 015 ± 0.002**↓	0 016 ± 0 002**↓

[@] Values are expressed as Mean ± SE, * p≤0 05, ** p≤0 01

TABLE 3 9 The levels of Reduced Glutathione (GSH) and Malondialdehyde (MDA) in tissues of control and antiFGF-2 treated lizards at BL stage

Tissue	GSH (Blood- mg/dl of blood; Tissue- µg/g of tissue)		
	Control	IP FGF-2	IL FGF-2
Blood	261 908 ± 11.046@	170 375 ± 8 958**↓	173 403 ± 7.716**↓
Liver	433 373 ± 10.298	281 023 ± 10 952**↓	258.785 ± 12.262**↓
Kidney	401 033 ± 6 653	256.458 ± 8 360**↓	274 615 ± 9.228**↓
Intestine	316 158 ± 1.337	267.328 ± 9 194**↓	259 715 ± 9 315**↓

Tissue	MDA (Blood- n moles MDA formed/ g Hb, Tissue- n moles MDA formed/ g tissue)		
	Control	IP FGF-2	IL FGF-2
Blood	25.893 ± 0 947	35.37 ± 1 135**↑	37.363 ± 1 681**↑
Liver	24.41 ± 1.059	38.933 ± 1 788**↑	39.093 ± 1 170**↑
Kidney	17 795 ± 0 864	24 955 ± 1 117**↑	23 99 ± 0 899**↑
Intestine	16.738 ± 0 822	23.323 ± 1 265*↑	23 308 ± 1 728*↑

@ Values are expressed as Mean ± SE, * p≤0 05, ** p≤0 01

TABLE 3.10 The activities of Superoxide dismutase (SOD) and Catalase (CAT) in antiFGF-2 treated and control regenerating lizards at BL stage

Tissue	SOD (Blood- SOD units/g Hb/min, Tissue- SOD units/mg protein/min)		
	Control	IP FGF-2	IL FGF-2
Blood	278 67 ± 5 729[@]	172 438 ± 9 883**↓	174 478 ± 8 228**↓
Liver	0 462 ± 0 002	0 194 ± 0 005**↓	0 192 ± 0.006**↓
Kidney	0.413 ± 0 002	0 150 ± 0 003**↓	0 145 ± 0 003**↓
Intestine	0 371 ± 0 004	0.168 ± 0 004**↓	0 166 ± 0 005**↓

Tissue	CAT (Blood- K/g Hb, Tissue- m moles H_2O_2 decomposed/sec/g tissue)		
	Control	IP FGF-2	IL FGF-2
Blood	1 169 ± 0 01	1 09 ± 0 010**↓	1 095 ± 0 011**↓
Liver	0 026 ± 0 001	0 015 ± 0 001**↓	0 016 ± 0.002**↓
Kidney	0 024 ± 0 001	0 012 ± 0 001**↓	0 012 ± 0 001**↓
Intestine	0 023 ± 0.002	0 012 ± 0 002*↓	0.011 ± 0 003*↓

[@] Values are expressed as Mean ± SE, * p≤0 05, ** p≤0 01

TABLE 3 11 Levels of Reduced Glutathione (GSH) and Malondialdehyde (MDA) in the antiFGF-2 treated and control lizards at DF stage of tail regeneration.

Tissue	GSH (Blood- mg/dl of blood, Tissue- μg/g of tissue)		
	Control	IP FGF-2	IL FGF-2
Blood	280 678 ± 5 453@	256.16 ± 12 201	260 475 ± 11 574
Liver	334.708 ± 7.558	337 49 ± 8 640	353 405 ± 9 561
Kidney	345 952 ± 5.908	362.465 ± 9 036	368 683 ± 9 913
Intestine	323 53 ± 4 543	328 553 ± 8 950	331.025 ± 13.826

Tissue	MDA (Blood- n moles MDA formed/ g Hb, Tissue- n moles MDA formed/ g tissue)		
	Control	IP FGF-2	IL FGF-2
Blood	13.838 ± 0 961	22 835 ± 1.881**↑	20 21 ± 1 949*↑
Liver	21 85 ± 0 605	37 475 ± 1 541**↑	38 03 ± 1 398**↑
Kidney	14 93 ± 0.846	25.11 ± 1 744**↑	24.988 ± 1 434**↑
Intestine	9 975 ± 0 598	17 413 ± 0 824**↑	18 65 ± 0 781**↑

@ Values are expressed as Mean ± SE, * p≤0 05, ** p≤0 01

TABLE 3 12 Activities of Superoxide dismutase (SOD) and Catalase (CAT) in the tissues of antiFGF-2 treated and normal lizards at DF stage of tail regeneration

Tissue	SOD (Blood- SOD units/g Hb/min; Tissue- SOD units/mg protein/min)		
	Control	IP FGF-2	IL FGF-2
Blood	276 183 ± 4 991[@]	171 748 ± 7.292**↓	168.683 ± 8 082**↓
Liver	0.398 ± 0 004	0 269 ± 0 008**↓	0.266 ± 0 006**↓
Kidney	0 385 ± 0.004	0.348 ± 0 019	0.361 ± 0.007
Intestine	0 290 ± 0 002	0 264 ± 0 006*↓	0 275 ± 0.006

Tissue	CAT (Blood- K/g Hb, Tissue- m moles H_2O_2 decomposed/sec/g tissue)		
	Control	IP FGF-2	IL FGF-2
Blood	1 223 ± 0 004	1 114 ± 0 003**↓	1 1165 ± 0 007**↓
Liver	0 025 ± 0 001	0.016 ± 0 001**↓	0.015 ± 0.001**↓
Kidney	0 018 ± 0 002	0 015 ± 0 002	0 013 ± 0 002
Intestine	0 011 ± 0 001	0.013 ± 0 002	0.017 ± 0 003

[@] Values are expressed as Mean ± SE, * p≤0 05, ** p≤0 01

Figure 3 1 Reduced glutathione (GSH) levels in the Blood (mg/dl) and Tissues(g/gm of tissue) in the FGF-2 treated and control lizards at WH stage

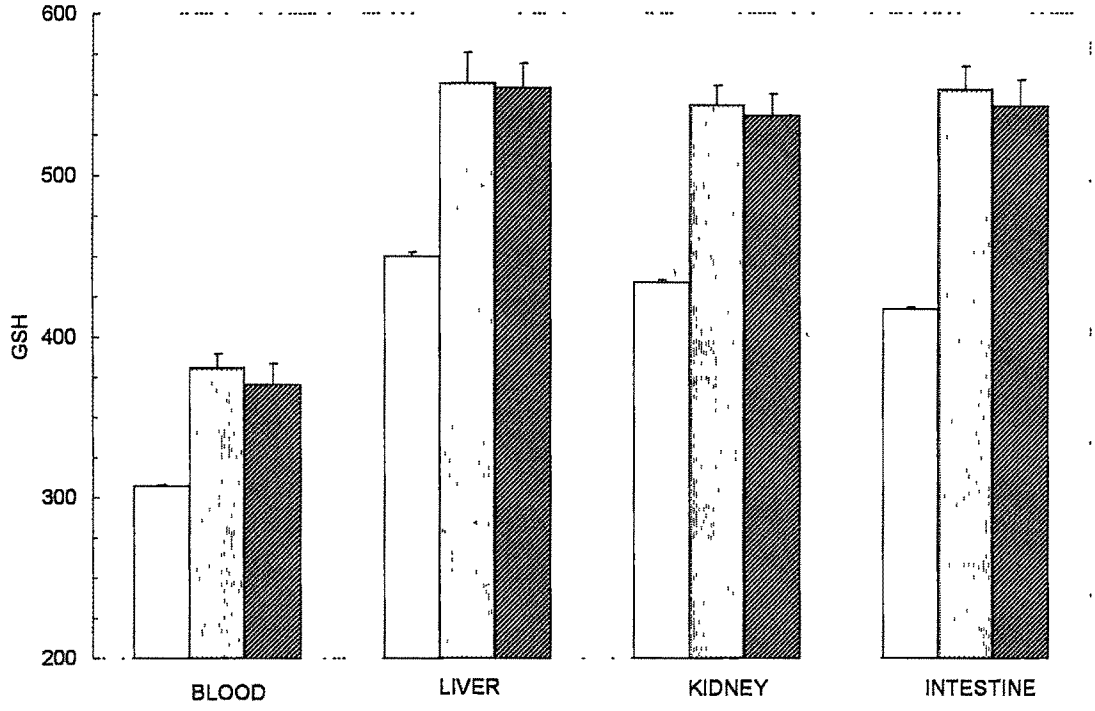

Figure 3 2 Malondialdehyde (MDA) levels in Blood (n moles MDA formed / gm Hb) and Tissues (n moles MDA formed/ gm Hb) in FGF-2 treated and control lizards at WH stage

