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EFFECTS OF BRODIFACOUM, ACETYLSALICYLIC ACID AND ITS COMBINATION ON HAEMOSTATIC, HEMATOLOGICAL AND HEPATIC MARKERS OF WILD RAT *RATTUS RATTUS*

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Development of resistance in rodents against rodenticides represents a major challenge for rat management strategies in the light of heavy damage and infestation of crop fields. The aim of this study is to examine the effects of brodifacoum (BDF), acetylsalicylic acid (ASA), and a novel formulation of both anticoagulants on haemostatic and hematological parameters, hepatic redox status and histoarchitecture. Forty eight adult female wild rats (*Rattus rattus*) were randomly and equally divided into three groups, eight rats each. BDF induced a significant increase in bleeding and prothrombine time, lipid peroxidation (LPO) and decrease in factor VII and IX and hepatic nitric oxide (NO) levels and catalase (CAT) activity, and eosinophils, lymphocytes and monocytes count. Rats in the combination group were characterized by a significant increase in clotting and prothrombin time and ionized calcium (Ca^{+2}) level together with a significant decrease in factors VII, IX and NO levels, platelet count, mean platelet volume, and hepatic CAT activity. ASA was responsible for a significant increase in bleeding time, factor IX level and hepatic CAT activity, and decrease in hepatic NO level. Adverse changes in hepatic histoarchitecture were observed following each of BDF and ASA exposure. The combination of ASA with BDF further aggravated the histopathological lesions of liver more than BDF alone. It was concluded that ASA induced a joint action with BDF especially regarding the haemostatic markers and hepatocellular features.

Keywords: Brodifacoum; acetylsalicylic acid; *Rattus rattus*; hematology; redox status; liver

INTRODUCTION

Because rodents pose a serious practical problem to crop fields not only by causing damage to various growth stages of plant but also by causing contamination in transportation and storage (Prakash, 2018), the control of rodents is an urgent necessity. Superwarfarins are very potent, long-lasting anticoagulant rodenticides inhibit the enzyme vitamin K epoxide reductase, thus reducing the recycling of Vit K, which is necessary for activation of several clotting factors (Feinstein et al., 2016). Amongst the superwarfarins typically incorporated into rodent bait, brodifacoum (BDF) is the most widely used. The biologic potency of BDF is thought to be attributable to their high lipid solubility and increased affinity for hepatic tissue and enzymes (Rauch et al. 1994; Vudathala et al. 2010). However, its inhibitory effect on blood clotting activity and Vit K cycle in the liver of rats seems to be transient (Mosterd & Thijssen, 1991). Oral administration of BDF to Sprague Dawley rats caused a dose-dependent transient hemoglobinuria accompanied by a transient decrease in hematocrit, gross hemolysis and an increase in free hemoglobinin the serum. At later times, rats developed true hematuria with presence of RBCs in the urine (Ware et al., 2015).The blood BDF concentration in Wistar rats showed a positive relation to the ingestion dose in a previous toxicological analysis. BDF exposure negatively affected the examined metabolic pathways indicating nephro- and hepato-toxicity (Yan et al., 2016). The hepatocytes of Wistar albino rats challenged with a single dose of BDF for four days showed increased numbers of lysosomes, enlargement of mitochondria, clumping of chromatin, accumulation of hemolyzed erythrocytes in sinusoid. Exposure to BDF for seven days caused formation of numerous vacuoles and lipid droplets, clumping of chromatin, and invagination of nuclear envelope (Gül et al., 2016).

An emerging genetic resistance against rodenticide represents one of the most challenges to eradication programs (Desvars-Larrive et al., 2017).Previous studies tried to overcome this practical issue by using combination baits which have low cost and ecotoxicological concern but from humanness viewpoint have long days-to-death (Witmer et al., 2017). Therefore, development of interventions that take another step wide jump on the road to enhance efficacy of anticoagulant rodenticide is highly recommended. The antithrombotic effect of acetylsalicylic acid (ASA) is well established and it is mediated *via* acetylation of blood clotting factors and suppression of its synthesis, inhibition of platelets, prevention of thrombin formation, and acceleration of fibrinolysis (Butenas et al., 2009; Lord, 2011; Undas et al., 2014; Mekaj et al., 2015). ASA treatment in rats reduced hematocrit value and RBC, WBC and platelet count (Vyas et al., 2016). Gastric intubation of rats with ASA resulted in a marked increase in hepatic lipid peroxidation level and decrease in SOD, CAT and glutathione peroxidase activities. The histological examination of liver section showed degenerations of hypatocytes, dilation of sinusoids, increased leukocyte infiltration, and congestion of the central portal vein (Bouzenna et al., 2016a).

The objective of this study was to identify the effects of BDF, and ASA, and a new formulation of rodenticide containing both constituents with respect to the potential changes in haemostatic and haematological, and oxidative stress parameters in hepatic tissue in addition to the histoarchitecture in a hope to translate the findings to the practical field.

MATERIALS AND METHODS

Animals

Forty eight adult female wild rats (*Rattus rattus*) weighing 100±20 gm were collected from three areas (houses, fields, and poultry farms) in Manfalut city, Assiut, Egypt using traps. They were collected and kept in separate cages in the Animal house, Zoology Department, Faculty of Science, Assiut University at

room temperature (25±3°C) with normal 12 h light/12 h dark cycle for a week before beginning of the experiment. All experimental protocols that held on animals were carried out according to regulations set by the Institutional Animal Care and approved by Assiut University.