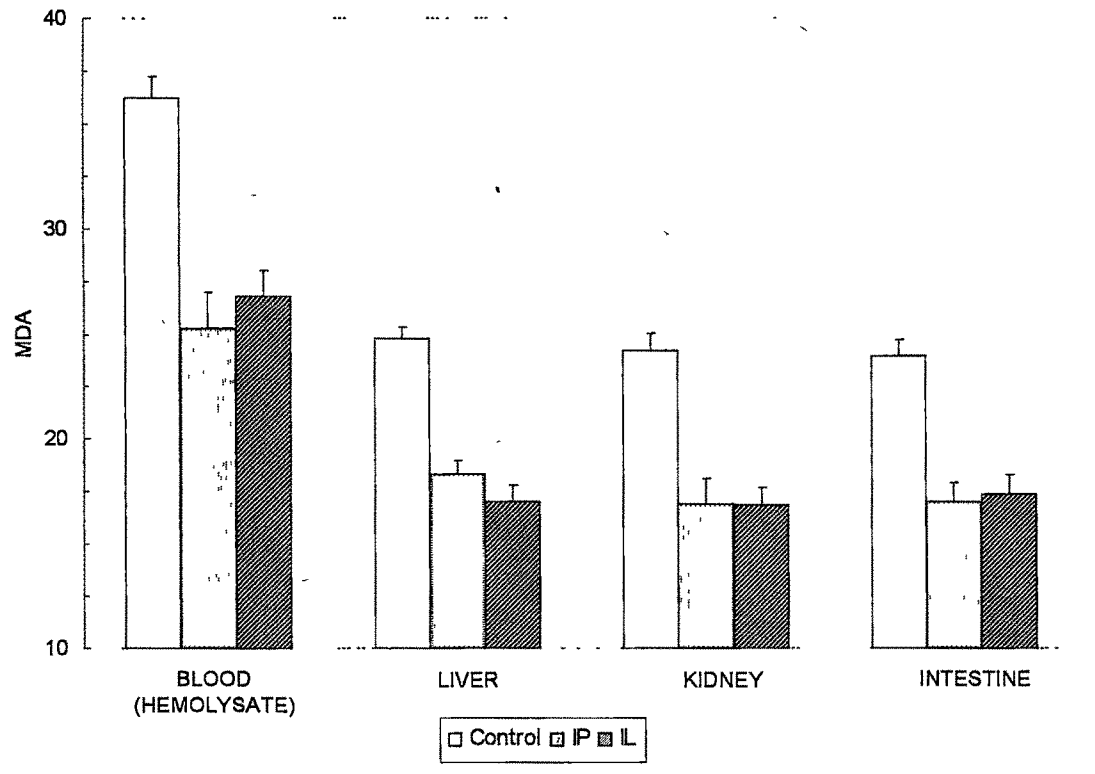

Figure 3 3 Superoxide Dismutase (SOD) activity in the Blood (SOD units/gm Hb/min) and Tissues (SOD units/mg protein/min) of FGF-2 treated and control animals at WH stage

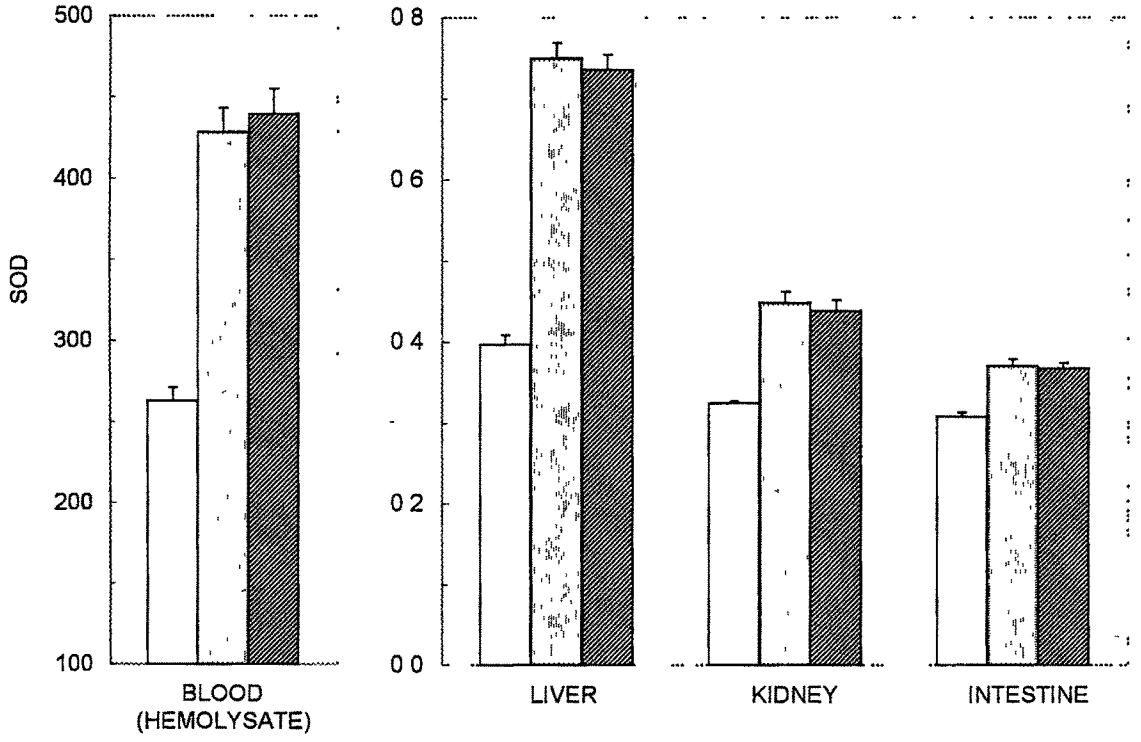

Figure 3 4 Catalase (CAT) activity in the Blood (K/gm Hb/) and Tissues (m moles H2O2 decomposed/sec/gm tissue) of FGF-2 treated and control animals at WH stage

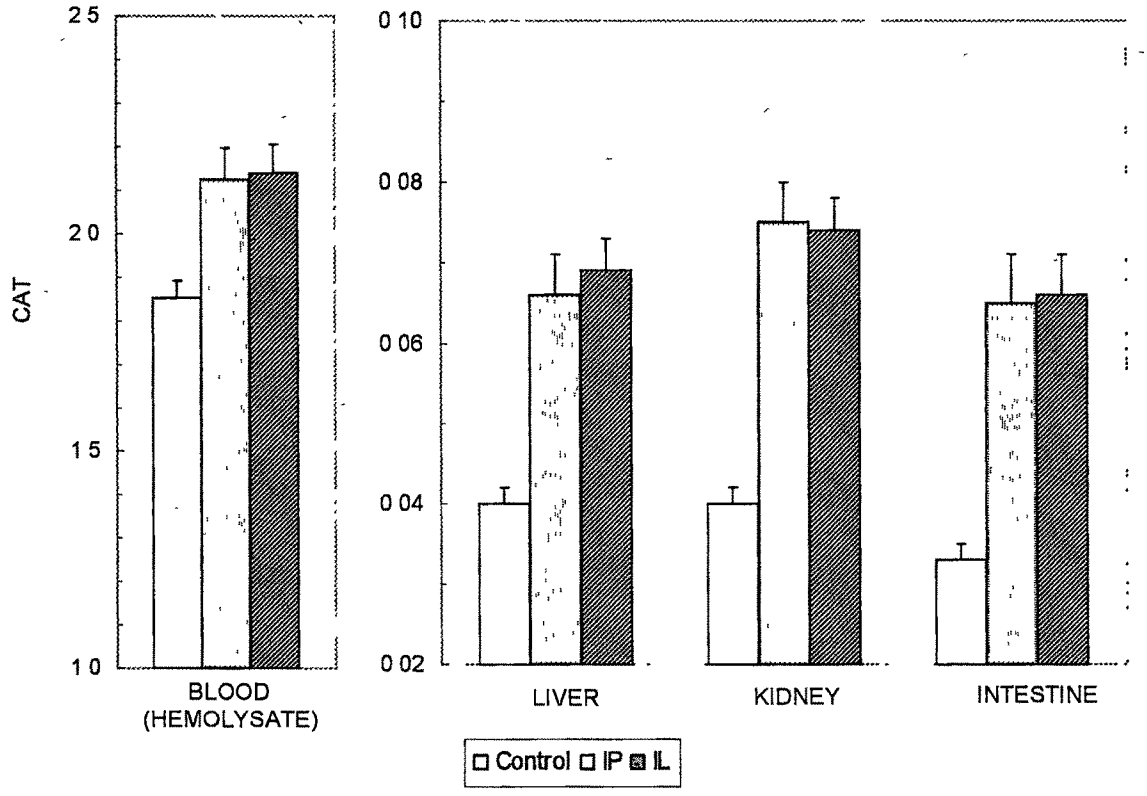

Figure 3 5 Reduced glutathione (GSH) levels in the Blood (mg/dl) and Tissues(µg/gm of tissue) in the FGF-2 treated and control lizards at BL stage

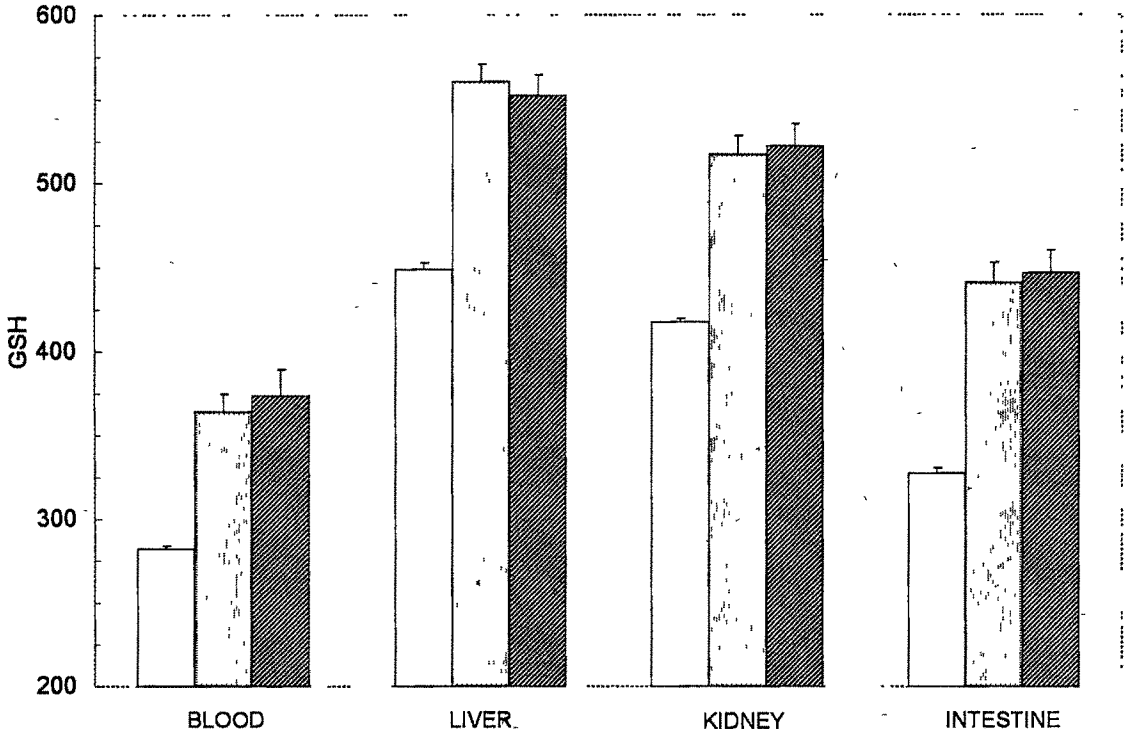

Figure 3 6 Malondialdehyde (MDA) levels in Blood (n moles MDA formed / gm Hb) and Tissues (n moles MDA formed/ gm Hb) in FGF-2 treated and control lizards at BL stage

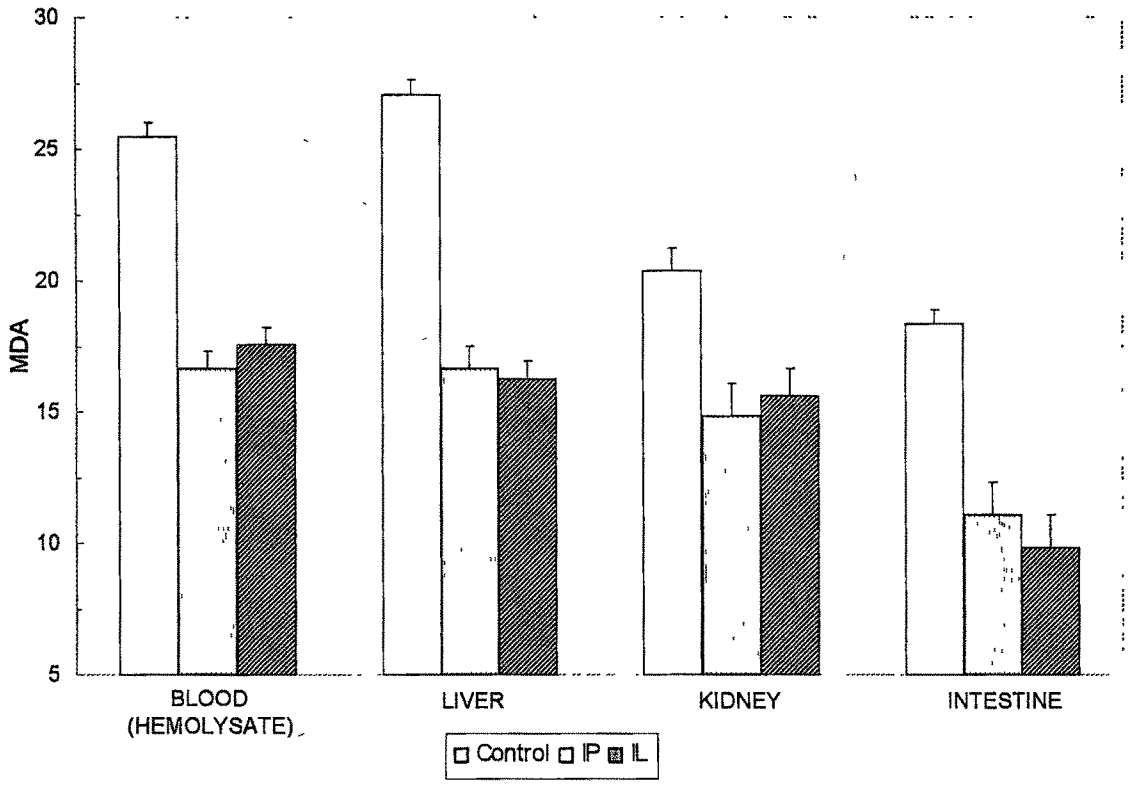

Figure 3 7 Superoxide Dismutase (SOD) activity in the Blood (SOD units/gm Hb/min) and Tissues (SOD units/mg protein/min) of FGF-2 treated and control animals at BL stage

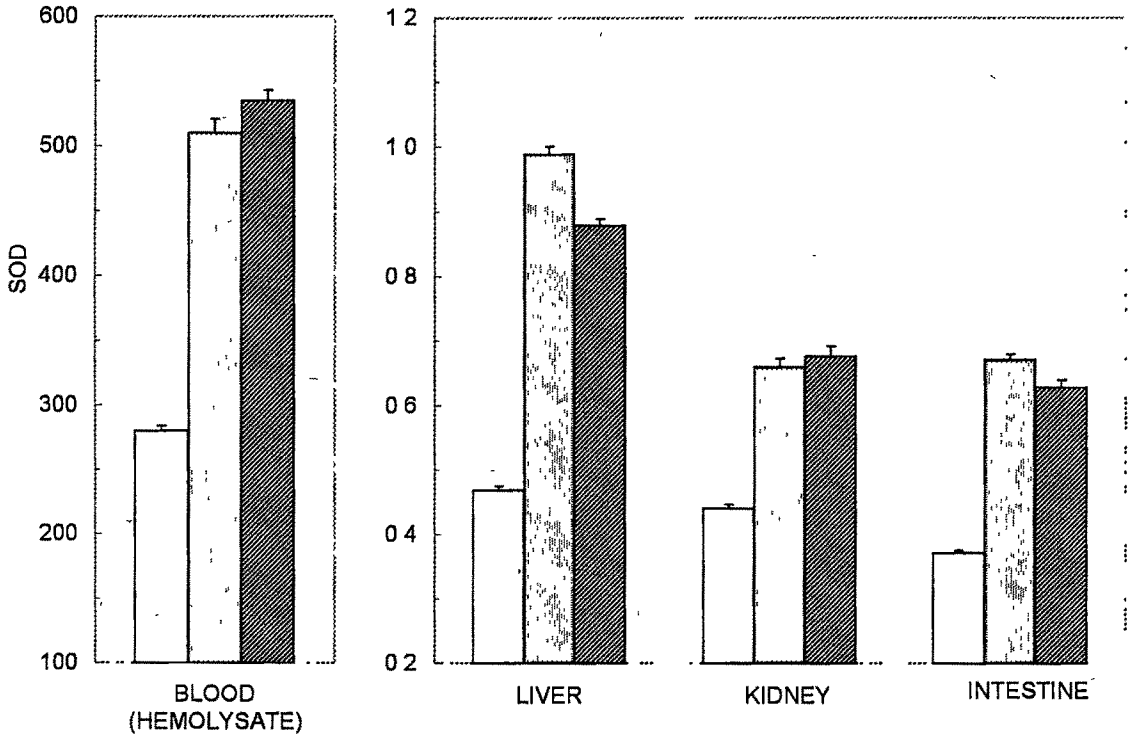

Figure 3 8 Catalase (CAT) activity in the Blood (K/gm Hb/) and Tissues (m moles H2O2 decomposed/sec/gm tissue) of FGF-2 treated and control animals at BL stage