Determination of LD50 of brodifacoum

LD50 was determined according to Finney (1971). Briefly, rats were divided randomly and equally into four groups (four rats each). Following overnight fasting, they caged individually and received serial doses of BDF (1.36, 1.81, 2.27 and 2.72 mg/kg BW) incorporated in wheat as single bait. Mortality and time to death were recorded after treatment. LC50 calculated based on this method was 1.9 mg/kg BW.

Experimental groups

After one week of acclimatization, rats were randomly categorized into three groups, eight rats each. A control group received no treatment. ASA group was exposed to 60 mg/kg BW dissolved in 10 ml distilled water for one day (Liu & Xu, 1995). BDF group was exposed to 2 mg/kg BW as single bait as determined by LD50.

Sample collection and preparation

Four blood samples were obtained from each rat. One of the blood samples was collected in EDTA containing tubes for evaluation of hematological and oxidative stress parameters. A second blood sample was collected in sodium citrate containing tubes for estimation of prothrombin time and factors VII and IX. A third blood sample was centrifuged at 3000 rpm for 10 minutes to obtain serum which was stored at -20°C until analysis of Ca^{+2} level.

Part of liver was homogenized in phosphate buffer (pH 7.4) using homogenizer (IKA Yellow line DI 18 Disperser, Germany) to prepare 10% w/v homogenate. The homogenates were centrifuged at 8.000 rpm for 15 minutes and the supernatants were kept frozen at -20°C for the subsequent biochemical assays.

Moreover, the other part of the liver were dissected out and preserved for the later histological examination .

Estimation of haemostatic parameters

To determine bleeding time, rats were anesthetized and a transverse incision was employed on the rat tail. Blood emerged from the incision was gently wiped away by a filter paper every 15 seconds until stop of bleeding. The elapsed time from beginning of bleeding until stop of bleeding was recorded by a stop watch (Wienen et al., 2007). One blood drop collected from retro-orbital sinus was added on a slide and timing immediately started with a stopwatch. The blood drop pricked lightly with a pin every 30 seconds. When blood fibers appeared, timer was stopped to record the blood clotting time (Zhou et al., 2014).

Prothrombin time and factors VII and IX were estimated by Sysmex CA1500 (Siemens Healthineers, USA). Ionized calcium was assessed by KD100 Electrolyte Analyzer (Shenzhen Kindle Medical Devices Co. Ltd, China).

Estimation of hematological parameters

Hematological variables were analyzed in the Clinical Pathology Laboratory, Department of Pathology and Clinical Pathology, Faculty of Veterinary Medicine, Assiut University, using Exigo Veterinary Hematology Analyzer (Boule Medical AB, Sweden).

Estimation of liver oxidant/antioxidant parameters

Lipid peroxidation (LPO) products as thiobarbituric acid reactive substances were estimated according to the method of Ohkawa et al. (1979). Nitric oxide (NO) was measured as nitrite concentration using the method of Ding et al. (1988). Catalase (CAT) activity was determined basing on its ability to decompose hydrogen peroxide (Lück, 1963). Total protein concentration in hepatic tissue was determined by the method of (Lowry et al., 1951). All hepatic oxidative stress parameters are normalized by total protein.

Histological examination

Small pieces of liver was quickly removed and fixed in 10% neutral formalin solution and routinely processed. Then sections (5μm) of different samples were mounted on slides and dried overnight at 37°C. Sections were de-waxed in xylene and hydrated in a graded series of alcohols and stained by hematoxylin and eosin for histological evaluation. Drury and Wallington(1980).

Statistical analysis

The data were expressed as mean ± SEM. The results were analyzed statistically using one-way analysis of variance followed by Newman–Keuls multiple comparison test using GraphPad Prism program, version 6.0 (Graph pad software Inc., San Diego, California, USA). Differences between the groups were considered significant if $P< 0.05$, 0.01, or 0.001.

Ethical approval

The study was carried out in accordance with the Egyptian laws and University guidelines for the animal care. All procedures of the current work had been approved by the National Ethical Committee of the Faculty of Science, Assiut University, Egypt.

RESULTS

1. Effects of brodifacoum, acetylsalicylic acid and their combinations on the haemostatic parameters

Versus the control group, rats exposed to BDF alone or ASA alone were characterized by a significant increase in the bleeding time, while only those exposed to the combination showed a significant increase in the clotting time. Relative to the prothrombin time of control group, a significant increase in BDF+ASA groups than that observed in BDF group indicated that ASA could potentiate the anticoagulant activity of BDF. A significant decrease in factor VII level was found when comparing BDF and combination groups with the control group. Regarding factor IX levels, BDF and combination groups showed a significant decrease, while ASA group showed a significant

increase as compared to the control group. Only the combination induced a significant increase in Ca^{+2} levels versus the control group (Table 1).

2. Effects of brodifacoum, acetylsalicylic acid and their combinations on the hematological parameters

Rats exposed to BDF in combination with ASA showed a high significant reduction in PLT count compared to untreated rats. In comparison with the control group, exposure of rats to combination of ASA with BDF caused a significant reduction in MPV.BDF group had significantly lower EOS count than the control group. Each of LYM and MONO count was significantly and, respectively) decreased in BDF group versus the control one (Table 2).