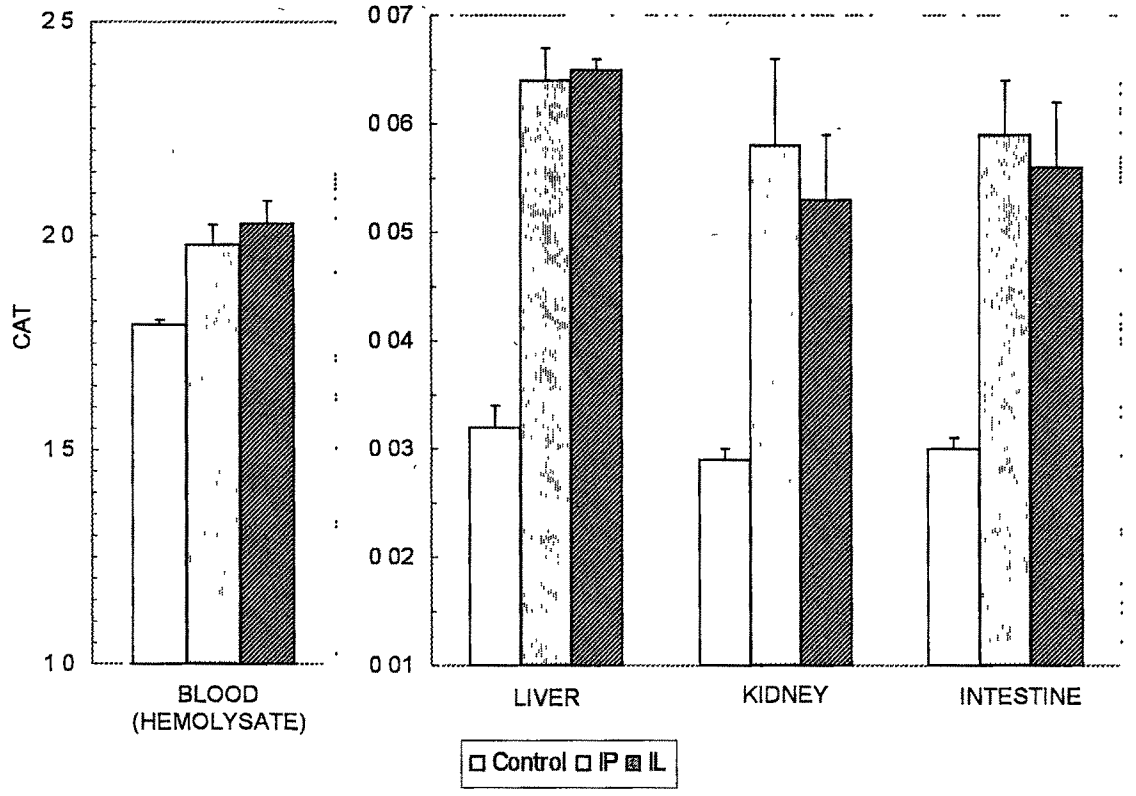

Figure 3 9 Reduced glutathione (GSH) levels in the Blood (mg/dl) and Tissues(µg/gm of tissue) in the FGF-2 treated and control lizards at DF stage

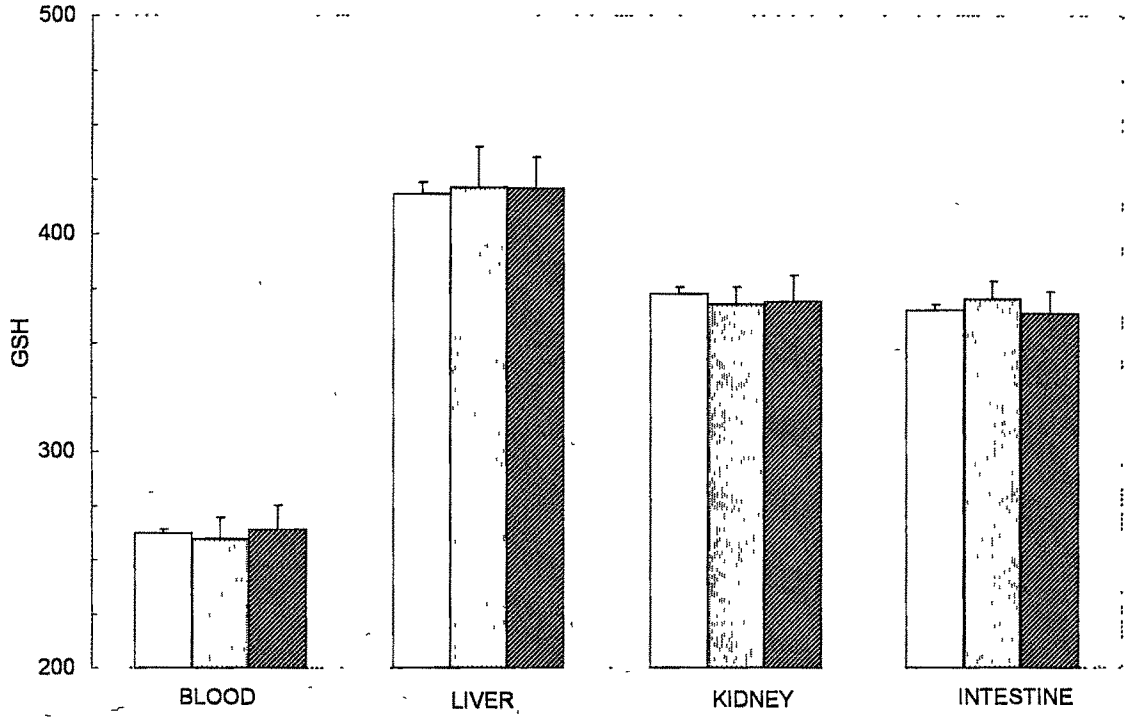

Figure 3 10 Malondialdehyde (MDA) levels in Blood (n moles MDA formed / gm Hb) and Tissues (n moles MDA formed/ gm Hb) in FGF-2 treated and control lizards at DF stage

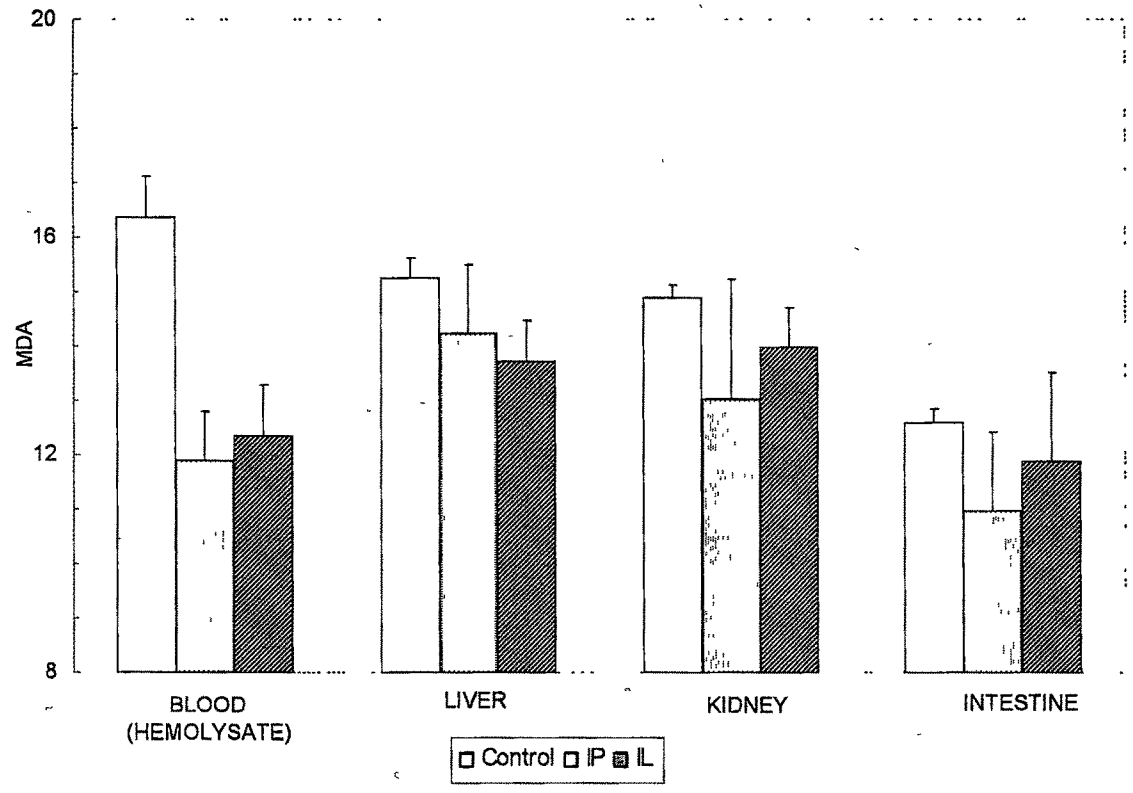

Figure 3 11 Superoxide Dismutase (SOD) activity in the Blood (SOD units/gm Hb/min) and Tissues (SOD units/mg protein/min) of FGF-2 treated and control animals at DF stage

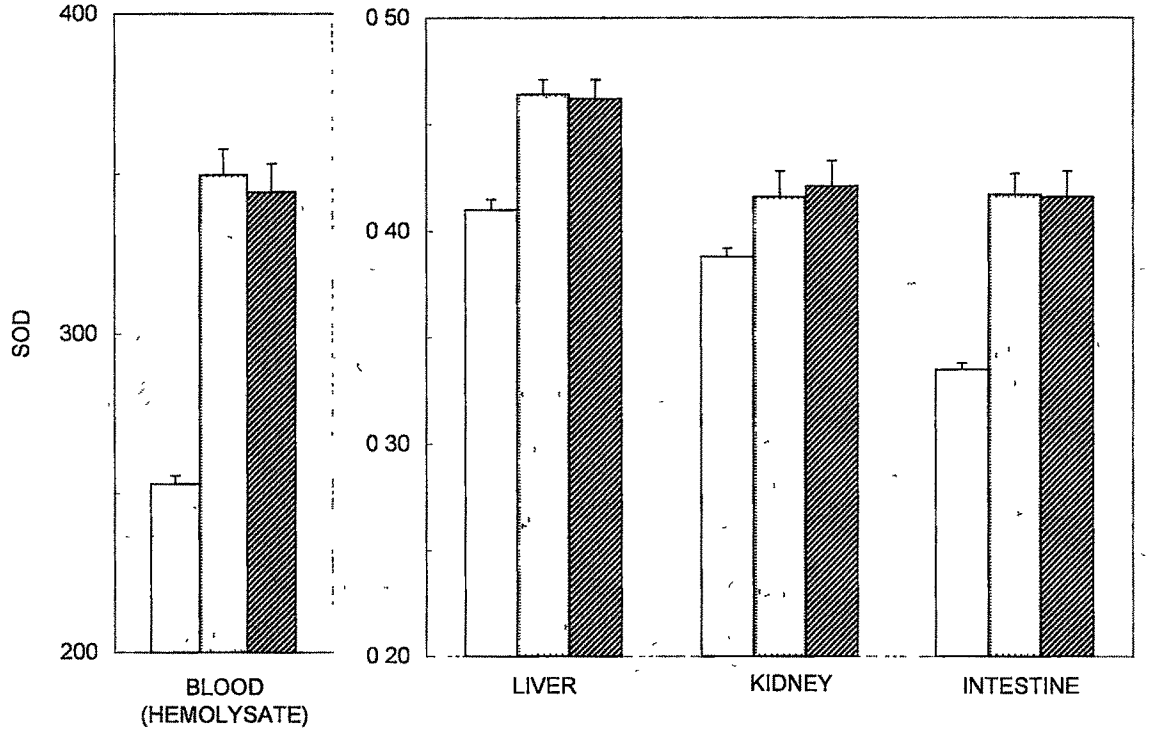

Figure 3 12 Catalase (CAT) activity in the Blood (K/gm Hb/) and Tissues (m moles H2O2 decomposed/sec/gm tissue) of FGF-2 treated and control animals at DF stage

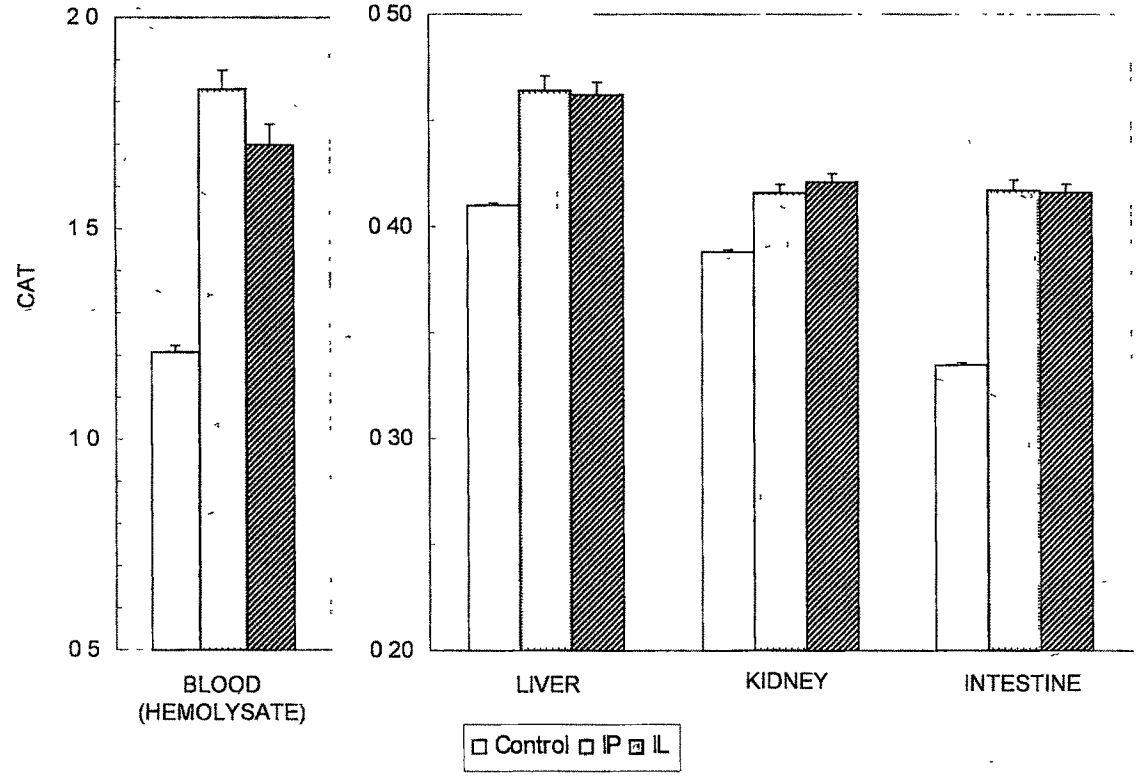

Figure 3 13 Reduced glutathione (GSH) levels in the Blood (mg/dl) and Tissueμg/gm of tissue) in the antiFGF-2 treated and control lizards at WE stage

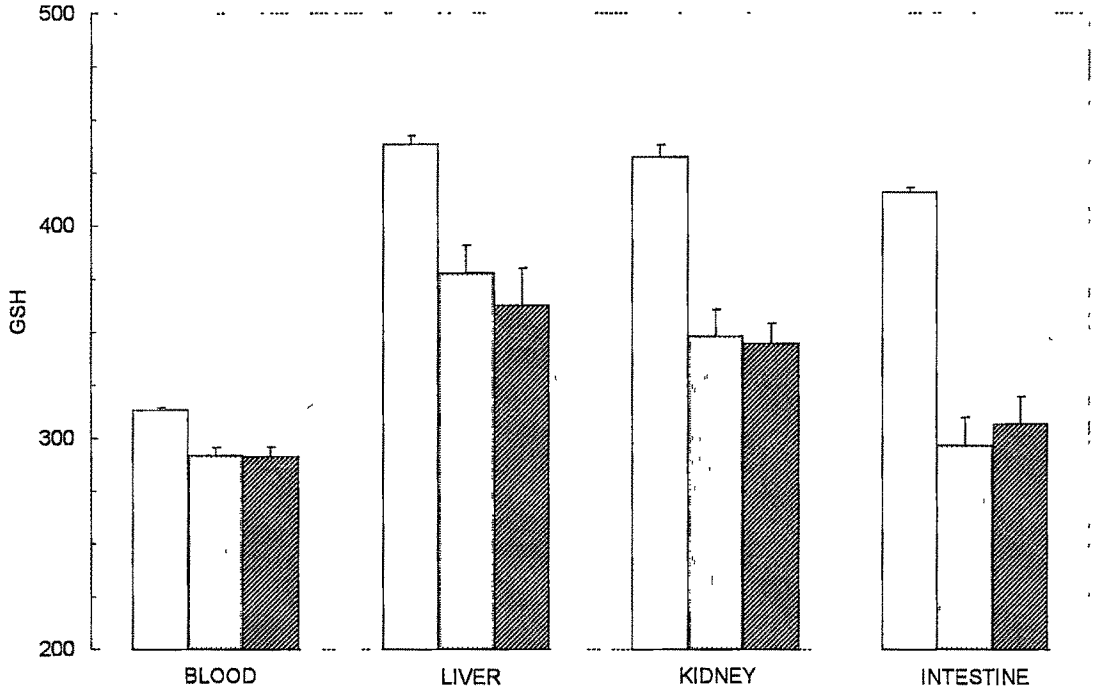

Figure 3 14 Malondialdehyde (MDA) levels in Blood (n moles MDA formed / gm Hb) and Tissues (n moles MDA formed/ gm Hb) in antiFGF-2 treated and control lizards at WE stage

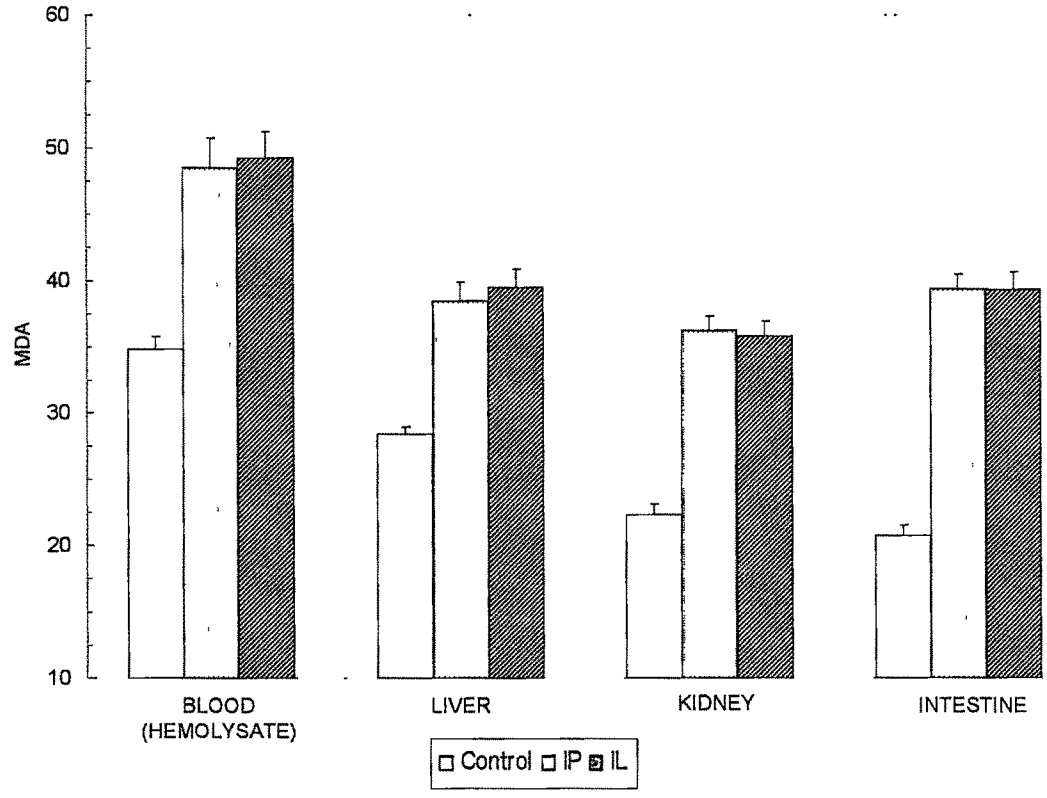

Figure 3 15 Superoxide Dismutase (SOD) activity in the Blood (SOD units/gm Hb/min) and Tissues (SOD units/mg protein/min) of antiFGF-2 treated and control animals at WE stage