3. Effects of BDF, ASA and their combinations on the liver oxidative stress parameters

Among all treated groups, only BDF group showed a significant increase in LPO level of liver as compared to the control group. In comparison with the control rats, a significant decrease in the hepatic NO level was observed when rats challenged with BDF alone or ASA alone or BDF in combination with ASA. Relative to the hepatic CAT activity, a significant decrease in BDF and the combination groups, and a significant increase in ASA group were found when compared with the control one (Table 3).

4. Histopathological changes in the liver

The liver of control group showed normal histoarchitecture (Fig. 1). On the other hand, central veins in the BDF group were markedly dilated and filled by hemolyzed blood (Fig 2).Hepatocytes with deeply stained nuclei and necrotic area were seen(Fig.3). Similarly, central vein of the ASA group was dilated and filled by hemolyzed blood and degenerated hepatocytes with deeply stained pyknotic nuclei (Figs. 4&5). In the combination group, disarchitecture of hepatic structure marked dilated central vein filled by hemolyzed blood , while

portal tracts was expanded and suroundrd by fibrotic area .Infilteration of inflammatory cells were seen in both fibrotic area and central vein (Fig 6&7).Few degenerated hepatocytes in peri-venular zone and hepatocytes with sign of apoptotic nuclei, containing patches of deeply stained chromatin were observed (Fig 7).

DISCUSSION

Up to our knowledge, this is the first study investigates the potential synergistic effect of BDF and ASA in single bait in rats. A major goal of this study is to develop a novel intervention to maximize the rodenticide efficacy of BDF by the possible potentiator effects of the gold standard anticoagulant drug, ASA, to overcome the health and economic threatening global problem of rodents. Examination of coagulation profile in the BDF group revealed a profound coagulopathy manifested by prolonged bleeding and prothrombine time as well as reduced factors VII and IX levels. Prolonged PT, a measure of the integrity of extrinsic and final common coagulation pathway, had been reported in response to BDF exposure in a wide array of animal species (Boermans et al., 1991; Kuhn & Hendrix, 2013; Webster et al., 2015). Superwarfarin rodenticides such as BDF produce their effect by interfering with Vit K1 epoxide reductase, resulting in the depletion of Vit K1 and subsequently impairment of the biosynthesis of the pro-coagulant factors II, VII, IX, and X and that of the anti-coagulant proteins C and S in the liver (Crāciun et al., 1998). Therefore, the hepatic histopathological findings in this study could provide a rational support for depletion of their quantities.

The bleeding time is used to evaluate the capacity of platelets to form a hemostatic plug (Paniccia et al., 2015). The more pronounced increase in the bleeding time following ASA treatment gives insight into its antiplatelet activity though irreversible inhibition of cyclooxygenase activity and suppression of platelet-associated substances secretion (Mekaj et al., 2015).

The more obvious prolongation in the clotting and prothrombin times in the combination group than BDF group can be emerged from the corresponding reduction in the examined blood clotting factors. Acetylation of blood clotting proteins, inhibition of platelet-associated substances secretion, inactivation of factors IIa, IX, X and XIII, acceleration of the rate of fibrinolysis, reduction in the synthesis of coagulation factors in the liver are the potential mechanistic avenues underlying the antithrombotic effects of ASA (Undas et al., 2003; Butenas et al., 2009; Lord, 2011; Undas et al., 2014). Ionized calcium is an indispensable prerequisite for multiple steps in the coagulation cascade and is necessary for the formation of fibrin from fibrinogen; the conversion of prothrombin to thrombin; as a cofactor for factors V, VII, VIII, IX, X, and XIII (Mann, 1999), and stimulator for platelet aggregation (Ware et al., 1987). In the present study, accumulation of Ca^{+2} in the serum of combination group may reflect failure to utilize it in the pathways of haemostasis and activation of blood platelet.

Platelets have a key role in hemostasis by forming plug that seal defects in the vessel wall (Nielsen et al., 2007). The clear reduction in PLT count and MPV in the combination group compared to the control one is consistent with that found in horse and rat (Boermans et al., 1991; Mirkov et al., 2016; Vyas et al., 2016). Warfarin interacts with polymorphonuclear leukocyte myeloperoxidase (Belij et al., 2012), which may induce hemolysis. The strong hydrophobic nature of BDF raises the possibility that it directly interacts with cell membranes, leading to cell injury (Ware et al., 2015). ASA have antithrombotic properties by inhibiting PLT function on several levels such as aggregation, granule release, and reactive substance secretion (Undas et al., 2014; Mekaj et al., 2015). Disruption in intracellular Ca^{2+} balance may play plays a pivotal role in the observed reduction in MPV as bromobenzene, the active moiety of BDF, suppresses mitochondrial Ca^{2+} sequestration (Casini et al., 1987). The decrease in MPV indicates that PLT became metabolically

and enzymatically less active and expressed low levels of prothrombotic substances and procoagulant surface proteins (Colkesen et al., 2008).

In the light of our findings, BDF is suggested to be immunosuppressor confirmed by obvious reduction in LYM, EOS and MON count. Nevertheless, BDF exposure did not influence the immune response of cat at the terms of antibody production and lymphocyte proliferation but it was associated with alternations in cytokine expression (Kopanke et al., 2018). This controversy may arise from the difference in experimental design as dose of BDF, species of animal model, and duration of exposure. Decreased release of granulocyte monocytes-colony stimulating factor may lie behind the immunosupressor effect of BDF as this factor induces granulocyte and macrophage populations from bone marrow precursor cells and increases regulatory T-cell numbers and function (Bhattacharya et al., 2015a; Bhattacharya et al., 2015b; Kopanke et al., 2018). In addition, suppression of lymph node activity and diapedesis of LYM out of the blood vessels to injured intestine might account for a decrease in LYM number (Mirkov et al., 2016).

In the current study, the induction of oxidative stress in liver following BDF exposure was indicated by a marked rise in LPO level together with a marked reduction in NO level and CAT activity. This outcome is in harmony with suggestion of Ware and his coauthors (2015) who assume that induction of oxidative stress is responsible for the early effects of BDF based on the ability of N-acetylcysteine to prevent the BDF-mediated hemoglobinuria. Bromobenzene moiety in BDF causes uncoupling of mitochondrial oxidative phosphorylation (Maellaro et al., 1990) which may be implicated in increased generation of reactive oxygen species leading to lipid peroxidation and alteration in oxidant/antioxidant homeostasis. Oxidative stress also can be evoked by the metabolites of bromine group, nitrous bromide or hypobromous, through their interactions with the mitochondria (Blondin & Green, 1970).The marked reduction in

the hepatic NO level and CAT activity in the combination group is matched with a broad spectrum of data indicated interruption of redox homeostasis under ASA challenge (Inkielewicz & Czarnowski, 2008; Bhattacharyya et al., 2014; Zeren et al., 2016; Bouzenna et al., 2016a; Bouzenna et al., 2016b). Increased LPO negatively impacted the physicochemical characters, fluidity and integrity of the cell membrane resulting in hepato-cellular damage and necrosis (Dobrzynska et al., 2008) as confirmed by the histopathological finding of this study. Reduction of NO may arise from suppression of inducible nitric oxide synthase by modulating NF-κB pathway following ASA treatment (Inaba et al., 2015).Under influence of ASA, a majority of NO may be consumed to adversely impact the haemostatic cascade including inhibition of platelet aggregation and reaction with superoxide anion in platelets to form peroxynitrite which inhibits platelet adhesion to collagen and fibrinogen, and activation of clotting plasma factors on the cell surface (Nowak & Wachowicz, 2002; Nowak et al., 2003; Banerjee et al., 2014).The drop in activity of CAT might results from consumption of this antioxidant in attempt to counterattack free radicals and limit the oxidative stress. Inhibition of CAT can led to excessive generation of hydrogen peroxide which is known to suppress the activities of enzymatic antioxidant and cause oxidative damage to all types of biological macromolecules culminating at cell death (Brewer et al., 2015; Yin et al., 2015).

Given that liver is the target organ of BDF action and accumulation, the histopathological examination of rat liver challenged with BDF was carried out. BDF induced a marked dilation in central vein which was filled with hemolyzed blood together with apotosis and karyomegally in the hepatocytes. Ultra-structural changes of hepato-cellular components were monitored previously by Gul and his collaborators (2016) using a window of three time point intervals revealing presence of increased numbers of lysosomes, enlargement of mitochondria, clumping of chromatin, accumulation of hemolyzed erythrocytes

in sinusoid, formation of numerous vacuoles and lipid droplets, and invagination of nuclear envelope. The hepato-cellular abnormalities in the current study could be attributed to the interference actions of BDF on the levels of mitochondrial electron transport chain, glucose and amino acid metabolism, and impairment of mitochondrial Ca^{2+} sequestration (Casini et al., 1987; Yan et al., 2016) resulting in a potential depletion of the cellular energy resources.

The combination of ASA with BDF further aggravated the histopathological lesions of liver more than BDF alone. A number of peer-review articles demonstrated the adverse impacts of ASA on the hepatic histoarchitecture (Huang et al., 2016; Bouzenna et al., 2016a; Bouzenna et al., 2016b). Shifting oxidant/antioxidant balance towards the oxidant side as evident by our findings and previous ones may contribute to the outcome by overproduction of ROS which attack critical macromolecules in the cells (Bhattacharyya et al., 2014; Bouzenna et al., 2016a; Bouzenna et al., 2016b). Salicylates have the ability to uncouple oxidative phosphorylation allowing increase in metabolic rate resulting in increasing oxygen consumption, glucose utilization, and heat production. They inhibit the Krebs cycle and alter lipid and amino acid metabolisms, producing lactic acid and ketones leading to metabolic acidosis (Bouzenna et al., 2016a). ASA also activated both cascades of extrinsic and intrinsic apoptotic pathways, as well as down regulated NF-kB activation and the phosphorylation of p38 (Bhattacharyya et al., 2014).The increased risk of bleeding was evident by a remarkable apoptosis in hepatic peri-venular zone in this study, and this finding was reinforced by the fact that one of side effects of ASA is to increase gastrointestinal and intracerebral hemorrhage (De Berardis et al., 2009; Sung et al., 2010).

In conclusion, the findings of this study indicated the adverse effects of BDF on all studied parameters and ASA on haemostatic and hepatic redox status and for the first time a possible synergistic action was found between BDF and ASA in the terms

of haemostatic markers and hepato-cellular features. Additional studies are suggested to search for other anticoagulant combinations to raise the potency of rodenticide.

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Table (1) Effects of brodifacoum, acetylsalicylic acid and their combinations on the haemostatic parameters .