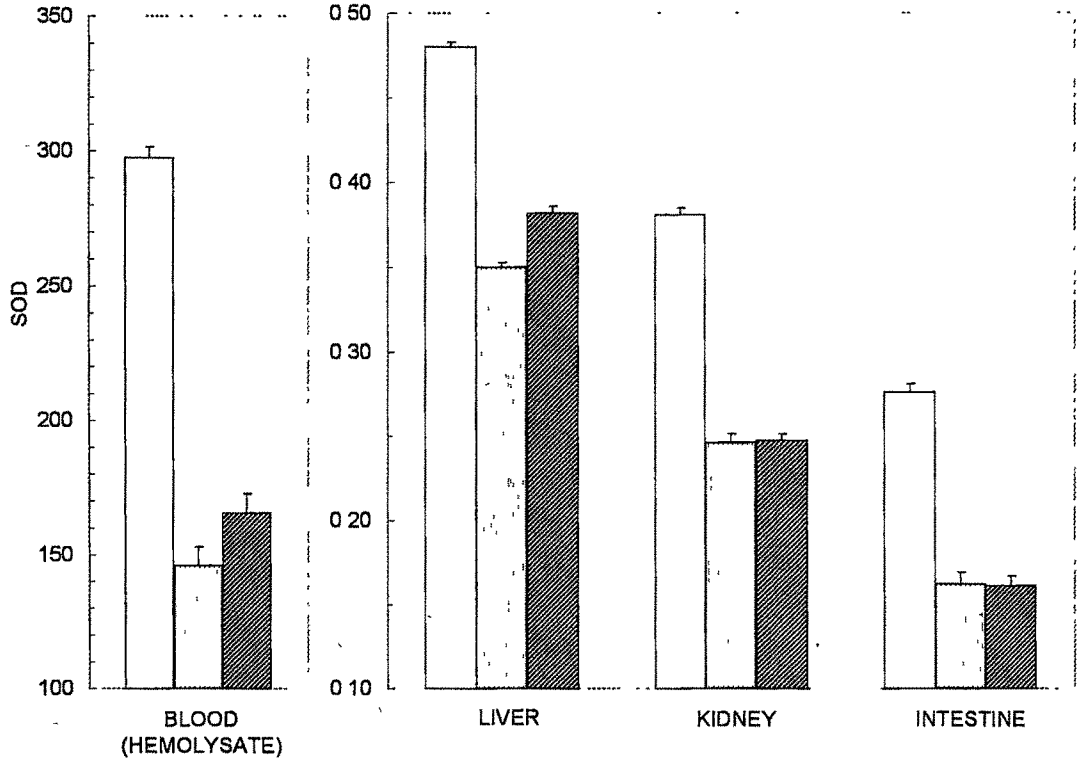

Figure 3 16 Catalase (CAT) activity in the Blood (K/gm Hb/) and Tissues (m moles H2O2 decomposed/sec/gm tissue) of antiFGF-2 treated and control animals at WE stage

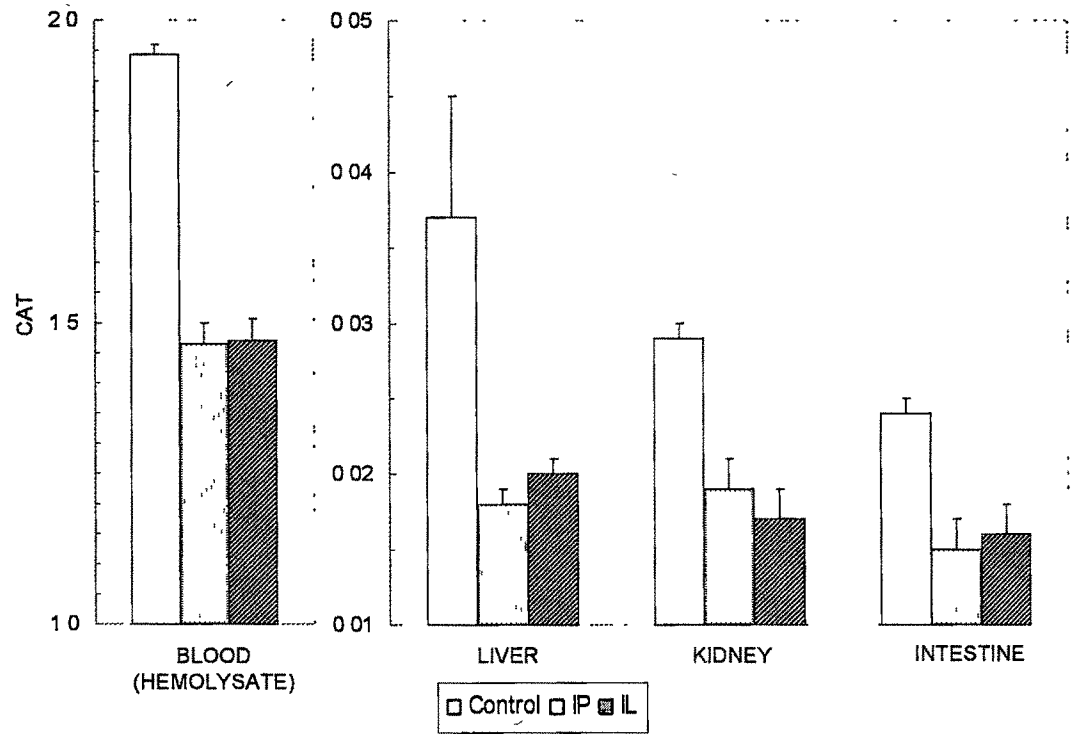

Figure 3 17 Reduced glutathione (GSH) levels in the Blood (mg/dl) and Tissues(µg/gm of tissue) in the antiFGF-2 treated and control lizards at BL stage

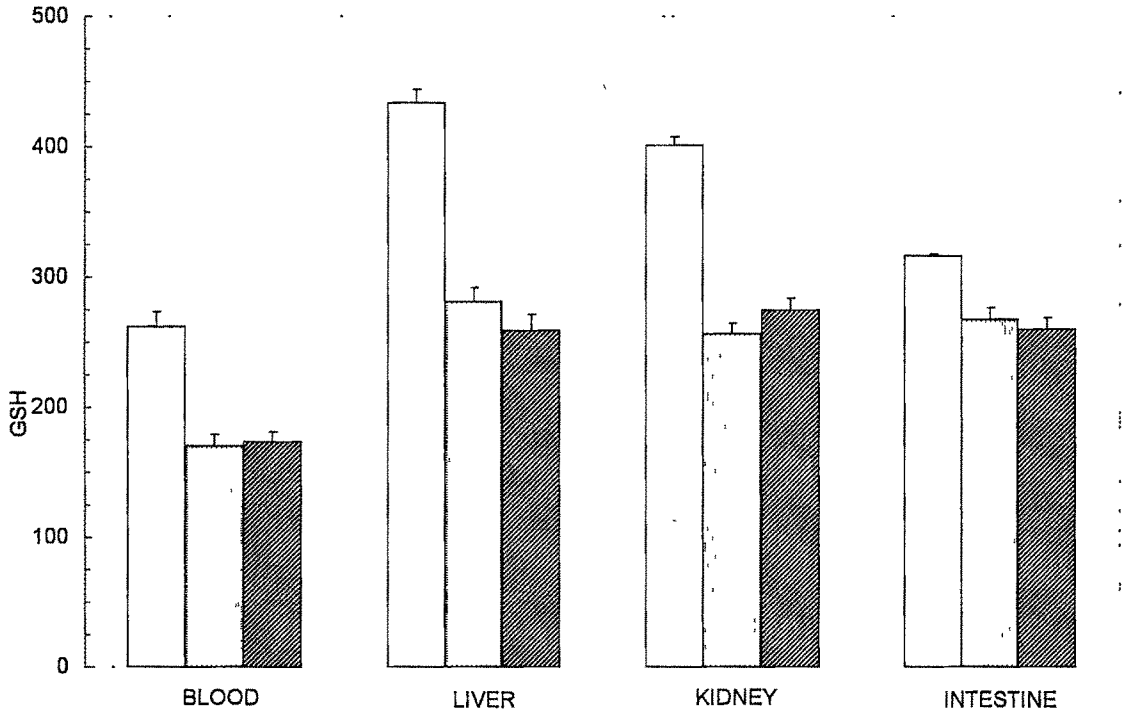

Figure 3 18 Malondialdehyde (MDA) levels in Blood (n moles MDA formed / gm Hb) and Tissues (n moles MDA formed/ gm Hb) in antiFGF-2 treated and control lizards at BL stage