Group / Parameter	C	BDF	ASA	BDF+ASA
Bleeding time (minutes)	0.48± 0.02	1.78± 0.24**	2.06± 0.36**	1.09± 0.03
Clotting time (minutes)	1.20± 0.26	2.97± 0.30	0.427± 0.04	3.58± 0.39*
Prothrombin time(seconds)	26.17± 6.03	182.6± 61.92**	21.80± 4.08	300.2± 0.03***
Factor VII level (%)	162.5± 25.69	6.40± 0.23**	184.0± 13.86	6.00± 1.79**
Factor IX level (%)	178.0± 37.53	28.00± 2.88**	269.5±* 35.51	13.38± 1.80**
Ca^{+2} level(nmol/mg protein)	0.89± 0.10	1.03± 0.03	0.76± 0.05	1.15± 0.03*

C: Control group; BDF: Brodifacoum group was exposed to 4 gm/kg BW as single bait; ASA: Acetylsalicylic acid group was exposed 60 gm/kg BW dissolved in 10 mL distilled water; BDF+ASA: Brodifacoum and acetylsalicylic acid group was exposed to the same previous doses of BDF and ASA in combination for one day. Results are expressed as mean ± SEM of six rats per group.

*= P<0.05, **= P<0.01, and ***= P< 0.001 versus the control group (one-way ANOVA followed by Newman-Keuls multiple comparison test).

Table (2) Effects of brodifacoum, acetylsalicylic acid and their combinations on the hematological parameters for all previous groups .

Group / Parameter	C	BDF	ASA	BDF+ASA
PLT count (10^9/l)	518.7± 49.03	382.8± 29.55	341.8± 58.75	112.5± 3.75***
MPV (fl)	8.70± 0.22	8.73± 0.19	9.50± 0.17	7.33± 0.44*
EOS (10^9/l)	0.50± 0.15	0.20± 0.04*	0.40± 0.11	0.25± 0.05
LYM (10^9/l)	3.53± 0.93	1.17± 0.04*	2.50± 0.37	2.83± 0.55
MON (10^9/l)	0.66± 0.06	0.28± 0.03**	0.80± 0.05	0.42± 0.13

PLT: platelet; MPV: mean platelet volume; EOS: eosinophils; LYM: lymphocytes; MON: monocytes.
Results are expressed as mean ± SEM of six rats per group.

*= P<0.05, **= P<0.01, and ***= P< 0.001 versus the control group (one-way ANOVA followed by Newman-Keuls multiple comparison test).

Table (3) Effects of brodifacoum, acetylsalicylic acid and their combinations on the hepatic oxidative stress parameters for all previous groups.

Group / Parameter	C	BDF	ASA	BDF+ASA
LPO level (nmol/mg protein)	0.17± 0.01	0.29± 0.03*	0.17± 0.02	0.19± 0.02
NO level (nmol/mg protein)	0.89± 0.09	0.58± 0.07**	0.55± 0.04*	0.52± 0.06**
CAT activity (U/mg protein)	0.61± 0.02	0.39± 0.06*	0.90± 0.05**	0.37± 0.06*

LPO: lipid peroxidation; NO: nitric oxide; CAT: catalase.
Results are expressed as mean ± SEM of six rats per group.
*= P<0.05 and **= P<0.01 versus the control group (one-way ANOVA followed by Newman-Keuls multiple comparison test).

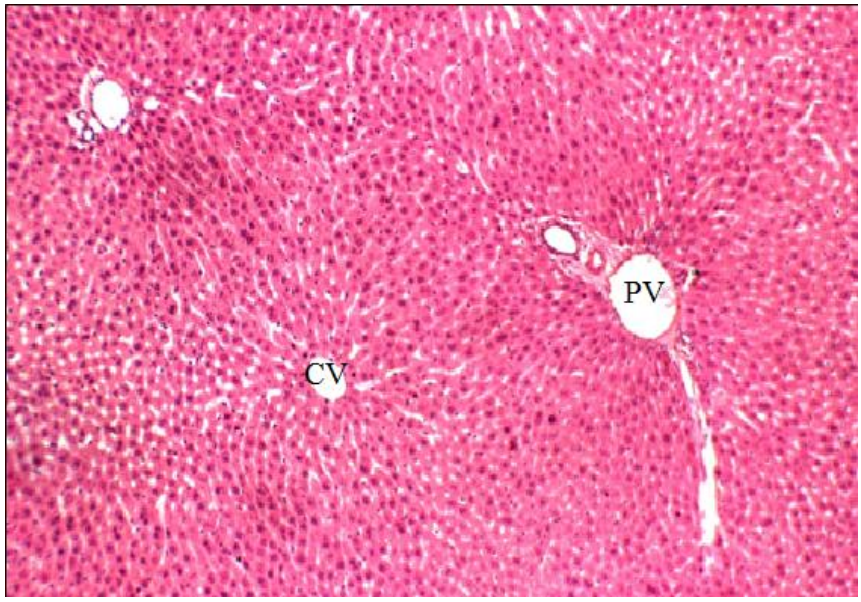

Fig. 1 Liver of control group, showing average central vein and portal tract (H&E X 200)