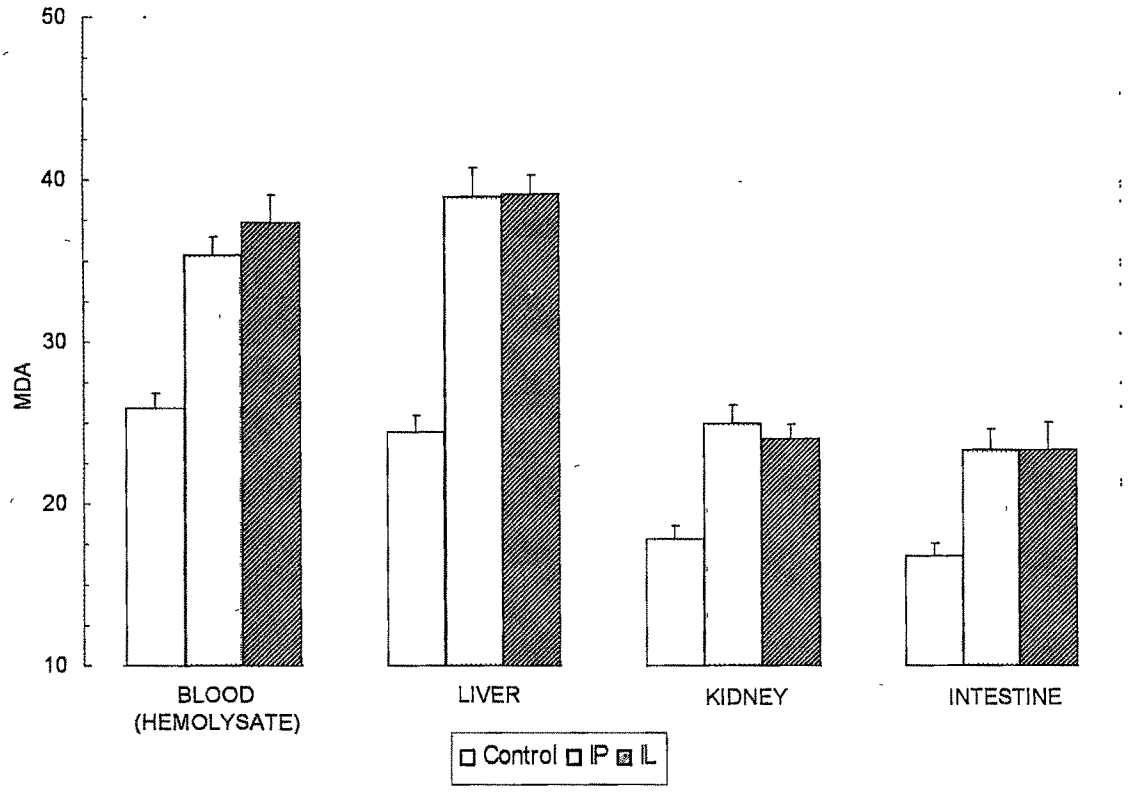

Figure 3 19 Superoxide Dismutase (SOD) activity in the Blood (SOD units/gm Hb/min) and Tissues (SOD units/mg protein/min) of antiFGF-2 treated and control animals at BL stage

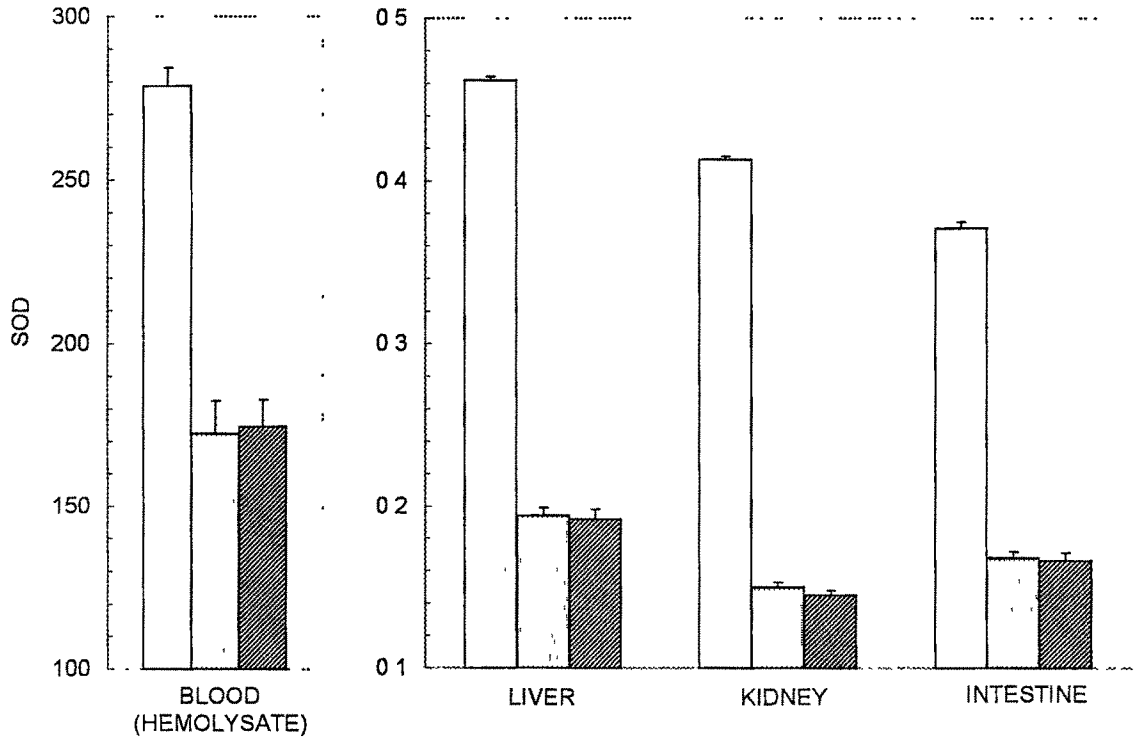

Figure 3 20 Catalase (CAT) activity in the Blood (K/gm Hb/) and Tissues (m moles H2O2 decomposed/sec/gm tissue) of antiFGF-2 treated and control animals at BL stage

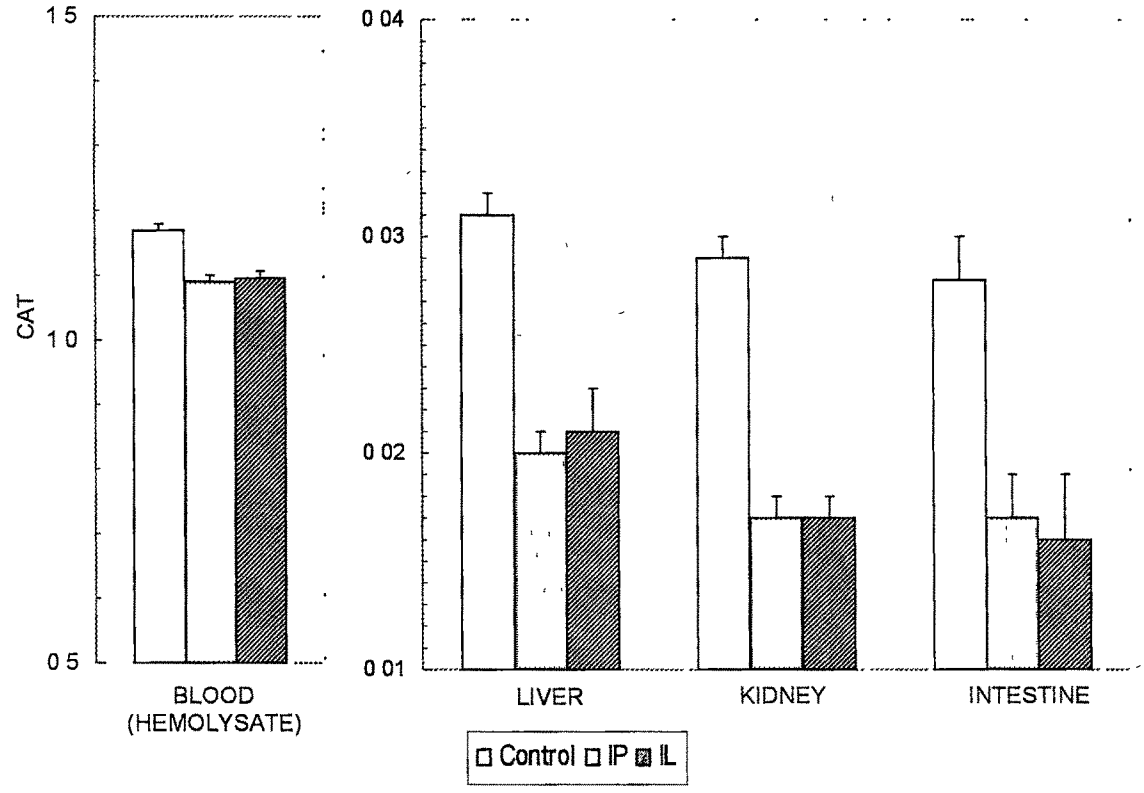

Figure 3 21 Reduced glutathione (GSH) levels in the Blood (mg/dl) and Tissues(µg/gm of tissue) in the antiFGF-2 treated and control lizards at DF stage