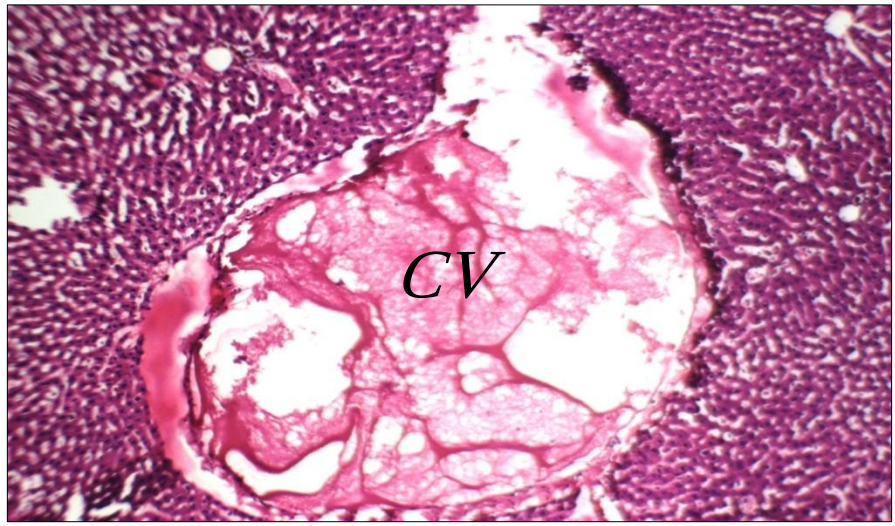

Fig. 2 Liver of BDF group,showing markedly dilated central vein filled by hemolyzed blood (CV) (H&E X 200)

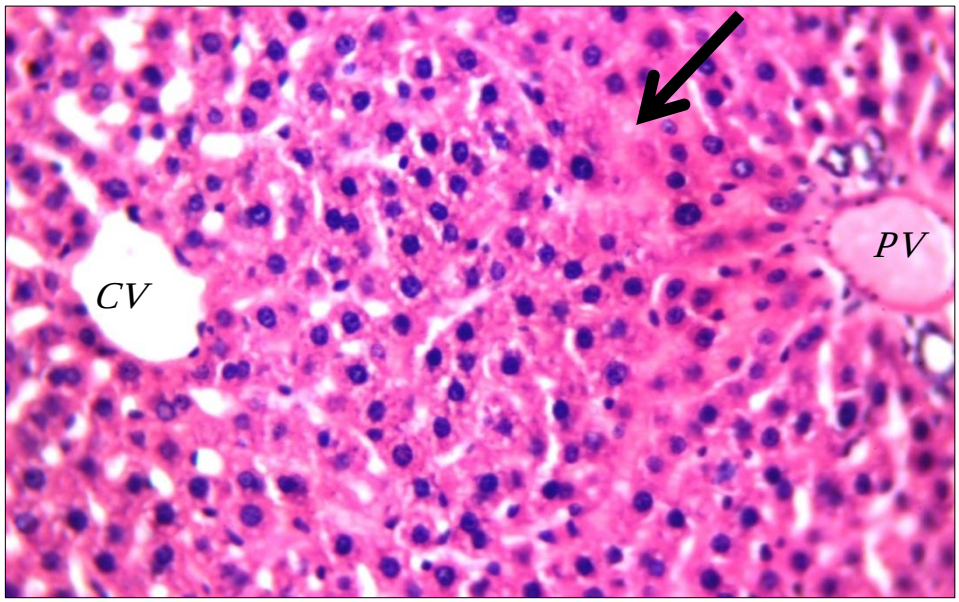

Fig. 3 Liver of BDF group,showing average central vein surrounded by hepatocytes with deeply stained nuclei .Note: Necrotic area were observed in region of portal area(Black arrow).(H&E X 400)

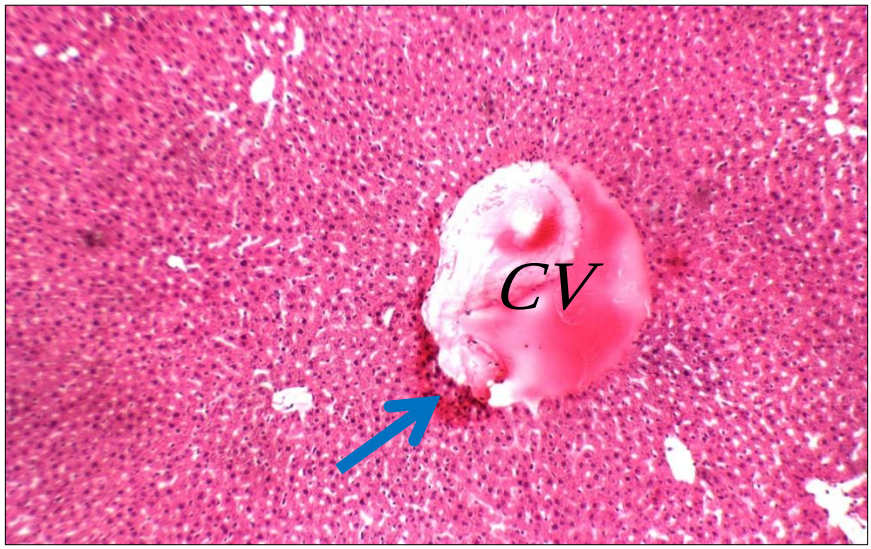

Fig. 4 Liver of ASA group, showing dilated central vein filled by hemolyzed blood (CV) Note:Patches of degenerated hepatocytes with deeply stained pyknotic nuclei were seen (blue arrows) (H&E X 200)