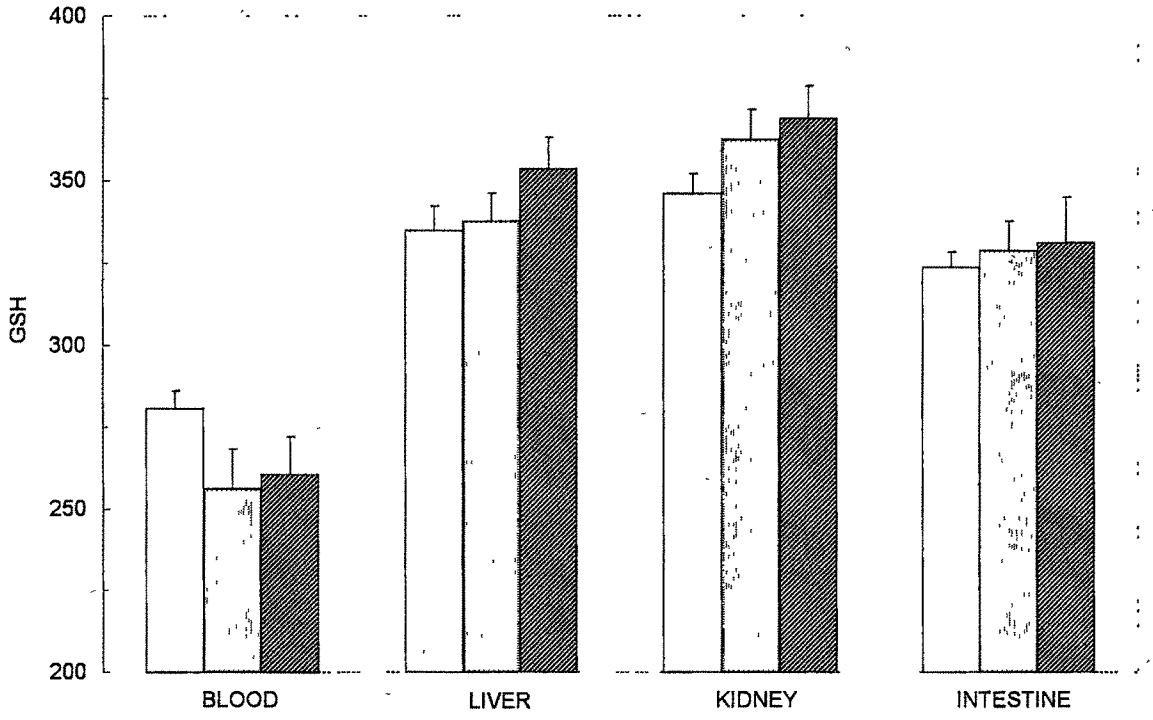

Figure 3 22 Malondialdehyde (MDA) levels in Blood (n moles MDA formed / gm Hb) and Tissues (n moles MDA formed/ gm Hb) in antiFGF-2 treated and control lizards at DF stage

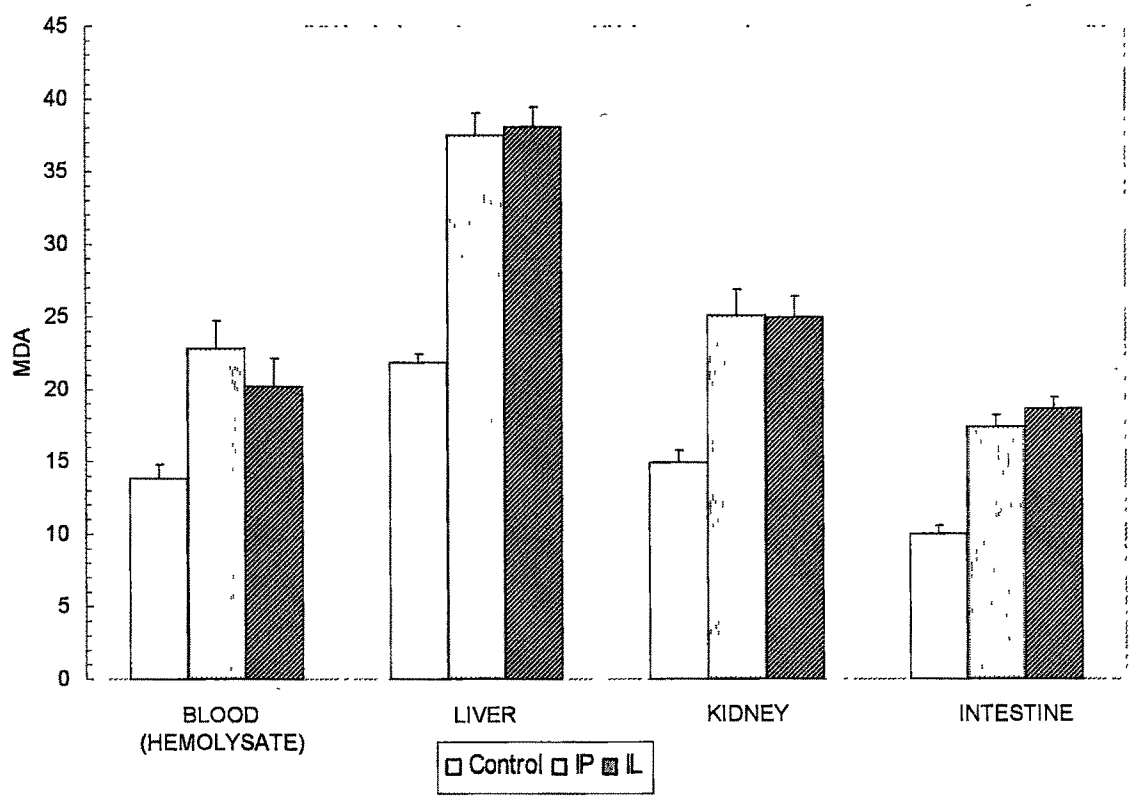

Figure 3 23 Superoxide Dismutase (SOD) activity in the Blood (SOD units/gm Hb/min) and Tissues (SOD units/mg protein/min) of antiFGF-2 treated and control animals at DF stage

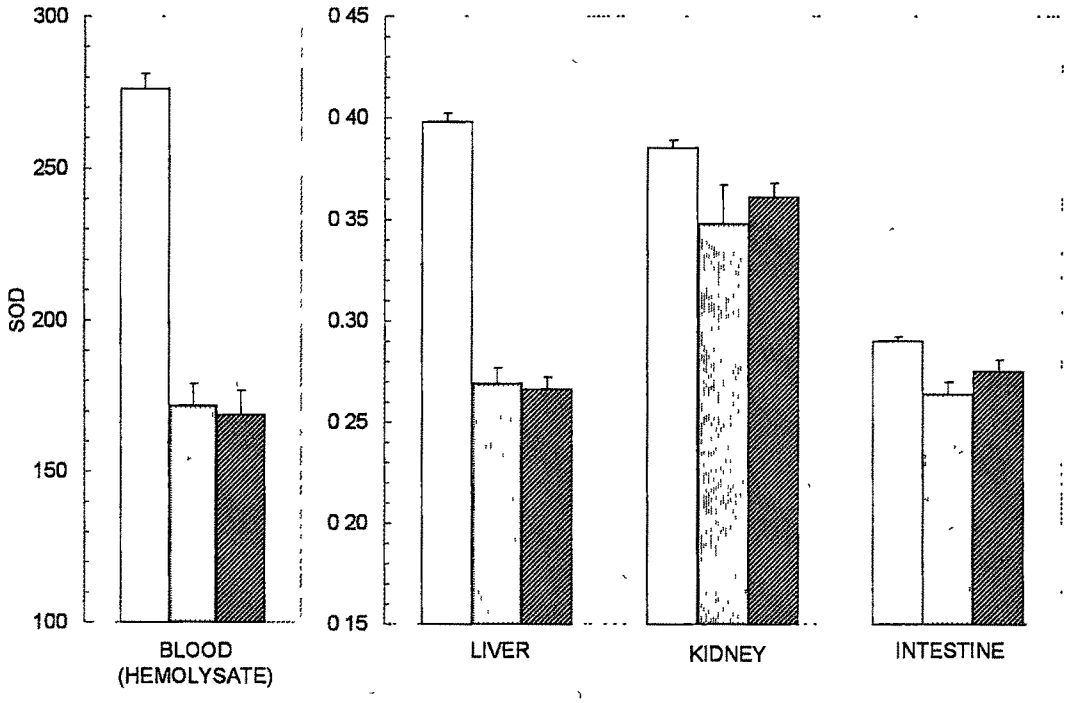

Figure 3 24 Catalase (CAT) activity in the Blood (K/gm Hb/) and Tissues (m moles H2O2 decomposed/ sec/gm tissue) of antiFGF-2 treated and control animals at DF stage