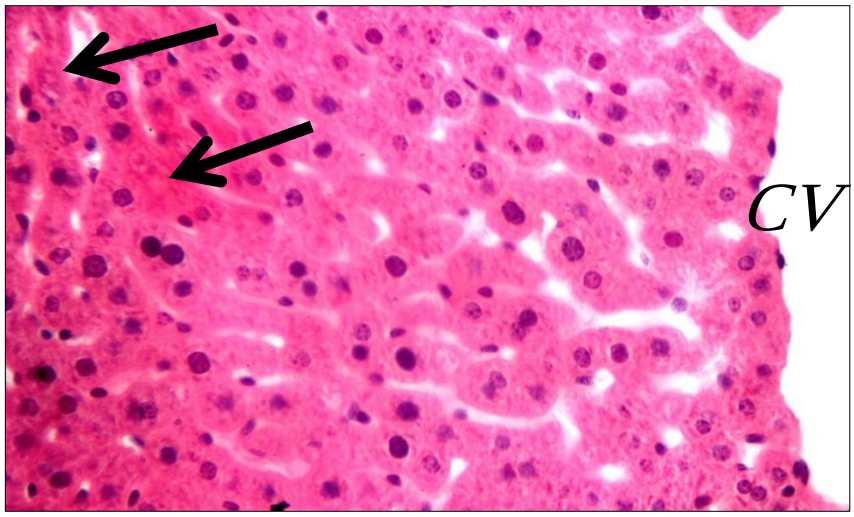

Fig. 5 Liver of ASA group, showing hepatocytes with eosinophilic cytoplasm .Note: Necrotic area (balck arrow)(H&E X 400)

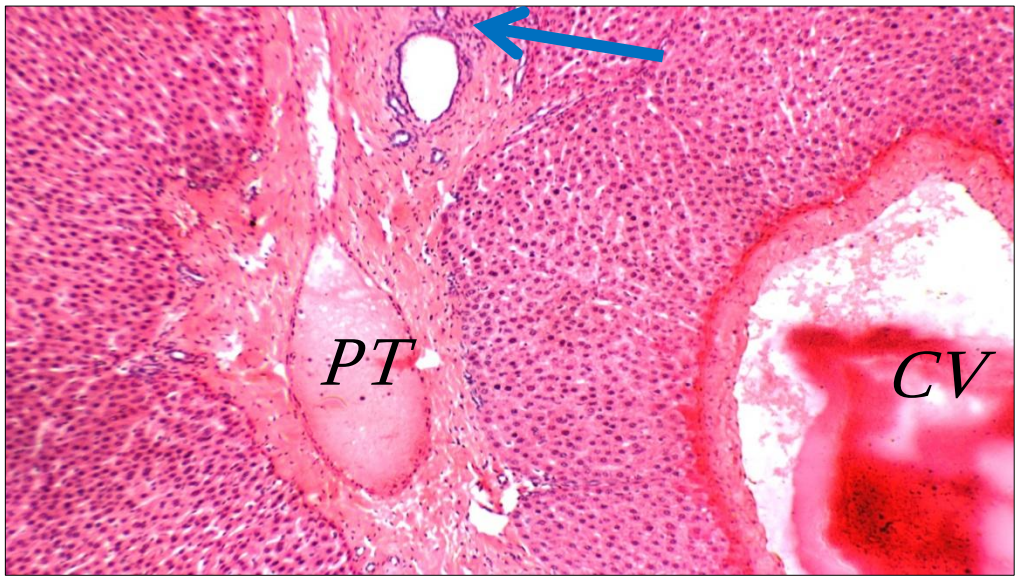

Fig. 6 Liver of (BDF+ASA) group,showing markedly dilated central vein filled by hemolyzed blood (CV) and expanded portal tract (PT) surrounded by fibrotic area . Note: Infilteration of inflammatory cells (blue arrow) (H&E X 200)

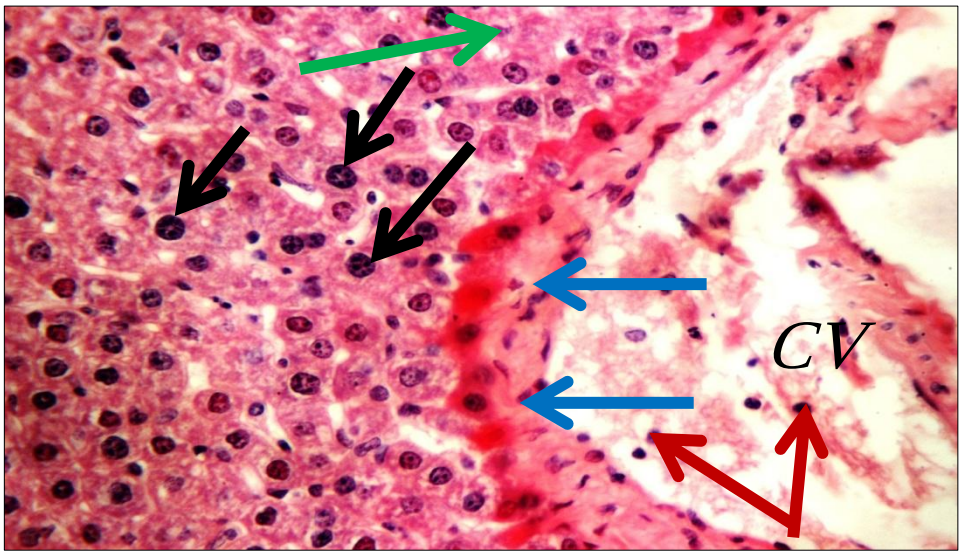

Fig. 7 Liver of BDF+ASA group, showing markedly dilated central vein filled by hemolyzed blood (CV) surrounded by degenerated hepatocytes with pyknotic nuclei.(blue arrows) Note:Necrotic area(green arroaw) , apoptotic nuclei (black arrows) and inflammatory cells (red arrows)(H&E X 400)

تأثيرات البروديفيكوم أو حمض الأستيل سلسيلك ومزيجهما علي دلالات تجلط الدم ، الدم والكبد في الجرذ الحقلي : Rattus rattus

لأن القوارض تشكل مشكلة عملية خطيرة لحقول المحاصيل ليس فقط عن طريق التسبب في أضرار لمراحل نمو النبات المختلفة ولكن أيضًا عن طريق التسبب في تلوث النقل والتخزين، فإن التحكم في القوارض يعد ضرورة ملحة.

تمثل المقاومة الوراثية الناشئة ضد مبيدات القوارض أحد أكثر التحديات التي تواجه برامج الاستئصال. حاولت الدراسات السابقة التغلب على هذه المشكلة العملية باستخدام الطعوم المركبة ذات التكلفة المنخفضة والقلق من ناحية السمية البيئية ولكن من وجهة النظر الإنسانية تستغرق أيام طويلة حتى الموت. لذلك، يوصى بشدة بتطوير التدخلات التي تتخذ خطوة واسعة أخرى على الطريق لتعزيز فعالية مبيدات القوارض المضادة للتخثر. إن التأثير المضاد للتخثر في حمض الأستيل سلسيلك

راسخ ويتم بواسطة عن طريق أستلة عوامل تخثر الدم وقمع تخليقها، تثبيط الصفائح الدموية، منع تكوين الثرومبين، وتسريع إنحلال الفيبرين.
السوبرورفريينز قوية للغاية كمبيدات قوارض طويلة الأمد تمنع تخثر إنزيم فيتامين ك، مما يقلل من إعادة تدوير فيتامين ك، وهو أمر ضروري لتفعيل العديد من عوامل التخثر. من بين السوبرورفريينز التي يتم دمجها عادة في طعم القوارض، يعتبر البروديفيكوم هو الأكثر إستخدامًا على نطاق واسع. ويعتقد أن القوة البيولوجية للبروديفيكوم تعزى إلى إرتفاع قابليتها للذوبان في الدهون وزيادة التقارب للأنسجة الكبدية والإنزيمات. ومع ذلك، يبدو أن تأثيره المثبط على نشاط تخثر الدم ودورة فيتامين ك في كبد الفئران عابر.
أجريت هذه الأطروحة لتحديد التأثير التآزري المحتمل للبروديفيكوم بالإقتران مع حمض الأستيل سلسيلك كصياغة جديدة لمبيد القوارض فيما يتعلق بالتغييرات المحتملة في وظائف تخثر الدم والدم والكبد والكلى ودلالات الإجهاد التأكسدي بالإضافة إلى البناء النسيجى لبعض الأعضاء المستهدفة بشكل خاص على أمل نقل النتائج إلى المجال العملي.
أظهرت نتائج هذه الدراسة أن:
١. تتميز الفئران المعرضة لمزيج من البروديفيكوم مع حمض الأستيل سلسيلك بإطالة كبيرة في زمن التجلط وزمن البروثرومبين، وإنخفاض في العوامل سبعة وتسعة ومستويات الكالسيوم المتأينة مقابل تلك المعرضة فقط للبروديفيكوم .
٢. أدى إعطاء حمض الأستيل سلسيلك مع البروديفيكوم إلى إستحثاث الإجهاد التأكسدي كما يتضح من زيادة مستوى اللبيد بيروكسيد فى البلازما وإنخفاض نشاط الكاتاليز وأكسيد النيتريك بالكبد مقارنةً بإعطاء البروديفيكوم وحده.
٣. تتميز الفئران المعرضة لمزيج من البروديفيكوم مع حمض الأستيل سلسيلك بانخفاض ملحوظ فى عدد الصفائح الدموية ومتوسط حجم الصفائح الدموية مقابل تلك المعرضة فقط للبروديفيكوم.
٤. التغيرات الهستوباثولوجية في الكبد حيث ادى البروديفيكوم أو من الأستيل سلسيلك أسيد الى اتساع الأوردة المركزية و احتوائها على دم متحلل وظهور الآنوية داكنة الاصتباغ وبينما المجموعة المعرضة لجرعة

مشترك لمزيج من الأستيل سلسيلك أسيد مع البروديفيكوم فقد كان لها تاثير كبير على التركيب الهستوباثولوجي للكبد من حيث اتساع الآوعية الدموية ووجود دم متحلل فى الوريد المركزى وانتشار الخلايا المناعية وظهور الآنوية داكنة الآصتباغ فى احدى مراحل الموت الخلوى حيث تحتوى على تجمعات من الكروماتين داكن الصبغة .

الخلاصة :

اضافة حمض الاستيل سلسيلك الى البروديفيكوم يحسن من كفاءته كمبيد للقوارض .